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REVIEW ARTICLE

Cannabis; Epidemiological, Neurobiological and Psychopathological Issues: An Update

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Abstract: Cannabis is the illicit drug with both the largest current levels of consumption and the highest reported lifetime prevalence levels in the world. Across different countries, the prevalence of cannabis use varies according to the individual income, with the highest use being reported in North America, Australia and Europe. Despite its 'soft drug' reputation, cannabis misuse may be associated with several acute and chronic adverse effects. The present article aims at reviewing several papers on epidemiological, neurobiological and psychopathological aspects of the use of cannabis. The PubMed database was here examined in order to collect and discuss a range of identified papers. Cannabis intake usually starts during late adolescence/early adulthood (15-24 years) and drastically decreases in adulthood with the acquisition of working, familiar and social responsibilities. Clinical evidence supports the current socio-epidemiological alarm concerning the increased consumption among youngsters and the risks related to the onset of psychotic disorders. The mechanism of action of cannabis presents some analogies with other abused drugs, *e.g.* opiates. Furthermore, it has been well demonstrated that cannabis intake in adolescence may facilitate the transition to the use and/or abuse of other psychotropic drugs, hence properly being considered a 'gateway drug'. Some considerations on synthetic cannabimimetics are provided here as well. In conclusion, the highest prevalence of cannabis use and the social perception of a relatively low associated risk are in contrast with current knowledge based on biological and clinical evidence. Indeed, there are concerns relating to cannabis intake association with detrimental effects on both cognitive impairment and mental health.

Keywords: Addiction, cannabimimetics, cannabis, delta-9-tetrahydrocannabinol, dopamine, gateway hypothesis, medical marijuana.

1. INTRODUCTION

The plant of cannabis, also known as *Canapa, Indian canapa, Marijuana, Mary Jane, Hashish, pot, herb, Maria, Hagga, Puf, Maconha*, *etc*., belongs to the family of the *Cannabinaceae* and it is native to Central Asia. According to its botanical profile, cannabis may include several subspecies and sub-varieties of the plant (*e.g*., *Cannabis sativa, C. indica, C. ruderalis*) [1]. It is found both as a naturally grown product but also as the result of direct cultivation. Some varieties of cannabis are specifically cultivated in order to obtain their resin extract, particularly in some countries with tropical climates (*e.g*., Caribbean, Latin America, Southeast Asia, India, *etc*.). Given its extreme adaptability to temperate climates and the spread/dissemination of its indoor hydroponic cultivation, its production has spread to both the United States and most European countries [2, 3].

Different preparations and extracts have been obtained from the *Cannabis sativa* plant. Marijuana appears to be a mixture of the inflorescences and the pressed/dried leaves of the plant, in which the larger proportion of its active ingredient delta-9-tetrahydrocannabinol (Δ9-THC) may range from

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0.5 to 5% (*sinsemilla*, variant without seeds) to 7-14% (*semilla*, variant with seeds), whilst the variant *Dutch Nederweed*, cultivated in the Netherlands, may contain higher concentrations (15-18%). Marijuana is usually smoked alone, mixed with tobacco or more rarely with hyoscyamus leaves. A more active product (called *Hashish*, or *Charash* or *cannabis resin*) is obtained from the resin extracted from the flowers and leaves located at the top of the female plant. Hashish retains variable Δ^9-THC concentrations, ranging from 2 to 20%. The *hashish oil* (also called *liquid cannabis*) is an extract of Δ^9-THC obtained through solvent extraction. The hashish oil usually contains Δ^9-THC concentrations ranging from 15 to 60%. It can be usually added as drops to the marijuana cigarettes in order to enhance the effects. *Bhang* is a mixed preparation, consisting of dried leaves and flowering tops of the overgrown plants. ~~Like marijuana,~~ it is It characterized by low levels of central effects, mainly due to its route of administration, since it is infused and ingested as a drink, almost exclusively in India. *Ganja* consists of a homogeneous mixture of resin and apical leaves of the flowering tops of the female plants. It has an average content of Δ^9-THC of about 3%.

Overall, cannabis represents the illicit substance with the highest consumption levels worldwide, particularly among adolescents with a mean age of first intake that has drastically decreased over time [2]. The overall prevalence of cannabis consumption, abuse and dependence is extremely variable across countries. Western Central Africa (12.4%), Oceania (12.4%), North America (10.7%) and Central Western Europe (7.6%) are the geographical areas that present with the highest consumption levels [2, 4, 5]. The growing trend prodrug of ~~pro drug~~ websites and of the online vending shops may well have recently contributed to the increasing trend of consumption in the population aged 15-19 [6]. Furthermore, recent studies seem to confirm that cannabis can be considered a 'gateway drug' (*i.e*., a 'bridge drug'). Indeed, the early cannabis consumption, through a process of learning and reinforcement, may cause a '*cerebral sensitization*' associated with a more positive attitude towards the consumption of drugs of abuse with higher levels of abuse potential [7-10].

The present paper aims at providing some scientific considerations relating to the epidemiological, neurobiological and psychopathological aspects of cannabis use. Further data, regarding the recently emerged synthetic cannabinoids' issues, will be here provided as well.

2. METHODS/LITERATURE SEARCH STRATEGY

A literature search on PubMed was carried out using the following MeSH terms: "*Cannabis*", "*Marijuana*", "*Synthetic cannabinoids*". No language or time restrictions were placed on the electronic search, covering the period up to January 2017. No filters were applied to limit the retrieval process, although the primary focus here was on human studies. The references indicated in the most relevant articles selected were used as further sources. The search was performed independently by MADL and CC; findings were then compared and discrepancies settled, if needed, with the supervision of GDC. Data were then collected in order to obtain relevant information regarding the epidemiological, neurobiological and psychopathological issues relating to the use and/or abuse of cannabis, its derivatives, and synthetic cannabinoids.

