

Keep off the grass? Cannabis, cognition and addiction

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Abstract | In an increasing number of states and countries, cannabis now stands poised to join alcohol and tobacco as a legal drug. Quantifying the relative adverse and beneficial effects of cannabis and its constituent cannabinoids should therefore be prioritized. Whereas newspaper headlines have focused on links between cannabis and psychosis, less attention has been paid to the much more common problem of cannabis addiction. Certain cognitive changes have also been attributed to cannabis use, although their causality and longevity are fiercely debated. Identifying why some individuals are more vulnerable than others to the adverse effects of cannabis is now of paramount importance to public health. Here, we review the current state of knowledge about such vulnerability factors, the variations in types of cannabis, and the relationship between these and cognition and addiction.

Psychosis

A mental disturbance characterized by aberrant perceptions (hallucinations) and thoughts (delusions) that causes an individual to lose touch with external reality.

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doi:10.1038/nrn.2016.28
Published online 7 Apr 2016

For millennia, cannabis has been used medically and for religious purposes, most notably in China and India. The plant and its many constituent cannabinoids are now becoming increasingly important in modern medicine, particularly in the treatment of chronic pain and spasticity¹. A much more widespread global use is for pleasure²: the 'stoned' experience varies widely across individuals but often includes euphoria, a heightened awareness of music and colour, and the tendencies to eat a lot and to giggle profusely². Despite its pleasurable effects, most scientific research has focused on adverse consequences of using the drug, such as addiction, cognitive impairment and a possible increased risk of psychotic illness^{3,4}.

How patterns of use will change as the legalization of cannabis proliferates is not known, but even a small percentage increase in the current 182 million users worldwide⁵ will mean a considerable surge in absolute numbers. Are we now able to use existing evidence about the less desirable effects of cannabis use to help us to look forward to the future?

This Review aims to survey our current state of knowledge about, and then pinpoint how we should be increasing our understanding of, the effects of cannabis, given its potentially soaring future use. We first summarize the variety of unique ingredients in cannabis and outline how its use affects cognition, learning and memory in the short and long term. We survey evidence of how the effects of the drug vary according to the maturational state of the brain and then go on to discuss cannabis addiction and the mental health problems that are often related to it. Finally, we identify the important gaps in our

current knowledge and look to the future in terms of both research and the current tide of changes to the legislation of cannabis.

Cannabis: a plant with many forms

The multitude of names for cannabis (such as Purple Haze, Northern Lights, charas, skunk, resin, grass, marijuana and weed) in part reflects variations in genetics, growing conditions, processing, and constituent cannabinoids and terpenoids in different strains of the plant. Of the roughly 100 unique ingredients in cannabis that are called cannabinoids, most research to date has focused on the two most prominent of these: Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD). These two compounds seem to have a range of opposing effects on the human brain and behaviour. For example, Δ^9 -THC acutely impairs learning, produces psychosis-like effects and increases anxiety⁶, whereas CBD can enhance learning⁷ and has antipsychotic⁸ and anti-anxiety⁹ properties in humans. When taken together, CBD may ameliorate the harmful effects of Δ^9 -THC^{10,11}.

Δ^9 -THC acts as a partial agonist at cannabinoid 1 receptors (CB1Rs), whereas CBD has a complex range of pharmacological actions. For example, although CBD has low affinity for CB1R, it can attenuate CB1R agonist effects in the brain even at low concentrations (that is, providing functional antagonism of CB1R signalling)¹². Conversely, CBD reduces the cellular reuptake and hydrolysis of the endogenous cannabinoid ('endocannabinoid' (eCB)) anandamide (AEA; also known as *N*-arachidonyl ethanolamide) in the brain^{12,13}.

Neuroimaging studies have documented opposing effects of Δ^9 -THC and CBD on blood oxygenation level-dependent (BOLD) signals during performance of several cognitive and emotional tasks, including the striatal response during memory retrieval and the amygdala response to fearful faces¹⁴.

Over the past two decades, the Δ^9 -THC content of street cannabis has risen dramatically, whereas its CBD content has decreased to negligible levels. For example, in the United States, the Δ^9 -THC content of street cannabis rose from 4% in 1995 to 12% in 2014 (REF. 15). In Europe^{16,17} and Australia¹⁸, high-potency cannabis containing ~15% Δ^9 -THC and less than 0.1% CBD now dominates the market. Thus, the type of cannabis available

years ago differs considerably from that sold today, limiting the relevance of older longitudinal cohort studies (for example, the New Zealand birth cohort study; see BOX 1) to the mental health and cognitive function of contemporary users. In the United States, the cannabis that the National Institute of Drug Abuse supplies to researchers for experiments generally has less than 4% Δ^9 -THC, and so findings from these experiments have limited implications for modern-day cannabis users.

Cognition, learning and memory

eCBs are, in a sense, the brain's own natural cannabis system, and Δ^9 -THC and other CB1R agonists alter brain levels of eCBs^{19,20}. eCBs are neuroactive lipids that participate

Box 1 | Does cannabis affect IQ or educational attainment?

Case-control and prospective cohort studies have found associations between cannabis use and both lower IQ and lower educational attainment. But do these associations reflect any causal relationships?

Does cannabis affect IQ?

To date, there have been three large prospective cohort studies that have assessed the relationship between cannabis use and IQ.

In a New Zealand birth cohort study of 1,037 38-year-old individuals born in 1972 or 1973, persistent cannabis dependence was associated with a decline of up to 6 IQ points from that measured at the age of 7–13 years³⁴. The decline was particularly evident for those who developed cannabis dependence in adolescence, and remained apparent even for those who, at the age of 38 years, used cannabis less than once a week.

By contrast, a UK birth cohort study of 2,235 15–16-year-old adolescents born in 1991 or 1992 found that cumulative cannabis use was not associated with a lower IQ compared with non-using controls when IQ measured pre-teen and various potential confounders (in particular, the adolescents' use of cigarettes and alcohol) were taken into account¹⁶³. However, cannabis use was relatively low in this study, with only 72 adolescents reporting more than 50 lifetime cannabis exposures.

A US prospective cohort study of 3,066 17–20-year-old individuals found no difference in IQ from that measured at the age of 9–12 years between monozygotic and dizygotic twins discordant for cannabis use¹⁶⁴. However, there were only 47 discordant twin pairs in which the cannabis-using twin had used cannabis frequently (more than 30 cumulative uses, and/or daily use), limiting the strength of any conclusions from this study.

The UK and US studies therefore both suggest that genetic or environmental factors drive the observed associations between lower IQ and cannabis use, although both cohorts included younger participants with fewer cannabis exposures than did the New Zealand study.

To date, all studies have relied on retrospective self-report of cannabis use, have ignored possible residual effects of the drug on IQ test performance and have not addressed the potency or variety of cannabis used (see the main article). Addressing these issues with confirmation of exposure to Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and other cannabinoids (such as cannabidiol (CBD)) using hair samples from participants may lead to more reliable and consistent findings¹⁶⁵.

Does cannabis use affect educational attainment?

Several case-control and longitudinal studies have provided fairly consistent evidence of associations between adolescent cannabis use and both early school leaving and poorer educational performance^{166–169}. But the mechanisms producing these relationships remain hotly debated¹⁷⁰.

Causal explanations have posited that heavy cannabis use results in cognitive and/or motivational deficits, which in turn result in poorer educational attainment. There are many anecdotes about an 'amotivational syndrome' resulting from heavy cannabis use, and a recent positron emission tomography study demonstrated that cannabis users had reductions in striatal dopamine synthesis that correlated with a measure of amotivation¹⁷¹.

Alternatively, reverse causality has been also suggested; that is, perhaps poorer educational attainment leads to cannabis use^{166,168}. However, the one study that addressed this hypothesis showed that the association between early school leaving and later cannabis use could be accounted for by cannabis use before leaving school early¹⁶⁶.

The other alternative is that educational attainment and cannabis use may not be causally related but instead share common risk factors^{168,170,172}. Reported associations between cannabis use and lower educational attainment have typically been robust to adjustment for some potential confounders such as early-life factors, baseline school performance or cognitive ability, social disadvantage and parental educational achievement^{167,169}. However, the potential role of teenage behaviours that typically occur alongside cannabis use — including use of other substances and other 'risky' behaviours such as truancy — remain relatively unexplored^{163,170}.

Recent analyses showed that adjusting for teenage use of other substances attenuated the association between cannabis use and school attainment^{163,173,174}. As such, the existence of unmeasured confounds is often posited to account for the negative associations with cannabis^{163,168,170}. Indeed, this idea is strongly supported by recent genetic studies that found no difference in early school leaving¹⁷⁰ or years of education¹⁷⁵ between both monozygotic and dizygotic twin pairs who were discordant for cannabis use^{170,175}.

in a range of physiological processes including reward, motivation, emotional homeostasis, pain processing, and synaptic plasticity that contributes to learning and memory. At present, the best-characterized eCBs are AEA and 2-arachidonoylglycerol (2-AG)^{21,22}, and both of these lipids exert agonist activity at CB1Rs and CB2Rs. Owing to their lipid nature, AEA and 2-AG are not stored in vesicles but are synthesized on an 'on-demand' basis and, as such, brain eCB levels are critically reliant on the balance between evoked biosynthesis and subsequent clearance by intracellular enzyme-mediated hydrolysis. eCBs are crucial in certain forms of neuronal plasticity, and Δ^9 -THC has been shown to disrupt long-term potentiation (LTP; a model for learning and memory) and long-term depression (LTD) in preclinical studies²³. In this section, we consider the acute and longer-term effects of cannabis on cognition, learning and memory, as well as effects potentially persisting after an individual has stopped using the drug. We also review evidence on the impact of starting cannabis use early in adolescence.

Acute effects. Acute effects are transient and seen in the time period during which the individual is intoxicated with the drug (that is, feeling 'stoned' for around 5–120 minutes when smoked). A single dose of cannabis or its main active ingredient Δ^9 -THC robustly and dose-dependently impairs working and episodic memory^{24,25}. Memory impairments occur however the drug is administered, but the onset of effect is more rapid when it is inhaled or given intravenously than when it is ingested orally. Specifically, the encoding of new memories is impaired during cannabis intoxication, and this leads to subsequent deficits in recalling these memories; by contrast, the retrieval of old memories that were consolidated when not under the influence is unaffected. Cannabis-induced deficits in working memory are seen more in the ability to manipulate information while it is 'online' (for example, when doing mental arithmetic) than in the ability to simply retain information for brief periods (for example, when remembering a telephone number before dialling it). Whereas on placebo, brain activity in the dorsolateral prefrontal cortex (DLPFC) increases linearly with the working memory load of a task, acute dosing with Δ^9 -THC prevents this load-associated increase in DLPFC activity²⁶.

