Chapter 12. Cannabinoids and drug addiction

Chapter · December 2015
DOI: 10.1016/B978-0-12-417041-4.00012-6

2 authors:

Maria Antonietta De Luca
Università degli studi di Cagliari
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Liana Fattore
Italian National Research Council
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CHAPTER 12

Cannabinoids and drug addiction

Maria Antonietta De Luca\textsuperscript{1,2} and Liana Fattore\textsuperscript{3}

\textsuperscript{1}Department of Biomedical Science, Neuropsychopharmacology Section, University of Cagliari, Italy \textsuperscript{2}National Institute of Neuroscience, University of Cagliari, Italy \textsuperscript{3}CNR Neuroscience Institute — Cagliari, National Research Council of Italy, Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Cagliari, Italy

DRUG ABUSE AND ADDICTION

Addiction is defined as obsessive thinking and compulsive need for something, like drugs, food or sex, despite the resulting negative consequences. In physiological conditions, the brain reward system reinforces behaviors required for species survival, including sexual activity, nursing, and eating. Drug abuse hijacks such a neural pathway and replaces normal reward-related behavior, leading over time to uncontrollable drug seeking and taking and, ultimately, to drug addiction. Yet, engaging in non-drug-related activities involving “natural” rewards such as food, sex, and playing activity could also activate the brain “pleasure circuit” and result in addiction. While it is quite intuitive to distinguish chemical from behavioral addictions, other important conceptual distinctions are necessary.

The terms “drug abuse,” “addiction,” and “dependence” are not interchangeable and cannot be used as synonyms, although they represent different ends of the same disease process.

While drug abuse refers to the taking of drugs for non-medical purposes, typically because of the drug’s positive subjective effects, “drug addiction” develops after repeated substance use and normally includes a pressing urge to take the drug and difficulties in controlling its consumption. Clinically, the occasional but limited use of a drug is different from escalated drug use and the emergence of chronic drug dependence. Yet, for some individuals drug addiction develops over time and usually begins with misuse, moving toward abuse and resulting in addiction (Figure 12.1). Once addicted, many drug users feel completely powerless and persevere in using the drug in the face of potentially dangerous health consequences.

Drug addiction is a chronically relapsing disorder characterized by (1) compulsion to seek and take the drug, (2) loss of control in limiting drug intake despite harmful consequences, (3) emergence of a withdrawal state and negative emotional
state (e.g., dysphoria, irritability, anxiety) when access to the drug is prevented, (4) greater importance given to obtaining the drug than to other activities or goals, and (5) development of tolerance. Addiction involves a complex neuropharmacologic behavioral cycle in which positive reinforcement exerted by the drug and the negative state of withdrawal drive the user to extremes to obtain the drug. Conversely, drug dependence refers to the need to continue taking a drug to avoid withdrawal effects on drug discontinuation, and is not necessarily associated with the rewarding properties of the drug nor is related to its abuse liability. Several drugs, such as the selective serotonin reuptake inhibitors (SSRIs), can induce dependence but do not possess positive reinforcing properties.

Closely linked to the concept of drug dependence, drug withdrawal (e.g., abstinence) is caused by chronic drug use cessation, is characterized by signs and physical symptoms usually opposite to the acute effects of the drug, and is associated with the emergence of negative emotional feelings. Even if it is possible for drug addicts to desist from drug use, maintaining abstinence is extremely difficult. The inability to remain abstinent is often referred to as relapse and consists of a process by which an abstaining individual falls again into old behavioral patterns and substance use, i.e., the return to drug use after a period of withdrawal. Relapse is the most common outcome of recovery programs treating addictive behaviors, for which craving (i.e., the strong, often uncontrollable desire to use the drug) represents a major risk factor. At the clinical level, craving and relapse are now considered major challenges in drug addiction treatment, and preventing relapse when an abstinent patient is exposed to the drug or drug-related stimuli is still demanding.
In human drug users, a variety of events or stimuli can precipitate drug craving and elicit the urge to use the drug, which ultimately results in relapse in abstinent individuals. The presentation of the drug itself, or stimuli previously associated with drug delivery (e.g., place, people, paraphernalia) or stressful events (e.g., loss of beloved person or employment, divorce), all increase the motivation to engage in drug taking and the likelihood of relapse (Bossert et al., 2013). Likewise, the same conditions that trigger relapse in humans are also able to reinstate drug-seeking behavior in laboratory animals, and include small doses of the drug itself, environmental stimuli (e.g., visual cues) previously associated with the drug delivery, and exposure to stressors such as electrical footshock or food deprivation (Shaham et al., 2003). Table 12.1 illustrates different factors triggering relapse in humans and drug-seeking reinstatement in laboratory animals.