3. PREVALENCE OF CANNABIS USE IN EUROPE

Based on the 2016 World Drug Report, global cannabis consumption has been stable in recent years. In 2014, about 3.8% of the world population had used cannabis in the previous year, a proportion that has remained stable since 1998. The largest amounts of cannabis herbal seizures which took place in the Americas, but Europe, North Africa and the Middle East remain the principal markets for cannabis, with the largest amounts of cannabis resin having been seized in 2014 in Western and Central Europe (about 40% of the total) [2].

According to the European Monitoring Centre [3], during 2016 in Europe, 22% (70 millions) of 15-64 years old individuals reported to have used cannabis at least once in their lifetime; 7% (23 millions) took it in the last year, and 4% (14 millions) during the last month. In the 15-24 years old group, the last-month figure increases to 17%. As a comparison, cocaine use is around 10 times lower than cannabis use. The ESPAD survey, conducted in 2015 [11] across 35 European countries amongst students with an average age of 15.8 years reported that approximately 19% of males (M) and 14% of females (F) stated that they had consumed cannabis at least once, with peaks of 48% M *vs.* 38% F in the Czech Republic and 39% M *vs.* 40% F in France. Moreover, an average last-month prevalence of around 8% in M and 5% in F has been reported, with an M/F ratio of 22.5% in France, 18% in the USA and 15% in the Czech Republic and Spain. In particular, according to "Monitoring the Future" study, *e.g*. a survey of the University of Michigan funded by NIH-NIDA, the annual prevalence of use for marijuana in the USA among full-time college students during 2015 was 37.9 % (40.2% in M and 36.6% in F) [12]. Furthermore, it has been demonstrated that the lifetime prevalence of cannabis use in Europe has increased, between 1995 and 2011, by 6%. According to the last EMCDDA report [3], cannabis represents the most common primary drug, after heroin, amongst those patients who undergo specialist treatment of drug dependence and it is the most frequently reported drug amongst those undergoing addiction treatment for the first time. In fact, in Europe, the number of cannabis consumers who started proper addiction treatment for the first time increased by approximately 45,000 in 2006 to 60,000 in 2011.

3.1. A Three-Stage Model: Availability, Use, and Abuse

Genetic epidemiology studies conducted in twins demonstrated that substance abuse depends on both genetic and environmental factors, with the latter being categorized into shared and non-shared between subjects. These three types of factors, in turn, may act on each of the stages that represent the natural history of substance dependence, *i.e*., availability, use, and abuse. Recently, Verweij *et al*. [13] conducted a meta-analysis of studies focusing on the use and abuse of cannabis in twins. They found that genetic factors accounted for about 50% of the variance, both for the initiation and the problematic use of cannabis. The remaining 50% was divided between the risk of environmental, shared and non-shared, factors. A typical issue in cannabis use is

that the drug is frequently offered by most experienced consumers to 'novices'. Hence, as expected, initiation into cannabis use is directly related to its availability [14]. Gillespie *et al.* [15] studied, in twins, the drug initiation and abuse issues and their relationship with cannabis availability, genetic and environmental, shared and non-shared, factors. Findings were consistent with a model in which cannabis availability may act on its initiation through shared environmental factors, whilst cannabis initiation may facilitate start of abuse through the non-shared environmental, and genetic, factors. Thus, prevalence of use and availability are inextricably linked and appear to influence each other. This relationship, magnified by the influence of the dynamics of the group, family, and social relationships which are typical of adolescence may, at least in part, explain the high prevalence of cannabis use amongst adolescents.

3.2. Global Burden of Disease

The notion of the Global Burden of Disease (GBD) was firstly introduced by the World Bank to assess the socio-economic impact of diseases and disorders. GBD is expressed in terms of years spent in disability (YLD), and years of life lost (YLL) that can be combined into years lost adjusted to disability (DALYs). Since GBD resulting from substance dependence is sensitive not only to the potential for abuse of a specific drug but also to its availability, the DALY expresses, better than any other indices, the socio-economic impact. Fig. (**1**) shows that cannabis dependence cause more DALYs than cocaine dependence. However, other data show that in countries with higher per person income the burden resulting from addiction to cannabis in 2010 was higher than that resulting from addiction to amphetamines (DALYs in millions of years: Australasia 9.3 to 5.8, Western Europe 4.3 *vs.* 3.4, North America 8.1 *vs.* 3.3 [16].

4. ABUSE POTENTIAL

'soft'

Cannabis is commonly considered a ~~"soft"~~ drug. This expression was proposed in the late 60s, when the cannabis began to spread amongst youngsters. At that time, it was known only that Δ9-THC represented the active ingredient of cannabis [17, 18]. It took 20 years to discover and understand its mechanism of action and its analogies with other misusing drugs [19, 20]. Unlike other illegal drugs, cannabis has been virtually considered devoid of lethal effects, a consideration which has significantly contributed to its connotation of being a "soft" drug, which is in contrast with the last 10-year evidence from both epidemiological and clinical studies. The ESPAD report (2015), in which the questionnaire was administered to students (average age: 15.8 years), found that, amongst those who had consumed cannabis in the last year, the 33% individuals (36% M and 29% F) showed a problematic pattern of use such as early onset and daily smoking [11]. These findings have been confirmed by a prospective study carried out by van der Pol *et al.* (2013) in a cohort of 600 daily cannabis users [21]. The authors observed a cumulative incidence of dependence, over three years, of around 37%.