These effects on memory are consistent with the extensive preclinical evidence of: the amnesic effects of cannabis in animal models; the high density of cannabinoid receptors in memory-associated brain regions such as the hippocampus, amygdala and PFC (FIG. 1); and observations that Δ^9 -THC induces disruption of plasticity (including LTP and LTD) in the hippocampus and decreases acetylcholine release in both the hippocampus and the PFC (FIG. 2a).

Some studies report acute Δ^9 -THC-induced impairment of behavioural inhibition and increases in impulsivity, but findings on attention, decision-making and risk-taking tasks are mixed and task dependent^{25,27}. There is also some evidence that acute effects may vary depending on an individual's previous level of use of the drug. Tolerance to the memory-impairing²⁸

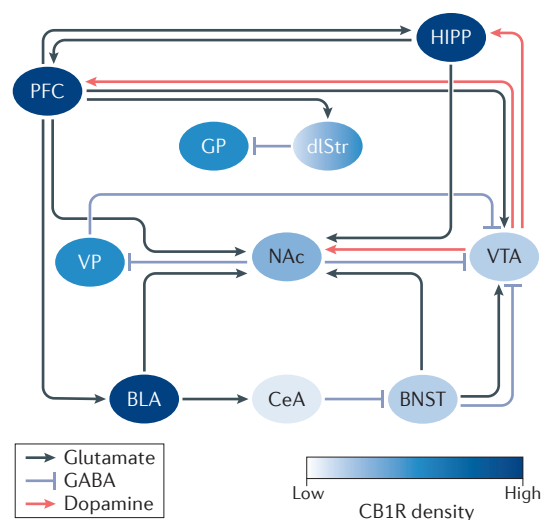


Figure 1 | Cannabinoid 1 receptor distribution within reward-, habit- and cognition-related circuits.

A simplified conceptualization of the major circuits implicated in reward (namely, the ventral tegmental area (VTA), nucleus accumbens (NAc)²⁰⁰ and ventral pallidum (VP)^{65,66,201}, stimulus–response habit formation (the dorsolateral striatum (dlStr) and globus pallidus (GP))⁶⁷ and cognition (the prefrontal cortex (PFC), hippocampus (HIPP) and amygdalar regions). Among these regions, cannabinoid 1 receptors (CB1Rs) are expressed with the following order of density^{202–206}, HIPP \approx basolateral amygdala (BLA) \approx PFC $>$ VP \approx GP \approx dorsolateral striatum (dlStr) $>$ NAc $>$ VTA \approx bed nucleus of the stria terminalis (BNST) $>$ central nucleus of the amygdala (CeA). Within the amygdala, CB1R expression is highest in the lateral and basolateral nuclei, with substantially lower expression in the central nucleus²⁰⁵. In the dorsal striatum, there is a comparable medial–lateral gradient of CB1R expression, with greater levels of expression evident in lateral aspects, and comparatively lesser CB1R expression is observed in the NAc²⁰⁶. The dense CB1R expression in HIPP, PFC and amygdalar regions underlies the effects of cannabis and CB1R agonists on cognitive and memory function, whereas the presence of CB1Rs within the mesocorticolimbic regions (VTA, NAc and PFC) contributes to the rewarding effects produced by cannabinoids.

and psychomotor²⁹ effects of acute Δ^9 -THC have been shown in individuals who use cannabis more than once a week, probably reflecting downregulation of cortical CB1Rs^{30,31} (FIG. 2b).

There is some evidence that acute effects of cannabis on memory depend on the particular type of cannabis ingested. Smoking cannabis with higher levels of CBD protected regular users against the acute memory-impairing effects of Δ^9 -THC¹⁰. Findings in cannabis-using volunteers replicated these protective effects of CBD on Δ^9 -THC-induced acute memory impairment¹¹. Indeed, CBD alone has been shown to enhance fear extinction learning in humans⁷. This further supports the notion that CBD and Δ^9 -THC may have opposing effects on some of the neural substrates of human memory³². A recent cross-sectional study found that CBD appeared to protect against hippocampal volume

Long-term potentiation (LTP). A lasting increase in the strength of neurotransmission at a synapse that is implicated in learning and memory.

Long-term depression (LTD). An enduring decrease in the strength of neurotransmission at a synapse that is implicated in learning and memory.

Episodic memory
Personal, contextualized autobiographical memory of past experiences.

Working memory
The capacity to hold information 'online' (maintenance) and manipulate it.

a Acute exposure

b Chronic exposure

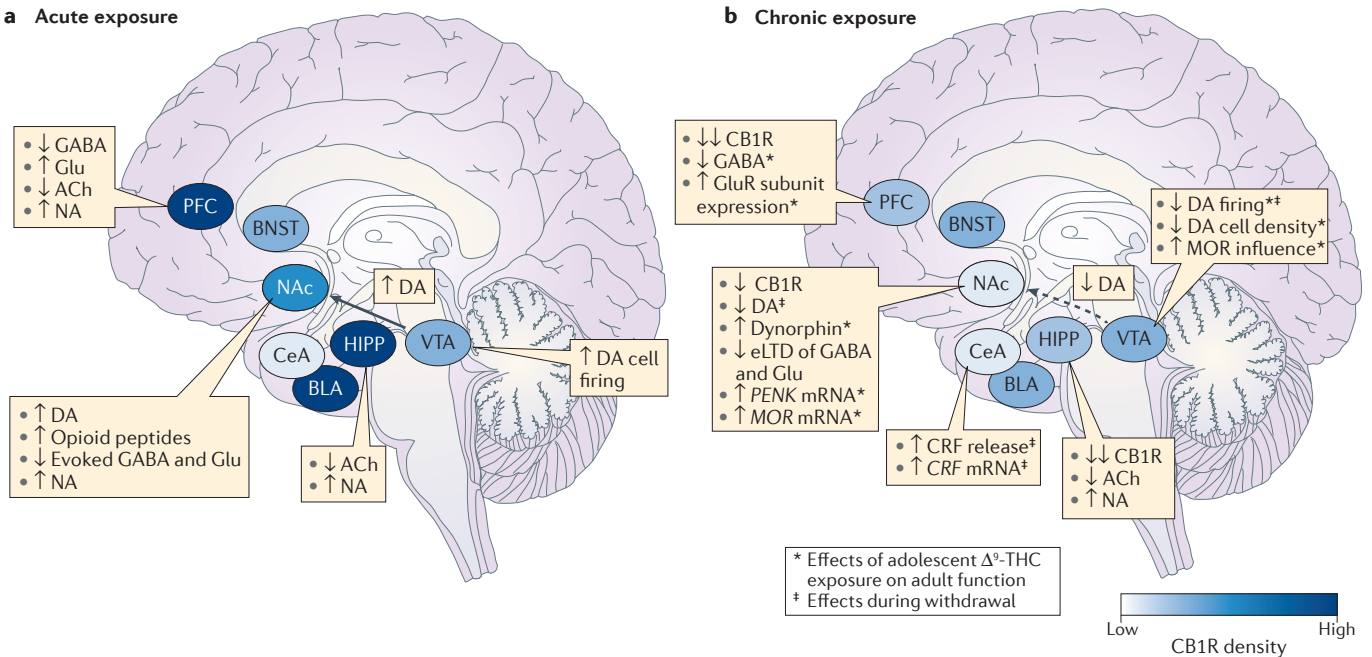


Figure 2 | Effects of acute or chronic exposure to cannabis on reward- and cognition-related circuits. Graphical summary of alterations in neurobiological function resulting from chronic exposure to cannabinoid agonists based on studies in rodents, non-human primates and humans. These collected observations should be viewed as a model of chronic cannabis exposure rather than an explicit representation of cannabis-induced alterations in human brain function. **a** | Acute cannabis or moderate-dose cannabinoid 1 receptor (CB1R) agonist exposure induces neurochemical events in the mesolimbic system that are similar to those produced by other drugs of abuse, including increased dopamine (DA) release and an attenuation of evoked GABA and glutamate (Glu) release in the nucleus accumbens (NAc)^{109,111–113,207,208}. The induced increase in opioid peptide release in the NAc^{118,119} probably also contributes to the acute rewarding effects of Δ^9 -tetrahydrocannabinol (Δ^9 -THC). Disruptions in cognitive function (including attention and memory impairments) probably result from: decreased acetylcholine (ACh) release in the hippocampus (HIPP) and prefrontal cortex (PFC)^{209,210}; reduced GABA release and increased Glu release in the PFC²¹¹; and increased noradrenaline (NA) release in HIPP and frontal cortical areas^{124–126}. **b** | Chronic Δ^9 -THC or CB1R agonist exposure results in decreased CB1R expression and function in many brain regions. Positron emission tomography imaging of daily cannabis users has revealed considerable decreases in CB1R levels, particularly in cortical regions^{30,31}, that correlate with years of cannabis use and withdrawal, and rapidly normalize during abstinence^{30,31}. Rodent studies demonstrate regional differences in the effects of chronic Δ^9 -THC on CB1R expression and function, with rapid and profound decreases evident in the hippocampus and layer VI of the frontal cortex, smaller but still statistically significant decreases in striatal and amygdalar regions, and nonsignificant disruptions in regions such as the globus pallidus (GP) and hypothalamic nuclei^{100,101}. The pronounced deficits in cortical and hippocampal CB1R function are consistent with memory and cognitive impairments associated with chronic cannabis use in humans. In this regard, chronic adolescent Δ^9 -THC exposure results in persistent disruptions of the hippocampal and cortical signalling that is critical for proper memory and cognitive functions. Chronic Δ^9 -THC exposure also disrupts reward-related signalling mechanisms in the mesolimbic system by reducing DA cell density in the ventral tegmental area (VTA)²¹² and by decreasing VTA DA cell firing and DA release in the NAc during both spontaneous and CB1R antagonist-precipitated withdrawal^{114,115}. Consistent with these observations in animal models, reduced striatal DA synthesis has been observed in human cannabis users, and this effect appears to be driven by individuals meeting cannabis use disorder criteria¹¹⁶. Together with increased stress-related signalling, such as dynorphin release in the NAc²⁰⁰ and corticotropin-releasing factor (CRF) release in the amygdala^{74,75}, these deficits in mesostriatal DA function may contribute to negative affective states associated with Δ^9 -THC abstinence. Adolescent Δ^9 -THC exposure also results in persistent increases in opioid peptide gene expression and influences the mesolimbic system of rats^{192,213,214}, possibly contributing to an increase in the rewarding effects of opiates and increased opiate consumption by these animals in adulthood^{191–193,213}. BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; eLTD, endocannabinoid-mediated long-term depression; GluR, glutamate receptor; MOR, μ -opioid receptor; *PENK*, proenkephalin.

loss associated with Δ^9 -THC³³. Future research should determine whether chronic exposure to CBD might be protective in the longer term.