### Table 12.1 Factors Triggering Relapse in Both Humans and Animals

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<thead>
<tr>
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<th>Humans</th>
<th>Animals</th>
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<tr>
<td><strong>Drug priming</strong></td>
<td>A single exposure to the previously abused drug</td>
<td>Not contingent, not reinforced acute drug injection</td>
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<tr>
<td><strong>Environmental cues</strong></td>
<td>People, places or things associated with past drug use</td>
<td>Context, cue light or tone previously associated with drug delivery</td>
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<td><strong>Stress</strong></td>
<td>Divorce</td>
<td>Intermittent footshock</td>
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<td>Loss of employment or beloved person</td>
<td>Acute 1 day food deprivation</td>
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<td></td>
<td>Violence</td>
<td>Yohimbine (chemical stress)</td>
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<td>Negative or challenging emotions</td>
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In human drug users, a variety of events or stimuli can precipitate drug craving and elicit the urge to use the drug, which ultimately results in relapse in abstinent individuals. The presentation of the drug itself, or stimuli previously associated with drug delivery (e.g., place, people, paraphernalia) or stressful events (e.g., loss of beloved person or employment, divorce), all increase the motivation to engage in drug taking and the likelihood of relapse (Bossert et al., 2013). Likewise, the same conditions that trigger relapse in humans are also able to reinstate drug-seeking behavior in laboratory animals, and include small doses of the drug itself, environmental stimuli (e.g., visual cues) previously associated with the drug delivery, and exposure to stressors such as electrical footshock or food deprivation (Shaham et al., 2003). Table 12.1 illustrates different factors triggering relapse in humans and drug-seeking reinstatement in laboratory animals.

THE MODULATING ROLE OF THE ENDOCANNABINOID SYSTEM IN DRUG-INDUCED REWARD

Cannabis is the most commonly abused illegal drug in the world and its main psychoactive ingredient, Δ⁹-tetrahydrocannabinol (THC), produces rewarding effects in humans (Hart et al., 2005), non-human primates (Tanda et al., 2000), and rodents (Braida et al., 2004). Over the last two decades, an endogenous system comprised of cannabinoid type 1 (CB₁) and type 2 (CB₂) receptors, several endogenous ligands (i.e., endocannabinoids) and enzymes responsible for their synthesis and degradation have been discovered and partially characterized (see elsewhere in this book).

Pleasurable behaviors such as sexual activity, eating, nursing, parenting, social interactions, and play activity are conserved strongly in evolution, are essential
for development and survival, and represent pleasant experiences with a high reward value. As such, they can act as robust rewarding stimuli in both humans and animals. Remarkably, rewarding behaviors activate the same brain circuits that mediate the positive reinforcing effects not only of drugs of abuse but also those of other forms of addiction, such as pathological gambling and food addiction. For decades, the dopaminergic and opioid endogenous systems have been considered the most important neurotransmission systems in mediating brain reward processes. Yet, given the involvement of the endocannabinoid system in a variety of physiological functions at both the central and peripheral level, it is not surprising that it takes part in the complex machinery that regulates gratification and perception of pleasure (Fattore et al., 2010a).

Experimental findings strongly suggest a major involvement of the endocannabinoid system in general brain reward functions including drug abuse, as natural and synthetic cannabinoids and endocannabinoids can produce rewarding effects in humans and laboratory animals (Fattore et al., 2001; Hart et al., 2005; Justinova et al., 2005; Seely et al., 2012). Accordingly, cannabinoid CB₁ receptors are present in brain areas involved in reward processes, and their activation by endogenous, plant-derived or synthetic agonists produces rewarding effects per se and also increases those of drugs of abuse (Vlachou and Panagis, 2014). Conversely, pharmacological or genetic blockade of cannabinoid CB₁ receptors reduces the rewarding effects of drugs of abuse and prevents their activation of dopaminergic neurotransmission (Cossu et al., 2001; Le Foll and Goldberg, 2005). Brain levels of the two best characterized endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), are altered either by rewarding activity (Fattore et al., 2010a) or drugs of abuse (Basavarajappa et al., 2000; Vigano` et al., 2003; Cippitelli et al., 2008). However, discrepant data concerning the facilitatory role played by the endocannabinoid system in brain stimulation reward have also been reported (Sañudo-Peña et al., 1997; Cheer et al., 2000). Actually, the influence of the endocannabinoid system on brain reward processes has been hypothesized to depend on the degree of activation of the different brain areas involved and to represent a mechanism for fine-tuning dopaminergic activity (Perra et al., 2005; Pillolla et al., 2007; Melis and Pistis, 2012). Notably, the endocannabinoid system appears to be also involved in the ability of drugs and drug-associated cues to reinstate drug-seeking behavior in animal models of relapse (Fattore et al., 2007a,b). Indeed, CB₁ receptor stimulation may elicit relapse not only to cannabinoid seeking (Spano et al., 2004; Fattore et al., 2010b) but also to heroin (De Vries et al., 2003; Fattore et al., 2003, 2005b) and nicotine seeking (Gamaleddin et al., 2012), all effects that are significantly attenuated, when not fully prevented, by pretreatment with the CB₁ receptor antagonist rimonabant. However, corroborating data on the involvement of the cannabinoid system in stress-induced reinstatement are still rather scarce (Vaughn et al., 2012).
ASSOCIATED POLYMORPHISMS IN THE CANNABINOID CB₁ RECEPTOR (CNR1) AND FATTY-ACID AMIDE HYDROLASE (FAAH) GENES

Many works have repeatedly associated substance dependence and drug-related behaviors with polymorphisms in the CNR1 and FAAH genes (López-Moreno et al., 2012). CNR1 is located on chromosome 6 in the 6q15 cytogenetic band and encodes a seven-transmembrane domain protein of 472 amino acids, whereas FAAH is located on chromosome 1 in the 1p33 cytogenetic band and encodes one transmembrane domain protein of 579 amino acids. A number of mutations described in these genes lead to altered mRNA stability and transcription rate or to a reduction of the activity of the encoded protein. Increasing evidence shows that these functional mutations are related to marijuana, cocaine, alcohol, heroin, and nicotine dependence (Hoenicka et al., 2007; Proudnikov et al., 2010; Bidwell et al., 2013).