The abuse potential of a drug can be expressed in terms of percentage probability of transition from use to 'problematic' use, *i.e.*, abuse and addiction. A German longitudinal study [22], carried out in a sample of subjects between 14 and 24 years-old subjects, observed a probability of transition from first use to dependence at 36% for nicotine, 11.2% for alcohol and 6.2% for cannabis. The probability of transition from use to abuse was of around 25.3% for alcohol and 18.3% for cannabis. Furthermore, Lopez-Quintero *et al.* (2011) evaluated all data coming from the USA National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) in terms of the cumulative probability of transi-

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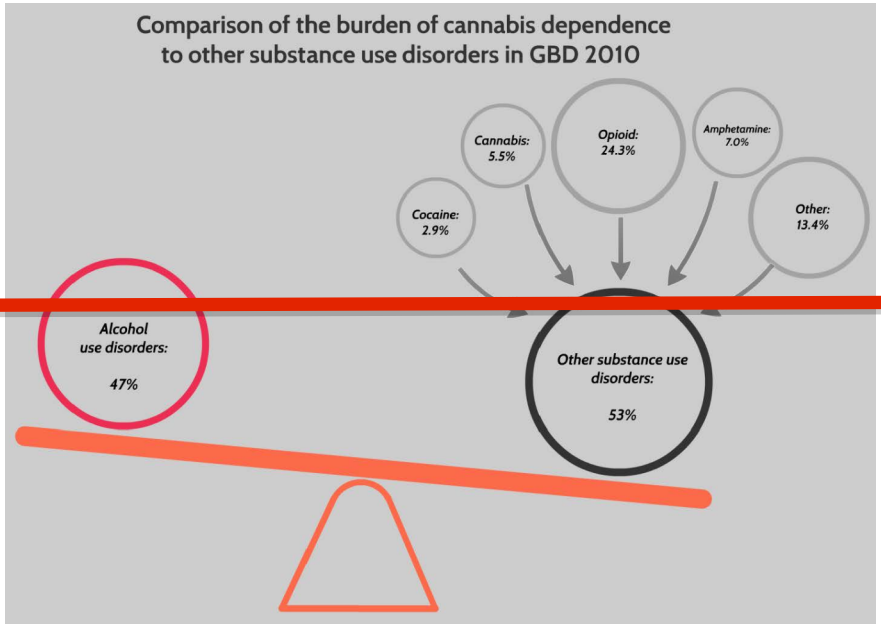

Fig. (1). Global epidemiology and contribution of cannabis use and dependence to the Global Burden of Disease (GBD). The proportion of years lost adjusted to disability (DALYs) due to cannabis dependence relative to other substance use disorders in GBD 2010. Adapted from Degenhardt *et al*. 2013 [16].

tion from first use to dependence for cannabis, nicotine, alcohol and cocaine by reporting a probability of around 2% for cannabis, nicotine and alcohol [23]. The authors reported a percentage of around 15.6% for nicotine, 14.8% for cocaine, 11.0% for alcohol and 5.9% for cannabis ten years after initiation of use. Overall, the authors reported as well a lifetime prevalence of transition of 67.5% for nicotine, 22.7% for alcohol, 20.9% for cocaine and 8.9% for cannabis. Wagner and Anthony (2007) evaluated the latency of cannabis, alcohol and cocaine addiction onset, in 15 to 44-years old subjects [24] (Fig. **2**). Regarding cannabis, dependence developed differently between M and F subjects, with a peak (max 4%) after three years of use in M and a lower plateau (max <1%) between 0.5 and 4.5 years in F (Fig. **2 A**). Similar results were observed for alcohol (Fig. **2 B**). Conversely, the latency of addiction to cocaine (Fig. **2 C**) had a similar pattern in both sexes, with an earlier (1 year) peak compared to cannabis and alcohol. Overall, in terms of cumulative probability of dependence, cannabis addiction may reach its highest prevalence levels (approximately 14%) during the 30s for M subjects, whilst cocaine addiction shows an increasing trend up to when the subjects are in their 40s (max 22%), and alcohol addiction may increase beyond the mid-40s (max 30%). A study by Wagner and Anthony (2007) [24] showed that the probability in the early onset of cannabis addiction has a very narrow 'time window' in M subjects, mainly due to a gender-specific genetic risk of developing cannabis dependence [25].

5. CANNABIS AS A "GATEWAY DRUG"

One of the most controversial issues in cannabis epidemiology is relating to the probability that its use in adolescence may facilitate the transition towards the use and/or abuse of other illicit drugs with a higher potential of abuse [26]. Several epidemiological studies showed that early cannabis consumption may be associated with a higher, later, risk of abuse and/or dependence not only to cannabis but also to other illicit drugs such as cocaine, methamphetamine, and heroin [27, 28]. Various mechanisms have been proposed to shed light on this correlation. Although not explicitly, the 'gateway hypothesis' states that the relationship between the intake of cannabis and that of drugs with a higher potential of abuse appears to be direct, mainly due to the properties of cannabis [26]. Conversely, others have suggested that the association between the use of cannabis and that of other drugs may result from a combination of individual factors such as genetic and environmental ones (*common liability*). For example, a reduced impulse control, or a greater tendency to seek gratification (*reward seeking*), due to genetic factors, could lead to a significant individual vulnerability towards the use of other drugs. Another hypothesis, proposed by Wagner and Anthony (2002) [24], suggested that people who use cannabis had a greater opportunity to be exposed to other drugs commonly sold in the same, shared, 'illegal market'. Overall, the 'causal relationship' hypothesis is very controversial because the existing genetic and environmental factors may act as 'potential confounders'. For this reason, some studies used statistical methods (*e.g.*, the 'Fitting model') [29], whilst others compared the risk of dependence within pairs of identical twins, with the same genetic background, who lived in the same family environment, but with a different pattern of cannabis use in adolescence [30, 31]. In both cases, the early use of cannabis resulted in a probability of abuse of other illicit drugs up to 2-5 times greater compared to subjects not exposed to cannabis. However, even though the approach of Linskey *et al.* [30] excluded the role of genetic and environmental (family) factors shared by both twins, it did not allow to exclude the relevance of the role of individual environ-