Long-term effects. Although in several countries legislation enables new studies of medical cannabis to use prospective, randomized controlled trial designs, to date

studies of the neurocognitive effects of repeatedly using cannabis (that is, the long-term or chronic effects) have relied mainly on retrospective, self-reported drug use by people who choose to use cannabis recreationally, and, in most cases, illicitly. More-objective indices of drug use can be obtained through hair samples, although such analysis has limitations (for example, they are influenced

by hair dyes)³⁴ and have been rarely used in studies. Long-term impairments in memory have been reported mainly in frequent, heavy users, but confounding factors make it difficult to establish cause–effect relationships between cannabis use and changes in neurocognitive function. Such factors include baseline cognitive function before drug use; use of other cognitively impairing drugs, such as alcohol; types of cannabis used; age at which use started; and mental health problems, including depression and cannabis addiction.

Case–control studies of non-acute effects of cannabis have produced inconsistent findings to date, but tend to mirror acute findings. The most consistently reported long-term effects in these studies are impairments in the encoding of new episodic memories, with some studies finding persistent deficits in the first few days of abstinence but little evidence of persisting deficits at 28 days after use²⁵. Meanwhile, the findings for measures of working memory, attention and impulsivity are mixed. One study³⁵ found no difference in decision making or risk taking between cannabis users and non-users who were matched for potential mental health confounds. However, poorer decision-making accuracy in users was significantly associated with the number of symptoms of cannabis addiction.

Functional neuroimaging studies often reveal subtle differences in brain activity between chronic cannabis users and controls during performance of cognitive tasks; again, the persistence and clinical importance of these differences remain to be determined. A systematic review³⁶ of 43 studies concluded that chronic cannabis use may alter brain structure and function in both adult and adolescent users (particularly in the medial temporal and frontal cortices and the cerebellum). However, the findings of the mainly cross-sectional studies displayed remarkable heterogeneity, and it was not possible to infer causation. Further, deficits are most consistently observed only among heavy users — those most likely to be addicted to cannabis. Cognitive changes related to chronic exposure to cannabis or Δ^9 -THC observed in humans and in animal model studies (such as reduced inhibitory control and impaired decision making)^{27,37,38} are implicated in the maintenance of addiction³⁹, in part by impairing the reversal of behaviours related to drug acquisition or drug taking that propel continued drug use despite negative consequences. It could thus be proposed that these cognitive changes may interact with genetic vulnerability factors to increase the risk of developing cannabis addiction.

Age-dependent effects: adolescence and adulthood. The eCB system has a major role in neurodevelopmental and maturational processes including synaptic pruning and white-matter development, and these processes are especially prevalent during adolescence. As exogenous cannabinoids affect the functioning of the eCB system, it is plausible that prolonged use during adolescence disrupts the neurodevelopmental maturational processes during this period⁴⁰. Thus, the human brain may be more vulnerable to drugs at the time when use of cannabis often begins.

Preclinical studies have shown that repeated exposure to Δ^9 -THC has a greater negative impact on the working memory, object recognition and pre-pulse inhibition of adolescent rodents than on adult rodents⁴⁰. Chronic administration of CB1R agonists or Δ^9 -THC to adolescent rats produces persisting impairments in object recognition memory that are not seen with the same treatment in adult rats^{41,42}, as well as greater alterations in the level of expression of various hippocampal proteins (which may account for adolescent-specific memory effects)⁴². A single dose of Δ^9 -THC has also been found to result in greater acute impairments in spatial and non-spatial learning in adolescent rats than in adult rats⁴³.

The age-related effects of cannabis use on cognitive function may therefore be dependent on the maturational state of the neural circuits that are affected by the drug. This may reflect the fact that Δ^9 -THC induces perturbations in the crucial influence of the eCB signalling that is involved in brain development, for processes including neural proliferation, morphogenesis, neural migration and synaptogenesis^{44–46}. Consistent with this interpretation were the results of a study in which Δ^9 -THC was repeatedly administered over 6 months to adolescent monkeys at doses that corresponded well to human self-administration (approximately 1–2 joints on 5 days per week). This repeated administration blunted the usual pattern of accuracy improvements on a test of spatial working memory (which matures after object working memory), but not on an object working memory task⁴⁷. Thus, the persistent effects of Δ^9 -THC on cognition in animals are more evident when exposure coincides with the developmental stage during which cannabinoid-affected neural circuits are actively maturing.

Similarly, there is also accumulating evidence in humans that neurocognitive function and aspects of brain architecture are more disrupted by cannabis when individuals start using it during adolescence, although there is a scarcity of direct comparisons with adult users. Some structural imaging studies in adolescent and young adult cannabis users have reported decreased volume in several cortical and subcortical regions³⁶, but findings across different studies vary considerably⁴⁸. For example, although structural differences between adolescent cannabis users and controls in orbitofrontal cortex (OFC) volume have been found, smaller OFC volumes at 12 years of age were shown to predict cannabis use at 16, suggesting that differences in the OFC may be a vulnerability factor for use rather than a consequence⁴⁹. And although smaller hippocampal volumes in cannabis users have been associated with age of onset of use, this association seems to be less consistent than does the association between reductions in the size of the hippocampus and the amount of use, suggesting that the structure of the hippocampus may be more affected by the duration and intensity of exposure rather than by early use specifically⁵⁰.

Diffusion tensor imaging (DTI) studies have found poorer white-matter integrity (indexed by both lower fractional anisotropy and higher mean diffusivity) in

adolescents who use cannabis frequently compared with control non-users⁴⁸. Further, reductions in those indices of white-matter integrity were found to correlate with deficits in measures of neurocognitive performance.

Several functional MRI (fMRI) studies suggest that there is an increased BOLD signal in task-related areas in young cannabis users compared with non-using controls⁴⁸. For example, Jager and colleagues⁵¹ assessed 13–19-year-old boys who had used cannabis at least 200 times in their lives and compared them with non-using, age-matched controls. The cannabis users showed greater activation in prefrontal regions during a working memory task than did controls. Overall, most functional imaging findings suggest that adolescent cannabis users show increased recruitment of neural resources — potentially reflecting compensatory activity — in brain areas subserving task-related processing.

In terms of neurocognitive function, individuals who started using cannabis during adolescence have been reported to have greater deficits in visuospatial attention⁵², verbal fluency⁵³ and inhibition⁵³ than do those who start in adulthood. Importantly, based on the Dunedin prospective cohort data, one study⁵⁴ concluded that having cannabis addiction that started during adolescence and persisted into adulthood was associated with a decline of around 8 IQ points (BOX 1). However, two recent large-scale studies cast doubt on a causal explanation (BOX 1).

One limitation of the studies to date assessing the effects of cannabis use on the adolescent brain is that they have focused on age (whereby onset of use before 15–17 years of age is considered to be ‘early’) rather than on adolescent pubertal markers or potential sensitive periods that may more accurately index the stage of brain development⁵⁵. As the eCB system interacts with gonadal hormones, and girls typically begin puberty earlier and reach pubertal maturation earlier than boys, pubertal stage may influence findings on sex differences. The few studies looking at acute effects of cannabis or Δ^9 -THC have found little in the way of age-dependent sex differences, although such differences may exist in chronic users²⁵. One study that did investigate this, however, found that a younger age of regular cannabis use onset predicted poorer memory in women but not men⁵⁶. Surprisingly, the same study found that an earlier age of cannabis use initiation predicted better decision-making performance in both male and female cannabis users. Maturation of the PFC and its connections with the limbic system occurs earlier in girls⁵⁷, and this difference may contribute to reported sex differences in the effects of adolescent cannabis use^{25,56}. The paucity of studies of sex differences in the effects of cannabis limits conclusions and should be addressed by future research.

Effects persisting after stopping use. Several studies of long-term effects after an individual stops using cannabis are converging to show that cognitive impairments do not persist beyond 4–6 weeks after abstinence^{58,59}. Using positron emission tomography (PET) imaging, one study³⁰ demonstrated that chronic cannabis users showed downregulation of cortical CB1Rs that correlated with

years of use. After ~4 weeks of continuously monitored abstinence from cannabis at a secure research unit, their CB1R density returned to control levels, further supporting recovery within 4 weeks, and even, according to one recent study, after as little as 2 days³¹. Reversible downregulation of brain CB1Rs after chronic exposure to cannabis has also been shown in rodent studies⁶⁰. Other studies of ‘persisting’ effects have used structural and/or functional imaging but with cross-sectional designs, different abstinence intervals and a range of confounds (including group differences in comorbid alcohol use and pre-cannabis level of functioning), which make it difficult to draw any causal conclusions. Longitudinal studies are thus needed to determine whether these effects of abstinence are seen even in those starting use in adolescence⁶¹.

Cannabis addiction

Much research on cannabis and mental health has focused on psychosis (BOX 2), although addiction is a far more common problem: we estimate that people who try cannabis are ninefold more likely to become addicted to it than to develop psychosis in their lifetime^{62–64}. In this section, we introduce the concepts of cannabis addiction and withdrawal, review the rewarding effects of cannabinoids in relation to the eCB, dopaminergic, opioid and noradrenergic neurotransmitter systems, and highlight vulnerability factors and possible treatments for cannabis addiction.

Cannabis addiction and withdrawal. The term ‘addiction’ is in a terminological quagmire; here, however, we define it as an acquired, chronic, relapsing disorder that is characterized by a powerful motivation to continually engage in an activity despite persistent negative consequences. Addictive drugs can all cause similar changes to brain circuits underpinning reward, salience, impulsivity, compulsivity, learning and memory^{39,65–67}, although these changes differ according to class of drug (including cannabis)^{68,69}. Clinical problems associated with cannabis use were previously diagnosed as cannabis abuse or cannabis dependence in the *Diagnostic and Statistical Manual for Mental Disorders*, fourth edition, text revision (DSM-IV-TR). In the most recent version (DSM-5), these categories were amalgamated into a single diagnosis of ‘cannabis use disorder’ (CUD), as described in BOX 3. The estimated chances of becoming addicted to cannabis after lifetime exposure is 8.9%, which is considerably lower than for cocaine (20.9%), alcohol (22.7%) or tobacco (67.5%)⁶⁴. Nevertheless, the clinical need for treatment of cannabis addiction is substantial and increasing in North America, Europe and Oceania⁵. Across Europe, cannabis now accounts for more first-time entrants to drug treatment services than any other illicit drug⁷⁰.

A specific cannabis withdrawal syndrome — one aspect of addiction — is well recognized and affects around 50% of daily users upon cessation of use, and typically begins 1–2 days after cessation, peaks at 2–6 days and remits at 1–2 weeks⁷¹. Prominent symptoms include craving, sleep problems, nightmares, anger, irritability, dysphoria and nausea⁷². Cannabis withdrawal symptoms correlate with reductions in CB1R availability during

Cannabis abuse

Cannabis use that is problematic for various aspects of an individual's life (for example, causing occupational, educational or social problems) or that is carried out in dangerous contexts.