One of the most compelling associations is with the C385A single nucleotide polymorphism (SNP), which is found in the human FAAH gene (385C to A) and in homozygous form is over-represented in subjects with problem drug use. This SNP, which converts a conserved proline residue in FAAH to threonine (P129T), suggests a potential link between functional abnormalities in the endocannabinoid system and drug abuse (Tyndale et al., 2007).

Another SNP in CNR1, rs2023239, has been associated with cannabis dependence diagnosis and intermediate phenotypes, including abstinence-induced withdrawal, cue-elicited craving, and parahippocampal activation to cannabis cues (Schacht et al., 2012). The rs2023239 G allele was shown to predict lower volume of bilateral hippocampi among cannabis users relative to controls, suggesting that CNR1 rs2023239 variation may predispose smaller hippocampal volume after heavy cannabis use (Schacht et al., 2012).

CB₁ RECEPTOR AND FAAH KNOCKOUT MICE IN CANNABINOID ADDICTION RESEARCH

The involvement of the endocannabinoid system in drug addiction was initially studied by a pharmacological approach, i.e., the use of compounds with different affinities for cannabinoid CB₁ or CB₂ receptors or for the enzymes involved in endocannabinoid inactivation. Genetically modified mice with selective mutations in specific components of the endocannabinoid system have been generated, which allowed essential progress in establishing the specific contribution of each component to drug addiction. In particular, mice lacking the CB₁ receptor or the enzyme responsible for catabolism of the endocannabinoid anandamide, i.e., fatty-acid amide hydrolase (FAAH), represent critical tools for the identification of the CB₁ receptor and anandamide as potential targets for drug addiction treatment.
As consequences of CB₁ receptor inactivation, CB₁ receptor knockout (CB1-KO) mice do not respond to cannabinoid drugs in the classical tetrad of behavioral test, demonstrating the exclusive role of the CB₁ receptor in mediating analgesia, hypothermia, hypolocomotion, and hypotension (Ledent et al., 1999). Also reinforcement resulted the number of affected, as CB1-KO mice do not self-administer CB₁ receptor agonists nor morphine (Ledent et al., 1999), while they readily self-administer cocaine, amphetamine, and nicotine to the same extent as in wild-type (WT) mice (Cossu et al., 2001). Yet, nicotine was reported to be unable to induce conditioned place preference (CPP) in CB1-KO mice (Castañé et al., 2002). CB₁ receptors were also shown to play an important role in alcohol preference, dependence, and stress-stimulated ethanol drinking, as CB1-KO mice fail to develop a clear alcohol CPP, display significantly lower baseline alcohol consumption compared to WT mice (Houchi et al., 2005; Thanos et al., 2005), and do not display ethanol withdrawal symptoms (Racz et al., 2003). Moreover, CB₁ receptors appear to be critical for the consolidation of cocaine reinforcement and, although to a lesser extent, for its acute rewarding effects (Soria et al., 2005). When compared to WT mice, CB1-KO animals display a significant reduction in the breakpoint level for cocaine self-administration under a progressive-ratio (PR) schedule of reinforcement (Xi et al., 2011). They also show reduced motor and striatal signaling responses to cocaine and amphetamines, and altered cocaine-induced sensitization (Corbillé et al., 2007).

These mutant mice were useful to also appreciate the involvement of the endocannabinoid system in the reinforcing effects of 3,4-methylenedioxymethamphetamine (MDMA). In fact, CB1-KO mice not only are less responsive than WT mice to the stimulating effect of MDMA on locomotor activity, body temperature, and anxiogenic-like responses, but they also fail to self-administer MDMA (Tourínol et al., 2008), and do not develop sensitization to the locomotor stimulant effects of amphetamine (Thiemann et al., 2008).

The involvement of the CB₁ receptor in drug-induced reward may be ascribed, at least in part, to the general modulatory role of the endocannabinoid system in hedonic reward perception and processing (Friemel et al., 2014). In support of this, CB2-KO mice (1) do not develop nicotine CPP (Ignatowska-Jankowska et al., 2013), (2) do self-administer significantly less nicotine than WT mice, and (3) do not exhibit somatic signs of nicotine withdrawal (Navarrete et al., 2013). Moreover, deletion of CB₁ receptors decreases operant behavior and motivation to obtain highly palatable isocaloric food in mice, without affecting the operant responses to obtain standard pellets (Guegan et al., 2013). Notably, cannabinoid receptor-dependent changes in drug- and food-oriented appetitive behaviors may reflect more general changes in reward-learning processes, including those whereby the incentive value of the drug or food is assigned to instrumental outcomes or outcome-associated stimuli. Accordingly, CB1-KO mice show significant deficits in outcome-selective instrumental devaluation (Crombag et al., 2009), suggesting a crucial role for the CB₁ receptor in the capability to represent
and use sensory-specific outcome representations to alter appetitive behaviors. In line with this hypothesis, a reduced sensitivity to reward in CB1-KO mice has been described (Sanchis-Segura et al., 2004).