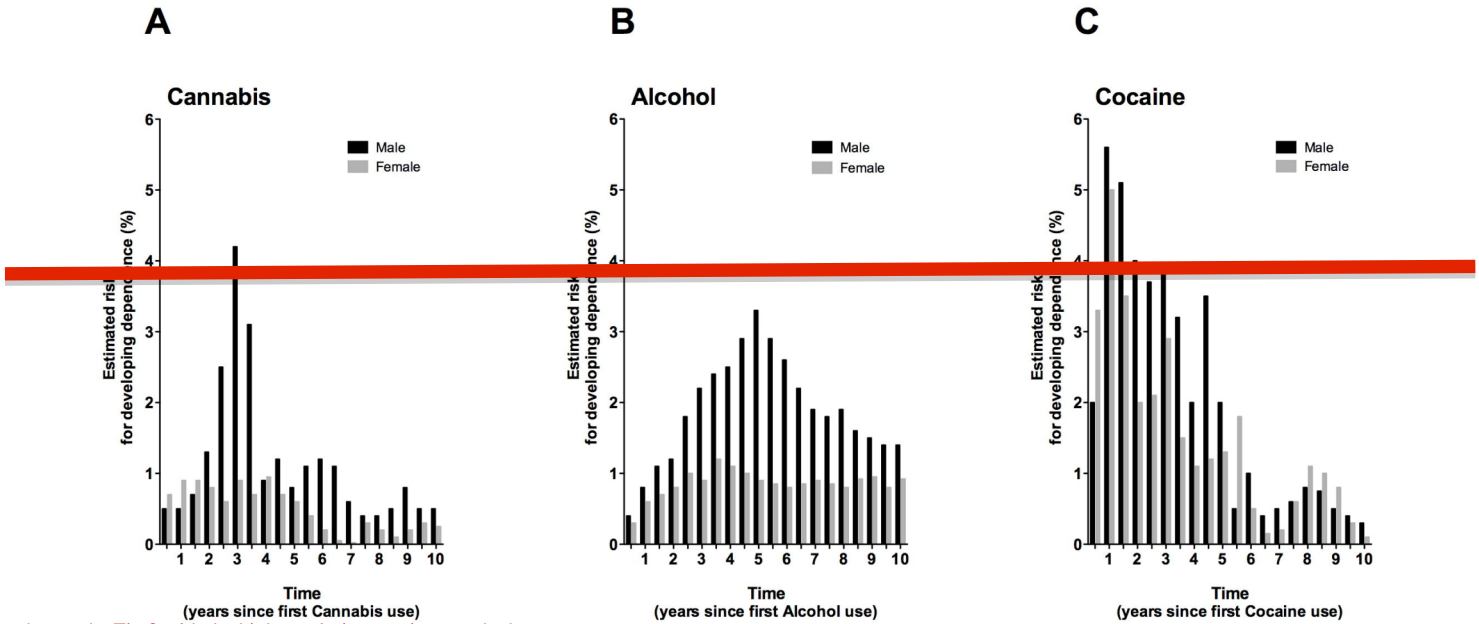

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Fig. (2). Estimated risk for developing cannabis (**A**), alcohol (**B**) and cocaine (**C**) dependence by males versus females and time since first use, among 15-44 years old cannabis users. Data from the U.S. National Comorbidity Survey, 1990-1992. Adapted from Wagner and Antony, 2007 [24].

mental factors not shared among twins. These limitations may have been better controlled by the longitudinal study of Lessem *et al.* [32], which included both twins and brothers/sisters and which confirmed the Linskey *et al*. findings [30]. Therefore, the current hypothesis is that the use and abuse of cannabis in adolescence may well predispose to the use and abuse of drugs with a higher potential of abuse. Lessem *et al.* [32] suggested that the use of cannabis may cause 'the fall of a taboo', paving the way for other, more powerful and dangerous, drugs. Whilst considering the hypothesis that the use of cannabis may predispose to the use of '*harder drugs*', the United States promoted a campaign for the prevention of cannabis availability and use in adolescence.

6. MEDICAL MARIJUANA

Recently, both several states of the USA and the District of Columbia (May, 2015) approved a range of laws to protect the prescription/sale/use of cannabis for therapeutic use from the federal justice. Some states also endorsed laws that protect the dispensaries of marijuana for therapeutic use from the federal justice. Kleber and DuPont (2012) highlighted the totally abnormal aspects of '*medical marijuana'* [33]. First at all, it is abnormal that it is a smoked medication. In fact, there is no FDA-approved drug administered by this way. The main reasons are obvious enough, since marijuana smoking is more dangerous than tobacco, and it is not possible to self-administer with precise dosages. Therefore, smoking does not deliver a reproducible and predictable dosage between different subjects as well as in the same subject, due to the differences in the Δ9-THC percentages in the different preparations of marijuana and Δ9-THC extraction efficiency among different people and situations. As reported by Kleber and DuPont (2012): "*Medical marijuana bypasses the century-old, scientifically based, drug approval procedure and the carefully regulated distribution of medications through licensed pharmacies'* [33]. The fact that marijuana has been introduced as a medical treatment after a referendum and a political decision, bypassing all the rules which drugs are subjected to, establishes a dangerous precedent for public health. The fact that smoking is a pharmacotherapeutic non-sense, whilst it is the common mode of cannabis use for recreational purposes, is symptomatic of the fact that the so-called '*medical marijuana'* is nothing more than a shortcut for legal recreational use of cannabis. In fact, less than 5% individuals authorized to purchase medical marijuana suffer from those conditions that may justify its compassionate use (*e.g*., HIV, cancer, multiple sclerosis) [34]. Currently, the most frequent justification for medical marijuana prescription is the treatment of pain (82-87%), anxiety (38%), and depression (26%) [34, 35]. Moreover, it has been reported that 96% of medical marijuana enthusiasts had used cannabis in their lifetime, whilst in the general population this figure is 4 times less. In addition, other medical cannabis preparations include cannabis tea and cannabis oil that, the most of the time, are not standardized preparation, thus not ensuring a homogeneous product of defined stability [36]. Recently, a systematic review of the side effects of cannabinoids for medical use including 79 clinical trials have shown an increased risk of short-term adverse events (*e.g*., dizziness, dry mouth, nausea, fatigue, somnolence, euphoria, vomiting, confusion, hallucinations) compared to associated benefits [37]. In Europe, cannabis preparations such as Sativex and Bedrocan are already available, and several European countries are able to supply patients with medicinal cannabis. Italy, after a trial period of two years, approved the pharmacies' sale of the whole-plant cannabis, or cannabis-based drugs produced by the state, upon presentation of a medical prescription.