Cannabis dependence

A group of severe consequences of repeated cannabis use, including tolerance to effects, withdrawal symptoms upon cessation, dysregulation of use, increased involvement with cannabis at the expense of other activities, and continued use despite the problems it causes.

Reinforcement

A learning process through which particular stimuli or events (such as familiar drug-taking environments, or pleasant drug effects) influence the likelihood or strength of behaviour, such as drug seeking.

Intracranial self-stimulation

(ICSS). An operant paradigm in which animals perform a behavioural response to receive brief electrical pulses into specific regions in the brain reward pathways.

Conditioned place preference

A Pavlovian conditioning procedure used to index the motivational properties of drug experience. Typically, the time spent in an environment associated with drug intoxication is compared with that spent in a neutral context.

acute abstinence³¹ and can be alleviated by Δ^9 -THC in a dose-dependent manner⁷³. Δ^9 -THC withdrawal is also associated with increased release of the stress peptide corticotropin-releasing factor (CRF) in the central nucleus of the amygdala^{74,75}. Similar increases in amygdalar CRF release are evident during withdrawal from most classes of recreational drugs (including nicotine, alcohol, psychostimulants and opiates) and contribute to negative affective states and decreased brain reward function⁷⁶. It is therefore noteworthy that cannabis is frequently rolled with tobacco in 'joints', and many users also smoke cigarettes (BOX 4). In daily users of cannabis and tobacco, individual withdrawal effects seem to be similar for both drugs; combined withdrawal produces stronger effects than does withdrawal for either one alone⁷⁷.

Cannabinoids and reward. Δ^9 -THC produces the effects that cannabis users seek; they report liking it and wanting more²⁴. In addition, cannabis with higher Δ^9 -THC content (for example, 3.5% versus 2.0%) produces stronger reinforcement in human choice paradigms⁷⁸. As the reinforcement of drug use is considered to be one component in the transition from voluntary to compulsive use⁶⁷, these findings suggest that cannabis with high

Δ^9 -THC content might increase vulnerability to addiction. However, it is difficult to extrapolate these findings to modern, high-potency cannabis with ~15% Δ^9 -THC¹⁶⁻¹⁸. Recent naturalistic studies indicate that people adapt to rising Δ^9 -THC concentrations by adding less cannabis to their joints⁷⁹ and/or inhaling less smoke⁸⁰. Nevertheless, cross-sectional data suggest that use of cannabis with high Δ^9 -THC content is associated with greater addiction severity⁸¹. It is therefore possible that recent increases in cannabis potency^{15,16} might have contributed to the rising demand for treatment of cannabis addiction^{5,70}.

The presence or absence of CBD in evaluations of Δ^9 -THC reward may also be relevant. One study found that people who smoked cannabis containing low levels of CBD were more prone to have their attention captured by cannabis-related stimuli than were those smoking cannabis with high CBD content⁸². This suggests that CBD could protect against addiction, as attentional bias towards drug-related stimuli correlates with craving and is sensitive to relapse-provoking manipulations⁸³. However, CBD does not influence the acute reinforcing effects of cannabis or the rewarding feeling of being 'stoned' (REFS 82,84,85).

The reinforcing effects of cannabinoids in animals depend on species, route of administration and experimental design. Rats will perform an operant behaviour to receive Δ^9 -THC infusions into the brain ventricular space and to receive CB1R agonist infusions into the nucleus accumbens shell and posterior ventral tegmental area^{86,87}, as with other drugs of abuse. However, there has been substantial difficulty in establishing operant intravenous Δ^9 -THC self-administration in rodents⁷⁸, possibly owing to the prolonged pharmacokinetic effects of Δ^9 -THC that impede the establishment of discrete response-reward associations. The higher cognitive function in primates may allow for clearer discernment as to whether lever-pressing behaviour is causal for the somewhat delayed alterations in reward state resulting from intravenous administration of Δ^9 -THC. It is also possible that the aversive and motor-depressant effects of Δ^9 -THC present greater impediments to self-administration in rodents than in primates.

Consistent with the human literature, studies in rats also demonstrate that Δ^9 -THC reward is dose dependent. These effects appear to follow an inverted-U-shaped curve, whereby high- Δ^9 -THC doses are less reinforcing than medium doses. For example, as indexed using the intracranial self-stimulation (ICSS) paradigm, reward-system function in the rodent brain is enhanced by low doses of Δ^9 -THC⁸⁸, whereas higher doses and more-potent CB1R agonists can decrease this function^{89,90}. Consistent with this, the rewarding effects of low doses of Δ^9 -THC or CB1R agonists in the conditioned place preference paradigm are supplanted by aversive effects at higher doses^{86,91,92}. An inverted-U-shaped profile is consistent with data from human studies that indicate that cannabis with high Δ^9 -THC content is preferred and associated with greater addiction severity than cannabis with low Δ^9 -THC content⁸¹, whereas extremely potent products (such as synthetic CB1R agonists) are less addictive than 'natural' cannabis, have more negative effects and are only preferred by 7% of users⁹³.

Box 2 | Cannabis and psychosis: cause, consequence or correlation?

Nearly 2,000 studies have been published on this topic since 1962, and the pro-psychotic effects of cannabis have dominated media reporting about this drug. But how clear is the link? Several longitudinal, population-based studies show an earlier first episode¹⁷⁶ and a roughly twofold increase in the risk of psychosis with regular cannabis use⁶². However, the vast majority of people who use cannabis do not develop psychotic disorders such as schizophrenia, and many people diagnosed with such disorders have never used cannabis.

More agreement is found in evidence that heavy cannabis use may mean that young people who are vulnerable to psychosis develop the disorder when they may not have otherwise done so. Converging data suggest that this may have a genetic basis, with certain polymorphisms of the gene encoding AKT1 potentially conferring risk of psychosis following smoking cannabis acutely¹⁷⁷ and chronically^{178,179}.

The type of cannabis used has recently been found to affect the risk of psychosis: self-reported hash use, even daily, is not associated with an increased risk of psychosis, whereas self-reported daily use of skunk (which contains high levels of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and negligible amounts of cannabidiol (CBD)) is associated with a fivefold greater chance of having schizophrenia¹⁸⁰. Several studies using objective biological markers of use have shown that CBD reduces the psychosis-like effects of Δ^9 -THC^{154,181}.

How cannabis interacts with the brain to increase the risk of psychosis is unclear. One confusing finding is that of reduced dopamine release in cannabis users¹¹⁶, which seems to be inconsistent with the higher levels of dopamine release observed in people with psychosis. Disruptions in the brain's endocannabinoid system, conversely, have been found in psychosis and may provide clues as to the pro-psychotic impact of cannabis. Higher levels of anandamide (AEA) in the cerebrospinal fluid have been associated with lower psychotic symptoms in individuals diagnosed with schizophrenia¹⁸², in individuals classified as having prodromal schizophrenia¹⁸³ who do not smoke cannabis and in cannabis users without a diagnosis of schizophrenia¹⁰⁷. AEA is known to have a neuromodulatory role in the brain; thus, during prodromal or first-episode psychosis, levels of AEA may be increased to attempt to control dysregulated brain dopamine¹⁸⁴.

In addition, *in vivo* positron emission tomography imaging and post-mortem receptor autoradiography studies have consistently reported higher levels of ligand binding to cannabinoid 1 receptors in several cortical regions of people with schizophrenia¹⁸⁵. Whether these alterations are part of the disease pathology or a compensatory response remains unclear, but they do suggest a molecular basis for a heightened sensitivity to cannabis of individuals with schizophrenia, and perhaps of those at risk.

Box 3 | Cannabis use disorder

Cannabis-related problems vary on a continuum, and it is important to define these not only for clinical diagnosis and treatment but also for research. The *Diagnostic Statistical Manual of Mental Disorders*, fifth edition (DSM-5)¹⁸⁶ has combined the definitions for 'cannabis abuse' and 'cannabis dependence' to provide criteria for the diagnosis of 'cannabis use disorder' (CUD). DSM-5 states that CUD is: "A problematic pattern of cannabis use leading to clinically significant impairment or distress, as manifested by at least 2 of the [symptoms from the three lists below], occurring within a 12-month period" (REF. 186). Mild CUD is associated with having two or three of these symptoms, moderate CUD, with four or five symptoms, and severe CUD, with six or more symptoms. One DSM-IV criterion — concerning persistent legal problems related to cannabis use — was dropped and not included in DSM-5 because it largely reflected sociocultural factors to do with policing and law enforcement rather than cannabis use factors.

One advantage of combining abuse and dependence criteria in CUD is to provide a clearer continuum between mild and severe, because previously all cases of dependence also met criteria for abuse. Another advantage is that dependence is often a normal bodily response to a substance (for example, a prescribed pain killer) that should not be confused with addiction (inasmuch as stopping use of that pain killer does not necessarily lead to drug seeking).

Cannabis dependence symptoms from DSM-IV

The following symptoms are taken from the DSM-IV criteria for cannabis dependence¹⁸⁷:

- Cannabis is often taken in larger amounts or over a longer period than was intended
- There is a persistent desire or unsuccessful efforts to cut down or control cannabis use
- A great deal of time is spent in activities necessary to obtain cannabis, use cannabis, or recover from its effects
- Important social, occupational, or recreational activities are given up or reduced because of cannabis use
- Cannabis use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by cannabis
- Tolerance, as defined by either [1] a need for markedly increased cannabis to achieve intoxication or desired effect or [2] a markedly diminished effect with continued use of the same amount of the substance
- Withdrawal, as manifested by either [1] the characteristic withdrawal syndrome for cannabis or [2] cannabis is taken to relieve or avoid withdrawal symptoms

Cannabis abuse symptoms from DSM-IV

The following symptoms are taken from DSM-IV criteria for cannabis abuse¹⁸⁷:

- Recurrent cannabis use resulting in a failure to fulfil major role obligations at work, school, or home
- Continued cannabis use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of cannabis
- Recurrent cannabis use in situations in which it is physically hazardous

New symptom in DSM-5

This item was added as a new symptom to DSM-5:

- Craving, or a strong desire or urge to use cannabis

In animals, CB1R antagonism produces a 'rightward' shift along this inverted-U-shaped reward-aversion dose-effect function, blocking the rewarding effects of low doses of CB1R agonists and preventing the aversive effects of high CB1R agonist doses. This underscores the bimodal effects of CB1R activity on brain reward processing. Squirrel monkeys voluntarily self-administer intravenous Δ^9 -THC⁹⁴, and rats reliably self-administer synthetic CB1R agonists^{95,96} — actions that are prevented by CB1R antagonism. Taken together, these studies suggest that CB1R activity has a crucial role in cannabinoid reinforcement and cannabis addiction.