Genetic deletion of FAAH is associated with a pattern of intense CB1 receptor-dependent behavioral responses, including hypomotility, analgesia, catalepsy, and hypothermia (Cravatt et al., 2001). Mice lacking this enzyme display (1) less severe THC precipitated withdrawal responses (Schlosburg et al., 2009), (2) an enhanced expression of nicotine CPP, (3) exacerbated physical somatic signs of spontaneous nicotine withdrawal, and (4) an increased conditioned place aversion (CPA) in a mecamylamine-precipitated model of nicotine withdrawal (Merritt et al., 2008). In line with the notion that the brain endocannabinoid system is critically involved in the neural circuitry regulating alcohol consumption and motivation to consume alcohol, FAAH-KO mice also show higher selective preference for, and higher intake of, alcohol than WT littermates (Blednov et al., 2007) as well as a reduction in the severity of handling-induced convulsions following withdrawal from chronic ethanol exposure (Vinod et al., 2008). Importantly, repeated anandamide administration in FAAH-KO mice causes smaller CB1 receptor down-regulation and desensitization and shows lesser dependence liability than repeated THC (Falenski et al., 2010), which suggests that pharmacotherapies targeting endocannabinoid degrading enzymes are less likely to cause development of tolerance and dependence than direct CB1 receptor stimulation.

When comparing findings from FAAH-KO mice with those from CB1-KO mice the increasing endogenous cannabinoid level amplifies, while lack of CB1 receptor signaling lessens, nicotine reward and withdrawal. In support of this, a decreased cortical FAAH expression and elevated endocannabinoid transmission were observed in alcohol-preferring (AA) rats as compared with non-preferring (ANA) rats, which were accompanied by a compensatory down-regulation of CB1 receptor signaling (Hansson et al., 2007).

THE ENDOCANNABINOID SYSTEM IN HUMAN DRUG ADDICTION

The endocannabinoid system is involved in reward mechanisms that facilitate the hedonic value of natural (Kirkham, 2003; De Luca et al., 2012) and drug (Fattore et al., 2008) rewards. This system participates in the primary rewarding effects of cannabinoids, nicotine, alcohol, and opioids (mostly through the release of endocannabinoids in the ventral tegmental area), and in the common mechanisms underlying drug addiction and relapse to drug-seeking behavior (by also mediating the motivational effects of drug-related environmental stimuli). In turn, many drugs of abuse, including cannabinoids, opioids, alcohol, and nicotine, can alter differently the levels of endocannabinoids in selected brain regions.
In rats chronically exposed to opiates, nicotine, ethanol, or cocaine, different changes in the brain contents of endocannabinoids and CB\(_1\) receptor levels have been described. In particular, alcohol and nicotine increased anandamide levels in the limbic forebrain (González et al., 2004b; Buczynski et al., 2013), while chronic morphine modulated the contents of 2-AG in the rat brain (Viganò et al., 2003).

In humans, recent studies have revealed diverse response by the endocannabinoid system to long-term exposure to several drugs of abuse, and cannabis, ethanol, opioids, nicotine, and cocaine were found to alter the endocannabinoid system regardless of their diverse pharmacological mechanism of action. This section of the chapter will explore clinical evidence of the alterations of the endocannabinoid system induced by the consumption of drugs of abuse.

**CANNABIS**

According to the last World Drug Report, cannabis is still the most widely used illicit drug worldwide (UNODC, 2013). A new generation of potent synthetic cannabinoids, which can induce serious health risks, have recently been reported in virtually all European countries (EMCDDA, 2013), and are dominating the US market under the brand names of “K2” or “Spice” (Fattore and Fratta, 2011; Seely et al., 2012). Cannabis induces dependence less readily than the majority of other illicit drugs. Yet, about 9% of marijuana users become dependent, and this proportion increases up to 17% among individuals who initiate use at a young age, and up to 25–50% among daily users (SAMHSA, 2012). It is worth noting that 23% of all substance abuse treatment admissions in the United States are for cannabis-related disorders, second only to alcohol-related disorders (SAMHSA, 2012).