7. THE IMPACT OF LEGALIZATION

In November 2016, California, Massachusetts and Nevada legalized the recreational use of cannabis. In Europe, none of the EU Member supports completely the legalization of cannabis sale for recreational use, but over one third (*e.g*., Czech Republic, Belgium, Denmark, Italy, Latvia, Lithuania, Luxembourg, Malta, Croatia, and Slovenia) act "depenalization" and "decriminalization" approach of its personal possessions, whilst others (*e.g*., Spain, Germany, Portugal, and the Netherlands) allow the use and sale of small amounts through special dispensaries, recreational stores (coffee shops) and clubs. From a scientific point of view, the legalization of cannabis is both a natural experiment and a large-scale business. However, it is likely that the legalization of cannabis will have consequences on physical and mental health, although the effects of cannabis legalization on health could be detected only after several years.A number of studies focused on some aspects of the cannabis market, such as the extent of demand and supply (production), the price, the Δ9-THC content and its consumption,. According to Pacula and Sevigny (2014), the differences in legislation between the States and the continuous legislative changes, in the same states, make it difficult to draw general conclusions [38]. Indeed, the available evidence suggests that the two markets, legal and illegal, are closely interrelated, especially when the control is loose. From this point of view, Rendon (2013, p. 147) wrote, "*the legalization of marijuana for medical use in California has changed everything about the market for pot and is pushing changes for growers, breeders, and the plant itself*" [39]. Substantial amounts of medical marijuana are produced in excess and diverted to the illegal market. In Colorado, a recent investigation documented dozens of cases of diversion of cannabis to the illegal market from dispensaries, patients and authorized physicians (Investigative Support Center, 2012). Data relating to the effects of the legalization on cannabis use, expressed as prevalence and therefore on the number of users, are not univocal, since the number of studies indicating an increase is equal to the number of studies that did not find any significant changes. However, there is no doubt that, by expressing the use in terms of quantity self-administered, legalization has been associated with an increase in consumption amongst regular users. Pacula and Sevigny (2014) observed that the effects of legalization on cannabis use depends on social (*e.g*. changes in social norms or in perceived risk), environmental (local presence of dispensaries and ease of access to cannabis), and market factors (scale of production and price) [38]. For example, an increase in demand due to the first two factors will produce an increase in price, which, in turns, will tend to limit the use. Conversely, an increase in the production of cannabis, by reducing the price, would promote its dissemination and use. Another possible consequence of the legalization of cannabis relates to the influence on alcohol con-

9 superscript

sumption. Alcohol and marijuana may be complementary, being consumed either jointly, or alternatively. In the first case, the effects on driving is quite negative, since alcohol and cannabis act on two different driving modes, respectively outcome-related and habitual, each of which can, in an asymmetric way, compensate in case of dysfunction of the other. In the second case alcohol, by impairing the executive functions, exerts the most severe effects. However, cannabis may force the subject to shift on the outcome-dependent mode, by interfering with the habit mode. In this case, the choice of alcohol or cannabis by adolescents/young adults depends on the price. According to Anderson and Rees (2014), the legalization of cannabis led to a substitution of alcohol with cannabis in those States in which a reduction of the cannabis price was reported, due to an excessive production, and a convenience of cannabis compared to alcohol [40]. However, this consideration has been questioned by Sevigny and Pacula (2014) [38]. Quite recently it has been reported that among 8th and 10th graders, in Washington state, perceived harmfulness of marijuana use decreased, and marijuana use increased, following the legalization of recreational marijuana use [39]. In contrast, Colorado did not exhibit any differential change in perceived harmfulness or past-month adolescent marijuana use following legalization. This difference may be related to the different degree of commercialization of marijuana prior to legalization in Washington and Colorado. Colorado had a very developed medical marijuana dispensary system prior to legalization, with substantial advertising, to which youths were already exposed. Washington, on the other hand, was not previously providing legal protection to medical marijuana stores. Therefore, the degree of commercialization and advertising of these dispensaries was substantially lower than in Colorado. In addition, rates of perceived harmfulness ~~in Colorado were already lower and rates of marijuana use were already higher than rates in Washington and non-legalizing states prior to legalization.~~ The longer-term effect of legalization implementation on adolescent marijuana use in Colorado is still to be determined [41].

of marijuana use were already lower in Colorado, while rates of marijuana use were higher before legalization compared to rates in Washington and non-legalizing states.

8. LEVELS OF Δ9-THC IN CANNABIS PRODUCTS

9 superscript

The effects of cannabis mainly depend on its Δ9-THC content. Indeed, several studies reported that, amongst cannabis consumers, the risk of occurrence of psychopathological disturbances, including anxiety, psychosis, and cognitive disturbances appears to be correlated to cannabis potency/Δ^9-THCcontent. Over the last 30 years, the Δ^9-THCcontent in cannabis herbal crops and products has progressively increased worldwide. In the United States, for example, the Δ^9-THC content in marijuana was 2% in 1980, 4.5% in 1997, 8.5% in 2007 and 9.6% in 2010.

space between THC and content

According to the latest EMCDDA report [3], the Δ^9-THC content in marijuana and hashish is of 7-8% (ranging from 2% to 16%) on average. However, the cannabis market appears to be more volatile than what described by the above data. In fact, the introduction of cannabis preparations obtained through unique, in house, cultivation techniques, *e.g.* by preventing female flowers pollination (sinsemilla), or obtained by breeding plants cultivated in artificial conditions (hydroculture), has radically modified the 'cannabis market' and, hence, its Δ9-THC content. Sifaneck and co-workers [42] described the cannabis scenario in New York City where, in addition to the open field-cultivated *commercial marijuana*, the so-called '*designer marijuana*', is available. Designer products (*e.g.*, 'Haze', 'Skunk', 'White Widow'), obtained by local crops in artificial conditions, are more expensive, but can be acquired by credit card with a toll-free call and delivered at home.