Similar to Δ^9 -THC and many synthetic cannabinoid agonists, eCBs exhibit agonist effects at brain CB1Rs. The eCB AEA takes its name from the Sanskrit word *ananda*, meaning 'bliss'. Although pharmacologically enhanced eCB signalling (for example, through the inhibition of eCB clearance) generally does not produce rewarding effects per se, persistent disruptions in eCB signalling appear to contribute to facets of drug dependence across drug classes^{97,98}. CB1R downregulation in chronic cannabis users has been reported in three studies^{30,31,99}; these effects subside within days to several weeks of sustained abstinence^{30,31}. Similarly, rodents chronically exposed to Δ^9 -THC or synthetic CB1R agonists exhibit a reduction in CB1R function throughout the brain^{100,101} that persists for days to weeks following Δ^9 -THC treatment, followed by a functional recovery that varies between brain regions¹⁰². eCB-mediated forms of synaptic plasticity in the nucleus accumbens and hippocampus are abolished following exposure to Δ^9 -THC or CB1R agonists^{101,103,104} — and this may substantially affect reward processing and memory processes mediated by these regions. Chronic exposure to Δ^9 -THC or CB1R agonists increases enzymatic clearance of AEA and reduces brain tissue AEA content in rodents^{19,105,106} and, consistent with these data, frequent cannabis smokers exhibit decreased AEA levels in cerebrospinal fluid¹⁰⁷. Although evidence is limited, serum AEA levels may be elevated in former users of the drug following prolonged cannabis abstinence¹⁰⁸.

Dopamine, opioids and noradrenaline. Human PET studies indicate that Δ^9 -THC can increase dopamine release in the striatum¹⁰⁹, although to a far lesser extent than do other recreational drugs, and not in all studies¹¹⁰. In rodents, Δ^9 -THC and CB1R agonists increase the firing rate and bursting activity of ventral tegmental area dopamine neurons, resulting in dose-dependent increases in the mesocorticolimbic release of dopamine^{111–113}. Conversely, withdrawal from chronic Δ^9 -THC or CB1R agonist exposure is associated with decreased firing of dopaminergic cells and decreased dopamine release in the nucleus accumbens^{114,115}. Consistent with these observations in animal models, reduced capacity to synthesize striatal dopamine was recently reported in human cannabis users, particularly among addicted individuals¹¹⁶. However, in humans, chronic cannabis exposure is not typically associated with abnormalities in striatal dopamine release or in D2 receptor expression¹¹⁷ and, together with the modest dopaminergic effects of acute Δ^9 -THC, the available human data provide only weak support for dopaminergic involvement in cannabis addiction.

Δ^9 -THC-induced increases in opioid peptide release^{118,119} may also contribute to the rewarding effects of cannabis. The opioid receptor antagonist naltrexone reduces Δ^9 -THC-induced increases in mesolimbic dopamine release, intravenous Δ^9 -THC self-administration and intracerebroventricular CB1R agonist self-administration in rats and monkeys^{120,121}. Sixteen days of naltrexone treatment reduced self-administration and some positive subjective effects of Δ^9 -THC in humans¹²².

Box 4 | The gateway theory

Although cannabis is traditionally considered to be a 'soft' drug, it is widely believed to act as a 'gateway' to harder drugs such as cocaine or heroin (with harm defined in terms of detrimental effects of using that substance on the individual and on society). According to this theory, there is a sequential progression from one drug to the next (for example, cannabis leading to cocaine, and then heroin)¹⁸⁸. This theory rests on evidence that use of one drug increases the likelihood of using the next drug in the 'sequence', which may be interpreted as causal if all other confounds are accounted for¹⁸⁸.

Evidence does support sequential progression and association between cannabis and other illicit drugs, and these effects increase with frequency of cannabis use and adolescent onset¹⁸⁹. In addition, a twin study¹⁹⁰ suggested that these effects cannot be attributed to shared genetic or environmental factors alone. Studies in rats demonstrate that adolescent Δ^9 -tetrahydrocannabinol (Δ^9 -THC) exposure results in increased opiate consumption and facilitated learning of cocaine self-administration in adulthood^{191–193}, and that the influence of adolescent Δ^9 -THC exposure on opiate reward may be transgenerational¹⁹⁴.

However, establishing causality remains challenging, and putative mechanisms remain speculative. For example, one possibility is that the wide availability of cannabis means that people are more likely to use it first, and this increases the chances that other illicit drugs are used because of contact with other drug users and with people selling illicit drugs¹⁹⁰. This formed part of the Netherlands' rationale for the regulated sale of cannabis; incidentally, the rates of cocaine use among people who have used cannabis are lower there (22%) than in the United States (33%)¹⁹⁵.

Biological explanations for the gateway hypothesis are supported by evidence that exposure to Δ^9 -THC enhances the reinforcing effects of other drugs. Intriguingly, however, prior Δ^9 -THC ingestion increases self-administration of nicotine and the reinforcing effects of nicotine in rats¹⁹⁶, but this does not occur with prior cocaine or heroin exposure^{197,198}. Indeed, one of the most potentially harmful and under-appreciated effects of cannabis is the 'reverse gateway': by smoking tobacco together with cannabis in 'joints', individuals may progress to nicotine addiction^{160,199}.

Δ^9 -THC and other CB1R agonists stimulate noradrenergic cell firing and increase levels of noradrenaline in multiple brain regions in which the neurotransmitter can affect motivated behaviours¹²³ — including the nucleus accumbens, prefrontal and cerebral cortices, hippocampus and hypothalamus^{124–126}. The behavioural importance of CB1R agonist-induced increases in noradrenaline release has not been carefully evaluated, although evidence suggests that such increases in noradrenaline release may contribute to the aversive (but not anxiogenic) effects of high doses of CB1R agonists¹²⁷. For example, the aversive effects of high doses of the CB1R agonist WIN 55,212-2 (as measured in the conditioned place aversion paradigm) are reduced by attenuation of noradrenergic signalling in the nucleus accumbens, although WIN 55,212-2-induced anxiety-like behaviour (as measured on the elevated plus maze) is not reduced by these manipulations¹²⁷.

Vulnerability factors. Irrespective of the drug constituents^{81,82}, only a minority of cannabis users become addicted; therefore, other factors must predict vulnerability. Concurrent tobacco use has been identified as a risk factor in several studies^{128–130}, as have early adolescent onset and frequent (especially daily) use^{128,131}. Males typically have an earlier opportunity to use cannabis, a greater risk of addiction and a faster progression from first opportunity of use to addiction^{64,128,129}. These findings are consistent with normative data from European treatment services: the mean age of first treatment is 24 years; the mean age at first cannabis use is 16 years; and 83% of treated individuals are male⁷⁰.

Interestingly, a 3-year prospective study of daily users found that variables related directly to cannabis use did not predict transition to addiction; more important were current factors such as living alone, coping motives and negative life events (such as having had a major financial crisis)²¹⁵. A meta-analysis of 24 twin studies¹³² suggested that genetic influences account for 55% of the vulnerability to cannabis addiction, with shared environmental factors and non-shared environmental factors accounting for much lower proportions (17.5% and 27.5%, respectively). Although isolated studies have identified specific gene variants associated with an increased risk of developing cannabis use disorder (reviewed in REF. 133), cannabis addiction phenotypes are likely to be polygenic, and genotypes probably overlap with those linked to substance addiction in general¹³⁴.

Possible treatments for cannabis addiction. Cannabis addiction is not easily treated by psychological therapies¹³⁵, and although many pharmacotherapies have been tested¹³⁶ — including antidepressants, anxiolytics, noradrenaline-reuptake inhibitors, anticonvulsants, glutamatergic modulators and CB1R agonists — none has been approved. Based on existing clinical trial data, a 12-week trial of the GABA mimetic gabapentin¹³⁷ and an 8-week trial of *N*-acetylcysteine¹³⁸ have shown promise for reducing cannabis use according to urine sampling during treatment. Gabapentin also improved several secondary outcomes, including withdrawal symptoms, executive function and self-reported depression¹³⁷. CB1R agonists such as dronabinol (an oral synthetic Δ^9 -THC)¹³⁹ and nabiximols (an oral spray containing Δ^9 -THC and CBD in equal ratio)¹⁴⁰ attenuated cannabis withdrawal symptoms and improved treatment retention, but did not reduce cannabis use compared with placebo. These findings suggest that substitution treatments can replace Δ^9 -THC in cannabis but are not sufficient to promote abstinence from it.

Few studies have evaluated the contribution of dysregulated eCB signalling to cannabis addiction and related physiological and behavioural disruptions. However, eCBs provide important homeostatic regulation over emotional state¹⁴¹ and sleep function¹⁴², and so it is plausible that Δ^9 -THC-induced impairment of eCB signalling contributes to the negative emotional states and sleep disturbances that are present during protracted cannabis abstinence^{71–73}. Intriguingly, studies in rodents demonstrate a palliative effect of 2-AG-clearance inhibitors on the somatic symptoms of CB1R-antagonist-precipitated Δ^9 -THC withdrawal¹⁰⁵. Collectively, these observations have led to the proposed use of eCB-clearance inhibitors as treatments for cannabis withdrawal, and perhaps addiction^{143,144}. Given the largely unmet clinical need, developing effective pharmacological treatments should be a top research priority.

Cannabis, anxiety and depression

Like most addictions, cannabis addiction is often comorbid with other mental health problems. Epidemiological evidence indicates a possible association between regular cannabis use and the development of anxiety and

depression. However, the evidence is more mixed and less consistent than that for an association between cannabis use and psychosis⁶². One recent study compared the mental health of individuals who were addicted to cannabis (according to the DSM-IV) with that of non-addicted cannabis users who had similar patterns of cannabis use. Only the addicted users had depression and anxiety problems¹⁴⁵. Compared with the general population, non-addicted frequent users were more likely to show externalizing disorders (such as attention-deficit hyperactivity disorder), which were likely to have predated their cannabis use. Otherwise, these individuals were similar in terms of mental health to the general population, suggesting that cannabis contributes to mental health problems only in those who are vulnerable for other reasons.

Depression and anxiety disorders not only are associated with cannabis addiction¹⁴⁶ but also are predictive of whether individuals transition from use to addiction¹⁴⁷. Strikingly, a high number of cases of depression and anxiety disorders were reported among obese individuals who were treated with the anti-obesity drug rimonabant, a CB1R antagonist. Many of these individuals had no prior history of these disorders^{148,149}, and so this led to the withdrawal of rimonabant from therapeutic use. These findings suggest that CB1R antagonists increase the risk of depression and/or anxiety. Moreover, pre-clinical studies have shown that mice that genetically lack CB1Rs show increased depressive-like symptoms¹⁵⁰ and, in wild-type mice, CBD has antidepressant effects¹⁵¹. Rodent studies have implicated the eCB system in the regulation of emotion¹⁵². Similarly, there are also data from rodent studies suggesting that impaired CB1R signalling leads to depression-like symptoms, and that enhancement of CB1R signalling produces antidepressant-like behavioural effects in rodents¹⁵³.