Epidemiological studies have demonstrated that chronic non-medical cannabis use can lead to severe adverse health effects such as dependence syndrome, impaired respiratory function, cardiovascular diseases, adverse effects on adolescent psychosocial development and mental health, and residual cognitive impairment (Hall and Degenhardt, 2009, 2014). For these reasons, alterations in endocannabinoid signaling have been extensively examined. It was found that after cannabis use, CB\(_1\) or CB\(_2\) receptor mRNA (Nong et al., 2002; Rotter et al., 2013) are increased in peripheral blood cells, and that 2-AG cerebrospinal serum levels are also increased while anandamide is absent (Morgan et al., 2013). Consistent with this study, among healthy people who had not used cannabis significantly in their lives (<five times) and those who were low-frequency users (<50 times in their lifetime) no differences in anandamide levels were observed (Leweke et al., 2007), suggesting that at least low-level cannabis use does not down-regulate endocannabinoid signaling. Another clinical study showed that in peripheral blood cells serum anandamide and CB\(_2\) receptor mRNA, but not CB\(_1\) receptor mRNA, are increased in high frequency users (who had smoked marijuana ≥20 times but had abstained from cannabis for ≥6 months) compared with no or infrequent (≤five times lifetime) cannabis use (Muhl et al., 2014). However, animals showed a depletion of anandamide following repeated THC administration (Di Marzo et al., 2000).
During frequent cannabis use a series of poorly understood neuroplastic changes occur, which lead to the development of dependence. Cannabis withdrawal in heavy users is commonly followed by increased anxiety, insomnia, loss of appetite, migraine, irritability, and restlessness (Haney, 2005). Tolerance to cannabis and cannabis withdrawal symptoms are believed to be the result of the desensitization of CB$_1$ receptors by THC. Abstinence in cannabinoid-dependent individuals elicits withdrawal symptoms that promote relapse into drug use, suggesting that pharmacological strategies aimed at alleviating cannabis withdrawal might prevent relapse and reduce dependence.

Cannabinoid replacement therapy and CB$_1$ receptor antagonism are two potential treatments for cannabis dependence that are under investigation (Huestis et al., 2001; Allsop et al., 2014). However, abuse liability and adverse side effects may limit the value of these approaches. A potential alternative stems from the recognition that (1) frequent cannabis use may cause an adaptive down-regulation of the brain endocannabinoid signaling, and (2) that genetic traits that favor hyperactivity of the endocannabinoid system in humans may decrease susceptibility to cannabis dependence. Altogether, these findings suggest that pharmacological agents that elevate the levels of the endocannabinoids in the brain might alleviate cannabis withdrawal and dependence. One such agent, the FAAH inhibitor URB597, selectively increases anandamide levels in the brain of rodents and primates. Preclinical studies showed that URB597 produces analgesic, anxiolytic-like, and antidepressant-like effects in rodents, which are not accompanied by evident signs of abuse liability, pointing to FAAH inhibitors as a possible therapeutic avenue for the treatment of cannabis withdrawal (Clapper et al., 2009).

**ALCOHOL**

Starting from the 1970s onward, a growing body of evidence has suggested a link between the neuropsychological effects of cannabis and ethanol consumption. Since then, the role of the brain endocannabinoid system in alcohol abuse and dependence as well as its comorbidity with mood disorders have been widely investigated (Schmidt et al., 2002; Wang et al., 2003; Vinod and Hungund, 2005; Pava and Woodward, 2012). A potential cross-tolerance to ethanol among cannabis users was corroborated by numerous studies investigating the cognitive and psychomotor effects of these two substances in humans (Jones and Stone, 1970; MacAvoy and Marks, 1975). In a clinical study conducted in adolescents with alcohol use disorders, over 70% reported use of cannabis within the past year, with a mean frequency of smoking marijuana ranging between 16 and 20 days per month (Martin et al., 1996). In turn, individuals in treatment for cannabis use disorders increased the frequency of alcohol drinking over a period of 1 year following treatment (Stephens et al., 1994). Accordingly, daily cannabis users significantly increased self-reported ethanol craving and consumption during a 2-week abstinence from marijuana (Peters and Hughes, 2010). Importantly, alcoholism often implies enhanced impulsivity and aggression that may induce suicide
Levels of endocannabinoids and CB₁ receptors are altered in the prefrontal cortex of depressed and alcoholic suicide victims (Ashton and Moore, 2011; Erdozain et al., 2014), which further strengthens the role of the endocannabinoid signaling in alcoholism and suicide (Vinod and Hungund, 2006).

Besides the high comorbidity between alcohol use disorders and cannabis use disorders, much evidence has also been provided in support to the notion that some of the pharmacological and behavioral effects of alcohol may be mediated through the endocannabinoid signaling system. Recent studies have demonstrated a down-regulation of CB₁ receptor function and signal transduction by chronic alcohol intake, which probably results from the persistent stimulation of CB₁ receptors by the endogenous agonists anandamide and 2-AG, the synthesis of which is increased in the limbic forebrain by chronic alcohol treatment (González et al., 2002). This enhanced formation of endocannabinoids may subsequently influence the release of neurotransmitters.

DBA/2 mice, known to avoid alcohol intake, display reduced brain CB₁ receptor function, in line with other studies where the CB₁ receptor antagonist SR141716A was shown to prevent voluntary alcohol intake in rodents. Similarly, CB₁ receptor activation promoted alcohol craving, suggesting a role for the CB₁ receptor gene in excessive drinking and development of alcoholism (Basavarajappa and Hungund, 2002).