Notably, in the Netherlands, an increase of Δ^9-THC content in cannabis products has been associated with cannabis legalization. Pijlman *et al.* [43] studied the Δ^9-THC percentage in both imported marijuana/hashish and in their derived preparations, Nederwijete Nederhasij, obtained from locally cultivated selected species, both sold in Amsterdam 'Coffee Shops'. Imported marijuana and hashish showed an increase in Δ^9-THC content (respectively: 5% and 11%, in 2000; 7% and 18.4%, in 2004), whilst the locally produced marijuana and hashish changed their Δ^9-THC content, respectively, from 8.6% and 20.7% in 2000 to 20.4% and 39.3% in 2004. Since 2004, the Δ^9-THC potency has remained at these levels [4]. In the US, a smaller level of increase (*e.g.*, of about 1%) in Δ^9-THC content has been observed in preparations sold in the illegal market in those States where legalization has been accompanied by establishing authorized dispensaries, nicknamed as 'Cannabis supermarkets' [44].

9. CANNABIS WITHDRAWAL SYNDROME

A chronic cannabis consumption may be conceived as a Cannabis Use Disorder (CUD) based on specific criteria of both DSM-IV and DSM-V. However, the cannabis capacity to induce both physical dependence and withdrawal syndrome after quitting its use was introduced only with the DSM-V [45]. A proper withdrawal syndrome may be observed in about one third of regular cannabis users and in around 50-95% of the individuals who consume high dosages of cannabis in experimental contexts [45]. The cannabis withdrawal syndrome is characterized by craving, irritability and aggressiveness, dysphoria, depressed mood, anorexia, sleep disturbances/weird dreams, and motor disturbances. Physical signs/symptoms are less intense when compared to those observed during an alcohol or heroin withdrawal syndrome, but the craving has been reported as possibly more intense [45-47].

Tolerance and physical dependence to cannabis may be rapidly induced in laboratory animals, where it may be precipitated by the administration of rimonabant, an ~~antagonist~~ of cannabinoid CB_1 receptors. In experimental animals, the behavioural features of a cannabis withdrawal syndrome are reminiscent of an opiate withdrawal syndrome. Like heroin and cocaine, the cannabis withdrawal syndrome may be associated with a reduction of dopaminergic turnover in the nucleus accumbens shell (*see below*) [48, 49].

10. CANNABIS REINFORCING PROPERTIES

The studies investigating the behavioural and reinforcing properties of the drugs of abuse in experimental animals have a fundamental importance in understanding the mechanisms by which these substances may induce a dependence status in humans.

A fundamental property, common to all drugs of abuse, is that cannabis may act as a behavioural reinforcer and can hence be self-administered by laboratory animals. Any effort made to obtain a self-administration of the active component of cannabis, Δ^9-THC, in different animal species, from primates to rodents, has proved to be a complex task. However, Δ^9-THC may be self-administered intravenously by monkeys (*squirrel monkeys*), *e.g*. both those previously trained to self-administer with cocaine and by naive ones as well [50, 51]. Δ^9-THC doses producing this effect are around 4 micrograms/kg. In the rat, the intravenous self-administration has been obtained with WIN 512,212-2, a synthetic agonist of cannabinoid CB_1 receptors, but not with Δ^9-THC itself [52, 53]. The reason of this discrepancy is likely to be due to the peculiar Δ^9-THC pharmacokinetics in the rat, which does not allow the maintenance of a strict temporal contingency between operant responding and the rewarding effects of the drug, which is an essential condition for the acquisition of a reinforced behaviour.

11. STIMULATION OF DOPAMINE TRANSMISSION

Δ9-THC ~~THC~~ is able to stimulate dopamine (DA) release in the ventral striatum, preferentially in the shell of nucleus accumbens. This property, relating to both Δ^9-THC and its synthetic analogue, WIN 512,212-2, has been demonstrated by means of different techniques both in rats, following passive (microdialysis and fast scan cyclic voltammetry) [49, 54-56], and active self-administration [53], and in humans (C11-raclopride binding measured by PET [57, 58]).

The maximal stimulation of DA release by Δ^9-THC administered intravenously is quantitatively similar to that induced by heroin [49]. These effects are blocked by an ~~an-~~ antagonist/inverse agonist ~~tagonist~~ of the CB_1 receptors as well as by an antagonist of opioid receptors, naloxonazine, at the same doses which are able to block the stimulation of DA transmission by heroin [49]. The stimulant effects of Δ^9-THC on DA transmission are due to a stimulation of firing activity of mesencephalic DA neurons projecting to the nucleus accumbens [59]. Given that opioid receptors blockade should also prevent the Δ^9-THC self-administration in monkey [60], these findings, together with the demonstration that Δ^9-THC releases beta-endorphins [61], may suggest that cannabis may release endogenous opioids acting on *mu* receptors. This effect may contribute to both Δ^9-THC reinforcing properties and the ability to release DA in the nucleus accumbens (Fig. **3**).

The ability of Δ^9-THC to stimulate DA transmission in the shell of nucleus accumbens is common not only to heroin and opiates but also to all drugs of abuse (*e.g*., psychostimulants/nicotine/alcohol) [62]. These findings may suggest that DA plays a role in the reinforcing properties of cannabis and, in general, of all drugs of abuse. The DA in the nucleus accumbens shell possesses different functions. Among these, there is the ability to facilitate acquisition and expression of incentive properties by conditioned Pavlovian stimuli. Experimental studies on behavioural and biochemical properties of Δ^9-THC and its synthetic analogues confirm the status of cannabis as a substance possessing the ability to induce dependence not differently from other drugs, commonly defined as '*hard drugs'*.

12. NEUROBIOLOGY OF CANNABIS AS A GATEWAY DRUG

The existence of a causal relationship between cannabis intake and the following use of other '*hard drugs*' may be studied and discussed in animal models as well.

From a neurobiological point of view, the more consistent relationship is between cannabis and heroin. Indeed, these drugs share a range of common pharmacological properties which are mediated by their stimulation of *mu* opioid receptors and which are in turn strictly correlated with their ability to induce dependence, facilitate *self-administration,* and the ability to stimulate DA release in the nucleus accumbens shell (see above). Moreover, previous exposure to Δ^9-THC or other synthetic cannabinoid agonists has been reported to sensitize to the stimulant motor effects (*behavioural sensitization*) of both Δ9-THC and morphine, but not 9 superscript of psychostimulants (cocaine and amphetamine) and vice versa [63, 64]. Similarly, previous exposure to morphine induces sensitization to morphine but also to Δ^9-THC (*cross sensitization*).