In our own studies of young (16–24-year-old) daily cannabis users, we have found that levels of Δ^9 -THC in hair are significantly associated with self-reported levels of both depression and anxiety¹⁵⁴. However, a recent epidemiological study¹⁴⁶ suggested that increases in self-reported depression in cannabis users are not long-lasting, as no consistent associations were found between adolescent cannabis use and depression at the age of 29 years. By contrast, the same study showed that daily cannabis use and cannabis addiction in early adulthood were associated with more than double the non-user control rate of anxiety disorders at 29 years of age. The association between cannabis use and anxiety may arise because the same factors that predispose people to use cannabis also predispose them to anxiety. Indeed, there is accumulating evidence that, in vulnerable individuals, cannabis is often ‘used’ to self-medicate social anxiety^{155,156}. This is interesting, as controlled studies of acute effects in humans have shown that Δ^9 -THC increases anxiety⁶, whereas CBD decreases it⁹. Furthermore, Δ^9 -THC and CBD acutely produce opposite but subtle effects on human facial affect recognition⁸⁴ and amygdala activation when viewing fearful faces³².

The interconnectedness of cannabis use, mental health problems and cognitive functioning is important. It is inherently difficult to determine causality

in the type of studies discussed above because factors besides cannabis use (for example, premorbid cognitive and emotional function) may be directly associated with risk of mental illness. Such factors could predispose an individual both to mental illness and to using cannabis³, and the combination of these disorders would in turn increase their impact on cognitive functioning.

Conclusions and future directions

Cannabis has been used for thousands of years for a range of medicinal purposes as well as for its desired psychological and social effects, which recreational users value. This use can, however, carry a penalty: a range of undesired effects that vary in the severity of their impact on the individual's life. Although evidence of clear causality is lacking, these undesired effects may range from mild cognitive impairment to disabling psychiatric disorders. However, most recreational and medical users appear to rate the benefits as outweighing the risks in choosing to continue their cannabis use. It should be noted that public health messages to users are distorted, because funding for research is often targeted to studying the harmful effects of cannabis rather than the benefits. Despite studies aiming to document negative effects, occasionally positive effects are noted, such as enhanced divergent thinking following either oral THC or smoked cannabis^{24,157}. Future research should evaluate perceived benefits to give a more balanced understanding; people clearly do not use cannabis only for its harms.

Throughout this Review, we have specified gaps in our knowledge. Although problems associated with cannabis use are mainly observed in heavy, frequent users, we are still not sure what level of use of what type of cannabis is non-problematic. We need such data for harm-reduction advice for both medical and recreational users.

Another question that needs answering is: how does repeated use of cannabis causally affect the human adolescent and adult brain given that most cannabis users also use alcohol, another cognitively impairing drug? A recent comparison of daily cannabis users who also drank alcohol and alcohol-intake-matched, non-using controls in both adolescents and adults found no differences in brain structure between the two groups¹⁵⁸, but longitudinal data are currently lacking. It is also still unclear exactly how the effects of cannabis vary across clinical populations. For example, a meta-analysis showed that people who are diagnosed with schizophrenia and use cannabis function better cognitively than individuals with schizophrenia who do not use cannabis¹⁵⁹. Finally, we need to understand more about how variants of the drug produce differential effects — variants not only in constituent cannabinoids but also in the new synthetic cannabis (such as ‘spice’)⁹³, as well as new forms of administration (for example, through vaping or edible forms)¹⁶⁰.

Future studies will help to fill these gaps of knowledge, especially if they incorporate methodological improvements. Most studies of long-term effects assess level of cannabis use through various self-report

measures (such as frequency, years of use and time to smoke a specific amount of the drug) and could benefit from improved biomarkers such as Δ^9 -THC and CBD levels in hair. We need longitudinal studies that follow young people from before puberty (and before drug use), through biologically defined puberty and into adulthood (while monitoring drug use) and again after subsequent abstinence or continued use. Such studies would use a comprehensive range of assessments (including: tests of cognition, motivation, brain function and mental health; biomarkers of different types; and quantities of cannabis or cannabinoids and other recreational substances used) so that the interactions among these factors may be monitored. This need should be addressed by the US National Institutes of Health-funded Adolescent Brain Cognition Development (ABCD) Study: a prospective, 10-year, longitudinal study of 10,000 individuals beginning at 9–10 years of age. This study is designed to assess the impact of substance use on brain development and neurocognitive function through a battery of measures obtained before cannabis use, following use at various levels and again following cessation of use. Given recent changes in the medical and legal status of cannabis in some countries, randomized controlled trials of the effects of different types and doses of cannabis can now be conducted. Work with animals will be important in delineating chronic effects, and studies administering doses and schedules within a human-relevant range (as exemplified in REF. 47) are the most helpful.

The number of cannabis addicts may well grow as cannabis use becomes more acceptable and the drug more accessible. Indeed, there is evidence that this is already happening in the United States¹⁶¹. We therefore urgently need to increase our efforts to understand what factors influence the development of addiction and build preventive measures against this. Currently, we lack an effective pharmacological treatment for cannabis addiction that can be used conjointly with psychosocial therapies to boost the currently low efficacy of treatment approaches. This need requires urgent research attention.

With hindsight, we can clearly see the enormous problems that have been caused to many individuals and to society by tobacco and alcohol. Unlike cannabis, these drugs are legal in most countries, despite the fact that, if asked to decide today which psychoactive drugs should be legal, cannabis (which rarely kills people) might well be judged as being comparatively benign. Legislative changes would help researchers, as current restrictive drug scheduling markedly hinders neuroscience research and the innovation of psychiatric treatments¹⁶². More importantly, if handled carefully from a harm-reduction standpoint, a regulated market might increase the control over the age of initiation of use and other vulnerability factors; inform accurately about dosage; and increase the availability of more-balanced cannabis (that is, with lower levels of Δ^9 -THC and higher levels of CBD) to maintain desired effects while reducing the incidence of harms.

- Whiting, P. F. *et al.* Cannabinoids for medical use: a systematic review and meta-analysis. *JAMA* **313**, 2456–2473 (2015).
- Curran, H. V. & Morgan, C. J. A. in *Handbook of Cannabis* Ch. 36 (ed. Pertwee, R. G.) (Oxford Univ. Press, 2014).
- Volkow, N. D., Baler, R. D., Compton, W. M. & Weiss, S. R. Adverse health effects of marijuana use. *N. Engl. J. Med.* **370**, 2219–2227 (2014).
- Hall, W. What has research over the past two decades revealed about the adverse health effects of recreational cannabis use? *Addiction* **110**, 19–35 (2015).
- United Nations Office on Drugs and Crime. *World drug report* (UNODC, 2015).
- D'Souza, D. C. *et al.* The psychotomimetic effects of intravenous Δ^9 -tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* **29**, 1558–1572 (2004).
- Das, R. K. *et al.* Cannabidiol enhances consolidation of explicit fear extinction in humans. *Psychopharmacology* **226**, 781–792 (2013).
- Leweke, F. *et al.* Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl. Psychiatry* **2**, e94 (2012).
- Bergamaschi, M. M. *et al.* Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology* **36**, 1219–1226 (2011).
- Morgan, C. J., Schafer, G., Freeman, T. P. & Curran, H. V. Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study. *Br. J. Psychiatry* **197**, 285–290 (2010).
- Englund, A. *et al.* Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. *J. Psychopharmacol.* **27**, 19–27 (2013).
- Pertwee, R. G. The diverse CB₁ and CB₂ receptor pharmacology of three plant cannabinoids: Δ^9 -tetrahydrocannabinol, cannabidiol and Δ^9 -tetrahydrocannabinol. *Br. J. Pharmacol.* **153**, 199–215 (2008).
- Muniyappa, R. *et al.* Metabolic effects of chronic cannabis smoking. *Diabetes Care* **36**, 2415–2422 (2013).
- Batalla, A. *et al.* Neuroimaging studies of acute effects of THC and CBD in humans and animals: a systematic review. *Curr. Pharm. Des.* **20**, 2168–2185 (2014).
- ElSohly, M. A. *et al.* Changes in cannabis potency over the last two decades (1995–2014): analysis of current data in the United States. *Biol. Psychiatry* <http://dx.doi.org/10.1016/j.biopsych.2016.01.004> (2016).
- Hardwick, S. & King, L. A. *Home Office cannabis potency study 2008* (Home Office Scientific Development Branch United Kingdom, 2008).
- Niesink, R. J., Rigtter, S., Koeter, M. W. & Brunt, T. M. Potency trends of Δ^9 -tetrahydrocannabinol, cannabidiol and cannabiolin in cannabis in the Netherlands: 2005–15. *Addiction* **110**, 1941–1950 (2015).
- Swift, W., Wong, A., Li, K. M., Arnold, J. C. & McGregor, I. S. Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. *PLoS ONE* **8**, e70052 (2013).
- Di Marzo, V. *et al.* Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of Δ^9 -tetrahydrocannabinol-tolerant rats. *J. Neurochem.* **74**, 1627–1635 (2000).
- Gonzalez, S. *et al.* Behavioral and molecular changes elicited by acute administration of SR141716 to Δ^9 -tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. *Drug Alcohol Depend.* **74**, 159–170 (2004).
- Hillard, C. J. Chapter one — the endocannabinoid signaling system in the CNS: a primer. *Int. Rev. Neurobiol.* **125**, 1–47 (2015).
- Mechoulam, R., Hanuš, L. O., Pertwee, R. & Howlett, A. C. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat. Rev. Neurosci.* **15**, 757–764 (2014).
- Zhu, P. J. Endocannabinoid signaling and synaptic plasticity in the brain. *Crit. Rev. Neurobiol.* **18**, 113–124 (2006).
- Curran, H. V., Brignell, C., Fletcher, S., Middleton, P. & Henry, J. Cognitive and subjective dose–response effects of acute oral Δ^9 -tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology* **164**, 61–70 (2002).
- Crane, N. A., Schuster, R. M., Fusar-Poli, P. & Gonzalez, R. Effects of cannabis on neurocognitive functioning: recent advances, neurodevelopmental influences, and sex differences. *Neuropsychol. Rev.* **23**, 117–137 (2013).
- Bossong, M. G. *et al.* Effects of Δ^9 -tetrahydrocannabinol on human working memory function. *Biol. Psychiatry* **71**, 693–699 (2012).
- Crean, R. D., Crane, N. A. & Mason, B. J. An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *J. Addict. Med.* **5**, 1–8 (2011).
- D'Souza, D. C. *et al.* Blunted psychotomimetic and amnesic effects of Δ^9 -tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology* **33**, 2505–2516 (2008).
- Ramaekers, J. G. *et al.* Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. *Psychopharmacology* **214**, 391–401 (2011).
- Hirvonen, J. *et al.* Reversible and regionally selective downregulation of brain cannabinoid CB₁ receptors in chronic daily cannabis smokers. *Mol. Psychiatry* **17**, 642–649 (2012).
- This was the first study to report downregulation of CB₁Rs in frequent cannabis users: the effects were reversed after 4 weeks of monitored abstinence, consistent with findings in rodents.**
- D'Souza, D. C. *et al.* Rapid changes in CB₁ receptor availability in cannabis dependent males after abstinence from cannabis. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **1**, 60–67 (2016).
- This paper reported a reversal of the downregulation of CB₁Rs in frequent cannabis users after only 2 days.**
- Bhattacharyya, S. *et al.* Opposite effects of Δ^9 -tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* **35**, 764–774 (2010).