In humans, alcohol dependence has been associated with a down-regulation of CB₁ receptors, while suicide is thought to be related to the up-regulation of these receptors in the ventral striatum (Vinod et al., 2010). Besides chronic alcohol use, other studies indicate that acute consumption of a moderate amount of ethanol affects the levels of the endocannabinoids in some brain regions (Feuerecker et al., 2012). Indeed, both anandamide and 2-AG levels were found significantly reduced in healthy volunteers after consumption of a moderate amount (28 g of ethanol) of red wine (Feuerecker et al., 2012).

Accumulating evidence continues to link certain aspects of the endogenous cannabinoid system with alcohol dependence, negative-reinforcement learning, and the modulation of stress responses. Specific alterations in brain regions related to stress and negative-reinforcement learning have been reported to exist in type 1 alcoholics, which are anxiety prone and characterized by adult onset alcoholism, and in type 2 alcoholics, which are characterized by impulsive, antisocial behavior and teenage-onset alcoholism (Cloninger, 1995).

Endocannabinoids have both anxiogenic and anxiolytic properties (Bortolato et al., 2006; Moreira and Wotjak, 2010), and different brain regions seem to be responsible for the anxiogenic and anxiolytic properties of the endocannabinoids (Rubino et al., 2008). In a recent study, endocannabinoid levels were measured in the amygdala and hippocampus of type 1 and type 2 alcoholic postmortem brains, and compared with analogous samples from a group of non-alcoholic controls (Kärkkäinen et al., 2013). A statistically significant difference between the groups was found in the level of the putative endocannabinoid docosatetraenoylethanolamine.
DHEA, an ethanolamide derivative of the omega-3 fatty acid docosahexaenoic acid (DHA), in the amygdala but not in the hippocampus. Another human postmortem brain study reported significant differences only in the levels of anandamide in the NAc and cortical regions (Lehtonen et al., 2010), confirming that in human alcoholism changes in 2-AG brain content may not be as important as suggested by animal studies (González et al., 2002, 2004b; Rubio et al., 2007, 2009; Malinen et al., 2009).

Notably, recent studies have suggested a possible protective effect of cannabidiol (CBD) in cannabis withdrawal syndrome and adverse psychological effects (Niesink and van Laar, 2013). A recent study described the case of a young woman with cannabis withdrawal syndrome treated with CBD for 10 days (Crippa et al., 2013). Daily symptom assessments demonstrated the absence of significant withdrawal, anxiety, and dissociative symptoms during the treatment.

NICOTINE

Nicotine is the primary psychoactive compound of tobacco smoke, and it determines and maintains tobacco dependence. Several lines of evidence suggest a functional interaction between central cholinergic and endocannabinoid systems (Castañé et al., 2005; Maldonado et al., 2006). Epidemiological, pharmacological, and behavioral studies in humans point out a clear link between cannabis and nicotine abuse (Viveros et al., 2006). Accordingly, many animal studies have shown that the pharmacological manipulation of the elements of the endocannabinoid system strongly influence important aspects of nicotine dependence (Scherma et al., 2008).

For example, CB₁ receptor activation and inhibition enhances and attenuates respectively the rewarding effects of nicotine, and nicotine addiction has been associated with altered endocannabinoid modulation of reward processing in the nucleus accumbens (Jansma et al., 2013). However, while FAAH inhibition results in increased brain anandamide levels and therefore increased endocannabinoid tone, distinct effects of FAAH inhibition on nicotine reward have been reported in mice and rats (Muldoon et al., 2013). A major PPAR-α receptor component appears to mediate the anti-nicotine reward effects of FAAH inhibitors in rats (Mascia et al., 2011).

In rats, nicotine and endocannabinoids seem to enhance the reinforcing effects of both systems (Viveros et al., 2006), and changes in endogenous cannabinoid levels have been observed in different brain regions chronically exposed to nicotine, such as the brainstem, hippocampus, cerebral cortex, and striatum (González et al., 2002). In mice, the administration of nicotine facilitates the pharmacological responses, tolerance, and physical dependence induced by THC (Valjent et al., 2002), while CB₁ receptor agonists decrease nicotine somatic withdrawal signs in mice (Balerio et al., 2004). Furthermore, no differences were reported on the effect of FAAH inhibition in nicotine somatic withdrawal signs in mice and rats. However, differences in affective signs of withdrawal, such as anxiety and aversion, after FAAH blockade seem to be emerging. More generally, the effects of FAAH inhibitors on nicotinic behavioral responses in animals may be influenced by procedural differences, species differences, level of nicotine exposure, and degree of FAAH inhibition. These factors
are likely to play an important role in understanding the physiological function of FAAH in nicotine reward and withdrawal. Pharmacological inhibition of FAAH blocks nicotine self-administration and prevent nicotine-induced reinstatement in rats, suggesting that FAAH is a promising molecular target for tobacco dependence (Scherma et al., 2008; Muldoon et al., 2013).

In humans, CB$_1$ receptor antagonists were evaluated as anti-smoking therapy, with some promising results (Cohen et al., 2005). For example, when used in combination with a nicotine patch, rimonabant was well tolerated and increased smoking cessation rates over rimonabant alone, with little weight gain after cessation in either group, even among weight-concerned smokers (Rigotti et al., 2009). However, evidence for using these antagonists in maintaining smoking abstinence remained inconclusive, mainly because of the adverse events observed, including nausea, upper respiratory tract infections and, more worryingly, depression and suicidal thoughts in people taking CB$_1$ receptor antagonists for weight control (Cahill and Ussher, 2007).