The most striking evidence of Δ^9-THC ability, administered during adolescence, to increase the rewarding and reinforcing properties of heroin has recently been provided [64]. Previous studies on this issue showed that Δ^9-THC exposure during adolescence [65] or adulthood [66] may increase rates of responding in intravenous heroin self-administration paradigms. The meaning of these findings is difficult to be interpreted since this might be suggestive of both an increase and a decrease of the reinforcing properties of heroin. Conversely, the Di Chiara *et al*. (2013) study used instead a progressive ratio paradigm, where the number of responses that the animal has to perform, in order to obtain an intravenous injection of the drug increased progressively [67]. The maximal number of responses the animal is able to emit (*breaking point*) is a measure of the ability of the drug to reinforce the behaviour. By using this paradigm, we observed that adolescent pre-exposure to Δ^9-THC increased the maximal number of responses the animals emitted and, thus, the heroin reinforcing properties. This effect was observed in the addiction-prone Lewis rat strain, but not in the Fischer 344 one [56], and appears to be associated with a potentiation of DA release stimulation in the nucleus accumbens shell [53]. These results suggest that, similarly to what was hypothesized for the psychopathological effects, cannabis intake may be associated with a vulnerability risk factor, likely genetic in nature, which may facilitate the use and abuse of other illicit drugs with higher levels of abuse potential.

13. CANNABIS AND PSYCHOPATHOLOGY

Cannabis intoxication, a transient condition that develops after the intake of the substance, is characterized by disturbances in level of consciousness, cognition, perception, affect, behaviour, or coordination. Common intoxication signs and features include conjunctival injection, increased appetite, dry mouth, tachycardia, euphoria, relaxation, perceptual alterations, suspiciousness, paranoia, intensification of ordinary experiences, increased sociability, impaired short-term memory and attention, and impaired motor activity [68-70]. A disorder of regulation of cannabis use can arise from its

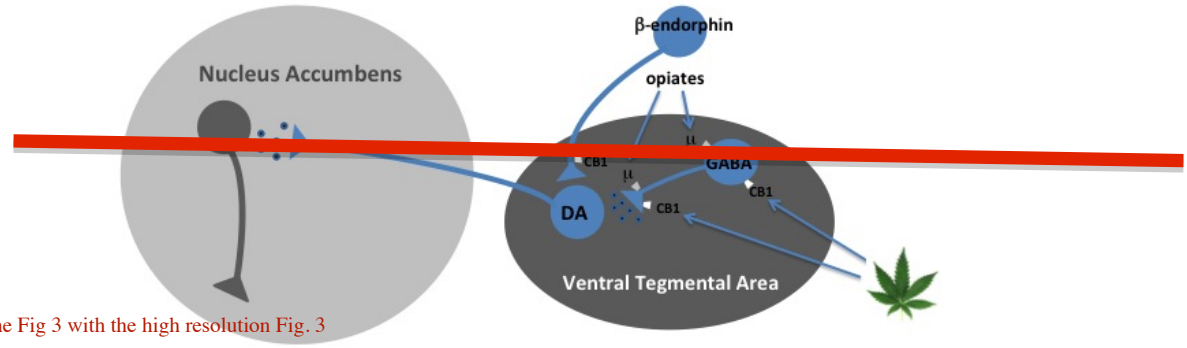

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Fig. (3). Effect of cannabis and opiates on dopamine (DA) transmission. The schema represents that opiates and cannabinoids stimulate DA release in the nucleus accumbens (NAc) by blocking the brake exerted by GABA neurons onto DA neurons in the Ventral Tegmental Area (VTA). CB1, cannabinoid receptors 1; MOR, μ-Opioid receptors. Adapted from Di Chiara et al., 2014 [105].

repeated or continuous use. A central feature of cannabis use disorder/dependence is a strong internal drive to use the substance, manifested by impaired ability to control use, increasing priority given to cannabis use over other activities, and persistence of use despite harm and adverse consequences [68, 70]. An *amotivational syndrome* has been described in long-term cannabis users; the syndrome includes lack of motivation, apathy, social withdrawal, lethargy, impaired memory and concentration. It may resolve or improve with continuous abstinence [70]. Δ^9-THC is known to possess psychotomimetic effects, and, indeed, its intravenous administration constitutes one of the current pharmacological models of psychosis [71-73]. The available evidence points to a causal role for Δ^9-THC in schizophrenia spectrum disorders [68] with a dose-response relationship between levels of Δ^9-THC ingested and risk for psychosis having been postulated [74]. The consumption of cannabis characterized by a high cannabidiol (CBD) content seems to be associated with fewer psychotic experiences [75]. On the other hand, the intake of highly Δ^9-THC concentrated cannabis strains (*e.g.* 12-18% of Δ^9-THC, and no CBD) is associated with an increased severity of cannabis dependence, higher risk for psychotic disorders, and a lower age at onset of psychosis [68, 76-78]. Acute cannabis-induced psychotic episodes are not uncommon in cannabis dependent individuals, and are characterized by rapid onset, and relatively benign course following cessation of use. Regular cannabis users may also experience repeated, short, episodes of psychosis and/or may remain in a pre-psychotic state. Cannabis use may precipitate psychotic symptoms in individuals vulnerable to psychosis, and in patients with schizophrenia may trigger a relapse/exacerbate symptoms even with stable antipsychotic medications [79]. There is some evidence to support the statistical association between cannabis use and the increased incidence of bipolar disorders. Cannabis use may lead to the onset of mania/hypomania symptoms, particularly among regular or daily users, and can moderate the course of bipolar disorder by increasing the time to recovery, relapse, and recurrence of manic phases [80]. A heavy use of cannabis is associated with a small increase in the risk of developing depressive disorders. Increased levels of both suicidal ideation and suicide attempts, and greater risk of death by suicide, are also related to cannabis use [80]. Anxiety and panic attacks are cannabis common adverse effects, and can often happen in long-term cannabis users.