33. Yücel, M. *et al.* Hippocampal harms, protection and recovery following regular cannabis use. *Transl. Psychiatry* **6**, e710 (2016).
This cross-sectional study used hair analysis to examine the relationship between cannabinoids and hippocampal integrity, and found that chronic Δ^9 -THC exposure is associated with reduced hippocampal volume and N-acetylaspartate concentrations; however, these effects are not found in those individuals with CBD as well as Δ^9 -THC in hair or after extended abstinence from the drug.
34. Han, E., Chung, H. & Song, J. M. Segmental hair analysis for 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid and the patterns of cannabis use. *J. Anal. Toxicol.* **36**, 195–200 (2012).
35. Gonzalez, R. *et al.* Performance of young adult cannabis users on neurocognitive measures of impulsive behavior and their relationship to symptoms of cannabis use disorders. *J. Clin. Exp. Neuropsychol.* **34**, 962–976 (2012).
36. Batalla, A. *et al.* Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS ONE* **8**, e55821 (2013).
37. Irimia, C., Polis, I. Y., Stouffer, D. & Parsons, L. H. Persistent effects of chronic Δ^9 -THC exposure on motor impulsivity in rats. *Psychopharmacology* **232**, 3033–3043 (2015).
38. Kucewicz, M. T., Tricklebank, M. D., Bogacz, R. & Jones, M. W. Dysfunctional prefrontal cortical network activity and interactions following cannabinoid receptor activation. *J. Neurosci.* **31**, 15560–15568 (2011).
39. Goldstein, R. Z. & Volkow, N. D. Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nat. Rev. Neurosci.* **12**, 652–669 (2011).
40. Lubman, D. I., Cheetham, A. & Yücel, M. Cannabis and adolescent brain development. *Pharmacol. Ther.* **148**, 1–16 (2015).
41. Schneider, M. & Koch, M. The effect of chronic peripubertal cannabinoid treatment on deficient object recognition memory in rats after neonatal mPFC lesion. *Eur. Neuropsychopharmacol.* **17**, 180–186 (2007).
42. Quinn, H. R. *et al.* Adolescent rats find repeated Δ^9 -THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* **33**, 1113–1126 (2008).
43. Cha, Y. M., White, A. M., Kuhn, C. M., Wilson, W. A. & Swartzwelder, H. Differential effects of Δ^9 -THC on learning in adolescent and adult rats. *Pharmacol. Biochem. Behav.* **83**, 448–455 (2006).
44. Berghuis, P. *et al.* Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. *Proc. Natl Acad. Sci. USA* **102**, 19115–19120 (2005).
45. Kim, D. & Thayer, S. A. Cannabinoids inhibit the formation of new synapses between hippocampal neurons in culture. *J. Neurosci.* **21**, RC146 (2001).
46. Mulder, J. *et al.* Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. *Proc. Natl Acad. Sci. USA* **105**, 8760–8765 (2008).
47. Verrico, C. D., Gu, H., Peterson, M. L., Sampson, A. R. & Lewis, D. A. Repeated Δ^9 -tetrahydrocannabinol exposure in adolescent monkeys: persistent effects selective for spatial working memory. *Am. J. Psychiatry* **171**, 416–425 (2014).
This study provided proof-of-concept evidence in non-human primates that chronic exposure to exogenous cannabinoids during adolescence selectively disrupts cognitive processes that were actively developing during the time window of exposure.
48. Jacobus, J. & Tapert, S. F. Effects of cannabis on the adolescent brain. *Curr. Pharm. Des.* **20**, 2186–2193 (2014).
49. Cheetham, A. *et al.* Orbitofrontal volumes in early adolescence predict initiation of cannabis use: a 4-year longitudinal and prospective study. *Biol. Psychiatry* **71**, 684–692 (2012).
50. Lorenzetti, V., Solowij, N., Fornito, A., Lubman, D. I. & Yücel, M. The association between regular cannabis exposure and alterations of human brain morphology: an updated review of the literature. *Curr. Pharm. Des.* **20**, 2138–2167 (2014).
51. Jager, G., Block, R. I., Luijten, M. & Ramsey, N. F. Cannabis use and memory brain function in adolescent boys: a cross-sectional multicenter functional magnetic resonance imaging study. *J. Am. Acad. Child Adolesc. Psychiatry* **49**, 561–572 (2010).
52. Ehrenreich, H. *et al.* Specific attentional dysfunction in adults following early start of cannabis use. *Psychopharmacology* **142**, 295–301 (1999).
53. Gruber, S. A., Sagar, K. A., Dahlgren, M. K., Racine, M. & Lukas, S. E. Age of onset of marijuana use and executive function. *Psychol. Addict. Behav.* **26**, 496–506 (2012).
54. Meier, M. H. *et al.* Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proc. Natl Acad. Sci. USA* **109**, E2657–E2664 (2012).
55. Blakemore, S. J., Burnett, S. & Dahl, R. E. The role of puberty in the developing adolescent brain. *Hum. Brain Mapp.* **31**, 926–935 (2010).
56. Crane, N. A., Schuster, R. M., Mermelstein, R. J. & Gonzalez, R. Neuropsychological sex differences associated with age of initiated use among young adult cannabis users. *J. Clin. Exp. Neuropsychol.* **37**, 389–401 (2015).
57. Lenroot, R. K. & Giedd, J. N. Sex differences in the adolescent brain. *Brain Cogn.* **72**, 46–55 (2010).
58. Pope, H. G., Gruber, A. J., Hudson, J. I., Huestis, M. A. & Yurgelun-Todd, D. Neuropsychological performance in long-term cannabis users. *Arch. Gen. Psychiatry* **58**, 909–915 (2001).
59. Schreiner, A. M. & Dunn, M. E. Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: a meta-analysis. *Exp. Clin. Psychopharmacol.* **20**, 420–429 (2012).
60. Sim-Selley, L. J. Regulation of cannabinoid CB₁ receptors in the central nervous system by chronic cannabinoids. *Crit Rev. Neurobiol.* **15**, 91–119 (2003).
61. Fried, P., Watkinson, B. & Gray, R. Neurocognitive consequences of marijuana — a comparison with pre-drug performance. *Neurotoxicol. Teratol.* **27**, 231–239 (2005).
62. Moore, T. H. *et al.* Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet* **370**, 319–328 (2007).
63. Kessler, R. C. *et al.* Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: results from the National Comorbidity Survey. *Arch. Gen. Psychiatry* **51**, 8–19 (1994).
64. Lopez-Quintero, C. *et al.* Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug Alcohol Depend.* **115**, 120–130 (2011).
65. Koob, G. F. & Volkow, N. D. Neurocircuitry of addiction. *Neuropsychopharmacology* **35**, 217–238 (2010).
66. Nestler, E. J. Is there a common molecular pathway for addiction? *Nat. Neurosci.* **8**, 1445–1449 (2005).
67. Everitt, B. J. & Robbins, T. W. Drug addiction: updating actions to habits to compulsions ten years on. *Annu. Rev. Psychol.* **67**, 23–50 (2016).
68. Badiani, A., Belin, D., Epstein, D., Calu, D. & Shaham, Y. Opiate versus psychostimulant addiction: the differences do matter. *Nat. Rev. Neurosci.* **12**, 685–700 (2011).
69. Fratta, W. & Fattore, L. Molecular mechanisms of cannabinoid addiction. *Curr. Opin. Neurobiol.* **23**, 487–492 (2013).
70. European Monitoring Centre for Drugs and Drug Addiction. *European drug report* (EMCDDA, 2015).
71. Budney, A. J., Hughes, J. R., Moore, B. A. & Vandrey, R. Review of the validity and significance of cannabis withdrawal syndrome. *Am. J. Psychiatry* **161**, 1967–1977 (2004).
72. Allsop, D. J., Norberg, M. M., Copeland, J., Fu, S. & Budney, A. J. The Cannabis Withdrawal Scale development: patterns and predictors of cannabis withdrawal and distress. *Drug Alcohol Depend.* **119**, 123–129 (2011).
73. Budney, A. J., Vandrey, R. G., Hughes, J. R., Moore, B. A. & Bahrenburg, B. Oral Δ^9 -tetrahydrocannabinol suppresses cannabis withdrawal symptoms. *Drug Alcohol Depend.* **86**, 22–29 (2007).
74. Rodriguez de Fonseca, F., Carrera, M. R., Navarro, M., Koob, G. F. & Weiss, F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* **276**, 2050–2054 (1997).
75. Caberlotto, L., Rimondini, R., Hansson, A., Eriksson, S. & Heilig, M. Corticotropin-releasing hormone (CRH) mRNA expression in rat central amygdala in cannabinoid tolerance and withdrawal: evidence for an allostatic shift? *Neuropsychopharmacology* **29**, 15–22 (2004).
References 74 and 75 were the first to demonstrate that Δ^9 -THC withdrawal is associated with increased levels of the stress neuropeptide CRF in the amygdala, which probably contributes to negative affective states and diminished brain reward function; this withdrawal-related effect is also common to many other classes of abused drugs, including nicotine, alcohol and opiates.
76. Zorrilla, E. P., Logrip, M. L. & Koob, G. F. Corticotropin releasing factor: a key role in the neurobiology of addiction. *Front. Neuroendocrinol.* **35**, 234–244 (2014).
77. Vandrey, R., Budney, A., Hughes, J. & Liguori, A. A within-subject comparison of withdrawal symptoms during abstinence from cannabis, tobacco, and both substances. *Drug Alcohol Depend.* **92**, 48–54 (2008).
78. Justinova, Z., Goldberg, S. R., Heishman, S. J. & Tanda, G. Self-administration of cannabinoids by experimental animals and human marijuana smokers. *Pharmacol. Biochem. Behav.* **81**, 285–299 (2005).
This comprehensive review considers the reinforcing effects and abuse liability of Δ^9 -THC and related cannabinoids as investigated in both human and animal studies.
79. Freeman, T. P. *et al.* Just say 'know': how do cannabinoid concentrations influence users' estimates of cannabis potency and the amount they roll in joints? *Addiction* **109**, 1686–1694 (2014).
80. van der Pol, P. *et al.* Cross-sectional and prospective relation of cannabis potency, dosing and smoking behaviour with cannabis dependence: an ecological study. *Addiction* **109**, 1101–1109 (2014).
81. Freeman, T. & Winstock, A. Examining the profile of high-potency cannabis and its association with severity of cannabis dependence. *Psychol. Med.* **45**, 3181–3189 (2015).
82. Morgan, C. J., Freeman, T. P., Schafer, G. L. & Curran, H. V. Cannabidiol attenuates the appetitive effects of Δ^9 -tetrahydrocannabinol in humans smoking their chosen cannabis. *Neuropsychopharmacology* **35**, 1879–1885 (2010).
83. Field, M., Marhe, R. & Franken, I. H. The clinical relevance of attentional bias in substance use disorders. *CNS Spectr.* **19**, 225–230 (2014).
84. Hindocha, C. *et al.* Acute effects of Δ^9 -tetrahydrocannabinol, cannabidiol and their combination on facial emotion recognition: a randomised, double-blind, placebo-controlled study in cannabis users. *Eur. Neuropsychopharmacol.* **25**, 325–334 (2015).
85. Haney, M. *et al.* Oral cannabidiol does not alter the subjective, reinforcing or cardiovascular effects of smoked cannabis. *Neuropsychopharmacology* <http://dx.doi.org/10.1038/npp.2015.367> (2016).
86. Braid, D., Iosue, S., Pegorini, S. & Sala, M. Δ^9 -tetrahydrocannabinol-induced conditioned place preference and intracerebroventricular self-administration in rats. *Eur. J. Pharmacol.* **506**, 63–69 (2004).
87. Zangen, A., Solinas, M., Ikemoto, S., Goldberg, S. R. & Wise, R. A. Two brain sites for cannabinoid reward. *J. Neurosci.* **26**, 4901–4907 (2006).
88. Gardner, E. L. *et al.* Facilitation of brain stimulation reward by Δ^9 -tetrahydrocannabinol. *Psychopharmacology* **96**, 142–144 (1988).
89. Vlachou, S., Nomikos, G. G. & Panagis, G. CB₁ cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. *Psychopharmacology* **179**, 498–508 (2005).
90. Vlachou, S., Nomikos, G. G., Stephens, D. N. & Panagis, G. Lack of evidence for appetitive effects of Δ^9 -tetrahydrocannabinol in the intracranial self-stimulation and conditioned place preference procedures in rodents. *Behav. Pharmacol.* **18**, 311–319 (2007).
91. Sanudo-Pena, M. C. *et al.* Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. *Neurosci. Lett.* **223**, 125–128 (1997).
92. Cheer, J. F., Kendall, D. A. & Marsden, C. A. Cannabinoid receptors and reward in the rat: a conditioned place preference study. *Psychopharmacology* **151**, 25–30 (2000).
93. Winstock, A. R. & Barratt, M. J. Synthetic cannabis: a comparison of patterns of use and effect profile with natural cannabis in a large global sample. *Drug Alcohol Depend.* **131**, 106–111 (2013).