**COCOAINE**

Cocaine use represents an important worldwide health problem due to the large number of physical, legal, social, cognitive, psychological, and psychiatric associated problems and comorbidity (O’Brien and Anthony, 2005; Karila et al., 2012). Cannabis is one of the most widely used illicit substances among users of psychostimulants such as cocaine and amphetamines. Interestingly, recent evidence points toward the involvement of the endocannabinoid system in the neurobiological processes related to stimulant addiction (Olìere et al., 2013).

In cocaine addicts, a link between exposure to cocaine and dysregulated brain endocannabinoid signaling has been reported. Indeed, CB$_1$ but not CB$_2$ receptor protein and G-protein coupled receptor regulatory kinases (GRK) were reported to be significantly reduced in the prefrontal cortex with a simultaneous receptor redistribution and/or internalization, i.e., they were decreased in membranes and increased in cytosol (Álvaro-Bartolomé and García-Sevilla, 2013). Thus, in cocaine-addicted subjects the reductions of CB$_1$ receptors and GRK determines a receptor desensitization, a notion further strengthened by the finding that chronic cocaine reduces CB$_1$ receptor protein also in the cerebral cortex of mice and rats (Álvaro-Bartolomé and García-Sevilla, 2013).

The evaluation of plasma-free endocannabinoids and circulating endocannabinoid-related lipids in abstinent cocaine addicts can be useful for the identification of biomarkers for cocaine addiction. Pavón and colleagues (2013) found that plasma acyl derivatives are altered in abstinent cocaine-addicted subjects. In the same subjects, free N-acyl-ethanolamines were found to be increased while 2-acyl-glycerols were decreased. Intriguingly, the monounsaturated N-oleoyl-ethanolamine and N-palmitoleoyl-ethanolamine were significantly elevated in cocaine addicts diagnosed with mood and anxiety disorders when compared with non-comorbid cocaine-addicted subjects (Pavón et al., 2013).
Human studies also show that cannabinoid receptor \textit{CNR1} gene polymorphisms might be related to cocaine addiction (López-Moreno et al., 2012). Two single nucleotide polymorphisms (SNPs) in \textit{CNR1}, i.e., rs6454674 and rs806368, have been associated with cocaine dependence (Zuo et al., 2009; Clarke et al., 2013). Notably, also the (AAT)\textit{n} triplet repeat polymorphism in the close proximity of the \textit{CNR1} gene has been associated with predisposition to cocaine dependence (Comings et al., 1997; Ballon et al., 2006), although non-significant association data have also been reported (Covault et al., 2001; Herman et al., 2006).

From a clinical perspective, much evidence suggests that cannabinoid ligands and endocannabinoid-level enhancers may be therapeutically useful against cocaine dependence (Tanda, 2007). For example, crack cocaine abusers reported smoking cannabis in order to get relief from cocaine-withdrawal symptoms (Labigalini et al., 1999). However, caution must be used before drawing any conclusions, especially in light of the potential negative clinical implications of cannabis use in cocaine-abstinent subjects (Aharonovich et al., 2005).

**AMPHETAMINES**

Amphetamine and its derivatives, i.e., methamphetamine (METH) and N-methyl-3,4-methylenedioxyamphetamine (MDMA), are stimulant drugs that increase feelings of arousal and euphoria, decrease fatigue, and enhance attention and feelings of alertness. Co-abuse of cannabis and amphetamines is very common among polydrug users in Western societies. Yet, there are only a few and somewhat contradictory studies examining the effects of smoking marijuana and concomitant use of amphetamines on cognitive parameters (e.g., impulsivity, memory, executive functions). Similarly, animal studies examining long-term effects of amphetamine and cannabinoid co-administration and underlying neurobiology are quite limited.

Ecstasy users are typically polydrug abusers, and marijuana is commonly smoked among regular ecstasy users (Sindicich et al., 2009). In ecstasy users, regular cannabis and/or METH use confers additional risk of poor mental health and high levels of psychological distress, particularly with regard to paranoia, over regular ecstasy use alone (Scott et al., 2012). Regular cannabis and METH use was also associated with earlier initiation to use ecstasy (Scott et al., 2012). Marijuana is frequently smoked also by METH abusers (Simon et al., 2002; Gonzalez et al., 2004a); yet, whether it is smoked for the purpose of enhancing METH subjective effects or attenuating its adverse effects (self-medication purposes) is still unknown. Regular cannabis abuse in METH-dependent individuals has been associated with frontal, temporal, and striatal metabolic abnormalities compared to subjects that use METH only (Voytek et al., 2005). However, cannabis use was not found to exacerbate the neurotoxic effect of METH (Gonzalez et al., 2004a). Actually, a recent animal study indicates that METH-induced neurotoxicity in the caudate putamen and PFC can be attenuated by pre- and post-treatment with THC through inhibition of nNOS overexpression and astrocyte activation (Castelli et al., 2014).
Adolescent methamphetamine and cannabis abusers were found to have increased regional striatal volume and show stronger novelty-seeking behavior as compared to healthy controls (Churchwell et al., 2012). In the same study, degree of methamphetamine exposure was found to be positively correlated with regional striatal volume and novelty seeking in methamphetamine and cannabis users. Another clinical study evaluated whether the subjective responses of healthy volunteers to controlled administration of amphetamine are influenced by polymorphisms in the FAAH gene (Dlugos et al., 2010). Genotypes at rs3766246 and rs2295633 were found to be significantly associated with increased ratings of “arousal” in response to amphetamines, suggesting that the endocannabinoid system influences variation in subjective response to amphetamines. Yet, other studies found no significant association between methamphetamine dependence and the synonymous polymorphism of the FAAH gene, Pro129Thr (Morita et al., 2005). Regrettably, no studies have been conducted so far to quantify the brain level of endocannabinoids in amphetamine users, or to evaluate the density and functionality of CB1 receptor in the brains of METH or MDMA users.