14. SYNTHETIC CANNABINOIDS

Herbal preparations containing synthetic cannabinoids (SC) have been marketed since 2004, mainly online, as 'legal alternatives' to cannabis, under different names ('Spice Gold', 'Spice Silver', 'nJoy', 'Blaze', 'Orange', 'Lilla', 'K2', *etc.*) [81-83]. In 2008, Spice/K2 products have become very popular in Germany, after several cases of intoxications [83, 84]. Spice preparations include inert or psychoactive plant materials (*e.g.*, Leonotis leunurus, Pedicularis densiflora) laced with various synthetic SC, which are CB_1 and CB_2 receptor agonists, chemically and pharmacologically different from Δ^9-THC. Indeed, Δ^9-THC is a partial CB_1 agonist, whilst SCs are full agonists with a higher potency and efficacy as compared to Δ^9-THC [85]. SC have been extensively studied and classified in three generations: the first includes Δ^9-THC analogues (HU-210) but also aminoalkylindole and cyclohexylphenoles (*e.g.* JWH-018 series, WIN-55,212-2, and CP-47,497); the second generation includes alkyl derivatives (AM-2201, MAM-2201, AM-694), N-metilpiperidines (AM-2233 and AM-1220) and benzoindoles (AM-679, RCS-4 and derivatives); ~~whilst~~ the third generation includes molecules in which the indole ring is replaced with an indazole or a benzimidazole group (AKB-48, 5F-AKB-48, FUBIMINA), or molecules where the carbonyl group is replaced with the carboxylic or carboxyamide functional group (APICA, SDB005), and quinolones (PB-22 or QUPIC, 5F-PB-22, BB-22 or QUCHIC) with secondary cyclic structures (ABDICA, AB- PINACA, 5F-AB-PINACA) and new nitrogen groups (AB-FUBINACA, AB-FUBICA) [86]. Smoking 'Spice' induces more intense and long-lasting euphoria compared to cannabis, frequently associated with panic/anxiety states, paranoia, psychosis, hallucinations, seizures, tachycardia, headache, nausea, vomiting and a severe withdrawal [82, 87, 88]. The first SC to be identified (in 2008) was JWH-018 (1-Pentyl-1H-indol-3-yl)-1-naphthalenylmethanone) which has been reported in more than 140 specimens of Spice both in Europe and in Japan [89, 90, 91], although in different amounts depending on the compound analysed. JWH-018 was synthesized in 1995 by

John William Huffman at Clemson University (USA) as part of a structure-activity relationship study of the structural determinants of the CB_1 versus CB_2 receptor binding and intrinsic activity. Compared to Δ^9-THC, JWH-018 exhibits an approximate four-fold higher affinity for CB_1 (Ki ~ 9 nM) receptor and ten-fold affinity for the CB_2 receptor (Ki ~ 3 nM) [85, 92-94]. *In vitro* studies on liver microsomes [95] and in urine [96] showed the presence of several pharmacological active metabolites [97]. The higher potency of JWH-018 compared to Δ^9-THC *in vivo,* and the formation of active metabolites, are likely to be the reason of Spice being able to induce a more severe dependence and withdrawal syndrome compared to cannabis. Recently, it has been suggested that rats and mice are able to consistently acquire levels of operant behaviour, resulting in JWH-018 self-administration [98]. This may well confirm the reinforcing properties of SC, which display a more robust intravenous self-administration compared to endocannabinoids or Δ^9-THC [99]. JWH-018 preferentially increased DA release in the NAc shell *in vivo via* CB_1 receptors, thus linking JWH-018 to both other cannabinoids with known abuse potential, and to other classes of misusing drugs that increase DA signalling in the NAc shell [98]. Furthermore, recent studies showed that third generation SC molecules (*e.g*., 5F-PB-22 and BB-22), compared to JWH-018, possess an even higher CB_1 receptor agonist potency, five- and seven- fold, respectively; higher efficacy levels; and a higher binding affinity (26- and 30-fold, respectively) at CB_1 receptors. Moreover BB-22, the SC with the highest affinity for CB_1 receptors among those tested, was able of stimulate DA release in the NAc shell at doses 10-fold lower compared to JWH-018 [100]. Other studies showed that SC impair sensorimotor reflexes and produce aggressive behaviour in mice [101, 102]. The availability and rapid spread of Spice compounds have largely contributed to the change of the global scenario of cannabis consumption. Despite the efforts to ban their sale, SC products continue to be easily purchased by anyone, irrespectively of age, and therefore represent a major risk for health [103, 104].

CONCLUSION

The issue of the legalization of cannabis can be looked up in different ways, depending on the perspective from which it is considered. From a social point of view, the legalization may well reduce the perception of cannabis as a danger, by increasing both its availability and consumption in adults. The economic liberalization, through the excise duty charged by the states, may well become a significant source of revenues for the states involved. However, this approach may not extinguish at all the illicit market, which could even become more competitive than the legal one due to the amount of taxes levied by the states. Paradoxically, under-age subjects, who are also the largest consumers and those at higher risk of possible long-term effects of cannabis, for legal reasons cannot directly access the legal cannabis. Hence, they could be more exposed to cannabis because of its diversion from the legal to the illegal market. Therefore, apart from the possible economic benefits and tax advantages, the liberalization of cannabis may not seem at all to reduce the dangers of long-term sequelae on adolescents' health.

CONFLICTS OF INTEREST

FS is both a Core Member of the Advisory Council on the Misuse of Drugs (ACMD, UK) and a member of the Specialist Advisory Group (Psychiatry) for the European Medicines Agency (EMA). No conflicts of interest are declared here that may have influenced the interpretation of the present data. JC is a co-opted member of the ACMD's Technical and Novel Psychoactive Substances Committees, as well as being a member of the Scottish Government's NPS Expert Group and Hertfordshire County Community Safety Unit's NPS Working Group.

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