## OPIOIDS

Cannabinoids and opioids share several pharmacological properties, including antinociception, hypothermia, sedation, and hypotension. Currently, a great body of evidence supports similarities of actions and interactions between central opioid and cannabinoid systems with reference to drug dependence and abuse (Fattore et al., 2004, 2005a; Spano et al., 2010; Scavone et al., 2013), including relapse phenomena (Fattore et al., 2003, 2005b, 2011; Spano et al., 2004). The first evidence for such an interaction in dependence-related phenomena, e.g., reward and withdrawal, dates to the middle 1970s, when it was reported that administration of THC attenuates naloxone-induced abstinence in morphine-dependent rats (Hine et al., 1975) and mice (Bhargava, 1976), whereas rats chronically treated with cannabinoids show opioid-like withdrawal signs following acute naloxone administration (Kaymakcalan et al., 1977).

Earlier clinical studies indicated that oral or smoked THC consistently induces changes in mood, usually euphoria, while higher doses are psychotomimetic producing, for example, marked distortion in visual and auditory perception (Isbell et al., 1967). When the effects of the opioid antagonist naltrexone on subjective responses to THC were examined in marijuana users, it was found that THC increases heart rate and self-reported drug effects (i.e., euphoria, marijuana-like effects) and decreases psychomotor performance, while naltrexone increases heart rate and decreases self-reported measures of vigor and hunger but does not alter any of the effects of THC (Wachtel and de Wit, 2000). Notably, more recent studies show that in morphine abusers cannabinoid receptors are up-regulated in the periphery blood mononuclear cells and that the expression of interleukin 4 (IL-4) mRNA in these cells is higher than that in healthy people (Zhang et al., 2012),
indicating that exposure to morphine can affect the expression of cannabinoid receptors and immune functions.

Cannabis is the most prevalent type of illicit drug used among heroin addicts. This implies at least two important issues to address, i.e., to determine the potential consequences associated with cannabis use in methadone-treated patients, and to evaluate the influence of concurrent marijuana use on treatment outcomes in opioid users on maintenance treatment. Concerning the first point, it was found that marijuana use does not impact on risk behavior for contracting acquired immunodeficiency syndrome (AIDS) (Saxon et al., 1990), but it can induce abnormalities in resting respiratory functions (Teichtahl et al., 2004). Moreover, when comparing outcomes of marijuana users and non-users enrolled in a methadone-maintenance program, no relation between cannabis consumption and the use of opioids was found (Saxon et al., 1990). Importantly, chronic marijuana smoking did not affect the normalization of the hypothalamic-pituitary-adrenal (HPA) axis induced by methadone in heroin addicts (Nava et al., 2007), which further supports the notion that marijuana use is not a risk factor for treatment outcome in methadone-maintenance treatment (Seivewright, 2003; Weizman et al., 2004).

Concerning the influence of opioid drugs on the brain endocannabinoid levels, it has been established that chronic morphine modulates the contents of 2-AG in the rat brain (Viganò et al., 2003), but no specific studies have been conducted so far on human opioid addicts. Similarly, it has been demonstrated that voluntary chronic intake of opioids or cannabinoids by rats induces reciprocal but differential regulation of μ-opioid and CB1 receptor density and activity in brain structures underlying drug-taking and drug-seeking behavior (Fattore et al., 2007c), but whether or not similar effects also occur in the human brain remains to be investigated.

**CONCLUSIONS**

The motivational and addictive properties of drugs of abuse are mediated through drug-induced changes in neurotransmitter and neuromodulatory signaling within the brain. Endocannabinoids mediate retrograde signaling in neuronal tissues and are involved in the regulation of synaptic transmission to suppress neurotransmitter release by the presynaptic cannabinoid receptors. This powerful modulatory action on synaptic transmission has significant functional implications and interactions with the effects of abused substances. Although there are differences in the central effects caused by various classes of abused drugs, accumulating evidence indicates a central role for the ubiquitous endocannabinoid physiological control system in the regulation of the rewarding effects of these substances. Cannabinoids and endocannabinoids appear to boost the rewarding effects of addictive drugs, including alcohol, nicotine, cocaine, amphetamines, and opiates, suggesting that the endocannabinoid system may represent an important target for the treatment of addictive disorders.
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