94. Justinova, Z., Tanda, G., Redhi, G. H. & Goldberg, S. R. Self-administration of Δ^9 -tetrahydrocannabinol (THC) by drug naive squirrel monkeys. *Psychopharmacology* **169**, 135–140 (2003).
95. Fattore, L., Cossu, G., Martellotta, C. M. & Fratta, W. Intravenous self-administration of the cannabinoid CB₁ receptor agonist WIN 55,212-2 in rats. *Psychopharmacology* **156**, 410–416 (2001).
96. Lecca, D., Cacciapaglia, F., Valentini, V. & Di Chiara, G. Monitoring extracellular dopamine in the rat nucleus accumbens shell and core during acquisition and maintenance of intravenous WIN 55,212-2 self-administration. *Psychopharmacology* **188**, 63–74 (2006).
97. Parsons, L. H. & Hurd, Y. L. Endocannabinoid signaling in reward and addiction. *Nat. Rev. Neurosci.* **16**, 579–594 (2015).
98. Serrano, A. & Parsons, L. H. Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol. Ther.* **132**, 215–241 (2011).
99. Ceccarini, J. *et al.* [¹⁸F]MK-9470 PET measurement of cannabinoid CB₁ receptor availability in chronic cannabis users. *Addict. Biol.* **20**, 357–367 (2014).
100. Breivogel, C. S. *et al.* Chronic Δ^9 -tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. *J. Neurochem.* **73**, 2447–2459 (1999).
101. Dudok, B. *et al.* Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nat. Neurosci.* **18**, 75–86 (2015). **This study used nanoscale imaging and electrophysiological techniques to demonstrate greater CB1R expression and influence on perisomatically projecting versus dendritically projecting GABAergic interneurons in the mouse hippocampus, and that persistent deficits in hippocampal LTP following chronic Δ^9 -THC exposure result from near-complete loss of CB1R at somatic synapses.**
102. Sim-Selley, L. J. *et al.* Prolonged recovery rate of CB₁ receptor adaptation after cessation of long-term cannabinoid administration. *Mol. Pharmacol.* **70**, 986–996 (2006).
103. Hoffman, A. F., Oz, M., Caulder, T. & Lupica, C. R. Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *J. Neurosci.* **23**, 4815–4820 (2003).
104. Mato, S. *et al.* A single *in vivo* exposure to Δ^9 -THC blocks endocannabinoid-mediated synaptic plasticity. *Nat. Neurosci.* **7**, 585–586 (2004). **References 103 and 104 were among the first to demonstrate that Δ^9 -THC exposure disrupts synaptic plasticity of nucleus accumbens neurons in rodents.**
105. Schlosburg, J. E. *et al.* Inhibitors of endocannabinoid-metabolizing enzymes reduce precipitated withdrawal responses in THC-dependent mice. *AAPS J.* **11**, 342–352 (2009).
106. Castelli, M. P. *et al.* Dysregulation of the endogenous cannabinoid system in adult rats prenatally treated with the cannabinoid agonist WIN 55,212-2. *Eur. J. Pharmacol.* **573**, 11–19 (2007).
107. Morgan, C. J. *et al.* Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. *Br. J. Psychiatry* **202**, 381–382 (2013).
108. Muhl, D. *et al.* Increased CB₂ mRNA and anandamide in human blood after cessation of cannabis abuse. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **387**, 691–695 (2014).
109. Bossong, M. G. *et al.* Further human evidence for striatal dopamine release induced by administration of Δ^9 -tetrahydrocannabinol (THC): selectivity to limbic striatum. *Psychopharmacology* **232**, 2723–2729 (2015).
110. Nutt, D. J., Lingford-Hughes, A., Erritzoe, D. & Stokes, P. A. The dopamine theory of addiction: 40 years of highs and lows. *Nat. Rev. Neurosci.* **16**, 305–312 (2015).
111. Cheer, J. F., Wassum, K. M., Heien, M. L., Phillips, P. E. & Wightman, R. M. Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J. Neurosci.* **24**, 4393–4400 (2004).
112. Chen, J., Marmur, R., Pulles, A., Paredes, W. & Gardner, E. L. Ventral tegmental microinjection of Δ^9 -tetrahydrocannabinol enhances ventral tegmental somatodendritic dopamine levels but not forebrain dopamine levels: evidence for local neural action by marijuana's psychoactive ingredient. *Brain Res.* **621**, 65–70 (1993).
113. Tanda, G., Pontieri, F. E. & Di Chiara, G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ 1 opioid receptor mechanism. *Science* **276**, 2048–2050 (1997).
114. Diana, M., Melis, M., Muntoni, A. L. & Gessa, G. L. Mesolimbic dopaminergic decline after cannabinoid withdrawal. *Proc. Natl. Acad. Sci. USA* **95**, 10269–10273 (1998).
115. Tanda, G., Loddo, P. & Di Chiara, G. Dependence of mesolimbic dopamine transmission on Δ^9 -tetrahydrocannabinol. *Eur. J. Pharmacol.* **376**, 23–26 (1999).
116. Bloomfield, M. A. *et al.* Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biol. Psychiatry* **75**, 470–478 (2014).
117. Ghazouli, R. & Abi-Dargham, A. Imaging dopamine transmission parameters in cannabis dependence. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **52**, 28–32 (2014).
118. Manzanares, J. *et al.* Chronic administration of cannabinoids regulates proenkephalin mRNA levels in selected regions of the rat brain. *Brain Res. Mol. Brain Res.* **55**, 126–132 (1998).
119. Valverde, O. *et al.* Δ^9 -tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. *Eur. J. Neurosci.* **13**, 1816–1824 (2001).
120. Braid, D., Pozzi, M., Parolaro, D. & Sala, M. Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. *Eur. J. Pharmacol.* **413**, 227–234 (2001).
121. Justinova, Z., Tanda, G., Munzar, P. & Goldberg, S. R. The opioid antagonist naltrexone reduces the reinforcing effects of Δ^9 -tetrahydrocannabinol (THC) in squirrel monkeys. *Psychopharmacology* **173**, 186–194 (2004).
122. Haney, M. *et al.* Naltrexone maintenance decreases cannabis self-administration and subjective effects in daily cannabis smokers. *Neuropsychopharmacology* **40**, 2489–2498 (2015).
123. Delfs, J. M., Zhu, Y., Druhan, J. P. & Aston-Jones, G. Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. *Nature* **403**, 430–434 (2000).
124. Moranta, D., Esteban, S. & Garcia-Sevilla, J. A. Chronic treatment and withdrawal of the cannabinoid agonist WIN 55,212-2 modulate the sensitivity of presynaptic receptors involved in the regulation of monoamine syntheses in rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **379**, 61–72 (2009).
125. Page, M. E., Oropeza, V. C. & Van Bockstaele, E. J. Local administration of a cannabinoid agonist alters norepinephrine efflux in the rat frontal cortex. *Neurosci. Lett.* **431**, 1–5 (2008).
126. Jentsch, J. D., Andrusiak, E., Tran, A., Bowers, M. B. & Roth, R. H. Δ^9 -tetrahydrocannabinol increases prefrontal cortical catecholaminergic utilization and impairs spatial working memory in the rat: blockade of dopaminergic effects with HA966. *Neuropsychopharmacology* **16**, 426–432 (1997).
127. Carvalho, A. F. & Van Bockstaele, E. J. Cannabinoid modulation of noradrenergic circuits: implications for psychiatric disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **38**, 59–67 (2012).
128. Coffey, C., Carlin, J. B., Lynskey, M., Li, N. & Patton, C. Adolescent precursors of cannabis dependence: findings from the Victorian Adolescent Health Cohort Study. *Br. J. Psychiatry* **182**, 330–336 (2003).
129. Hines, L. A. *et al.* Onset of opportunity to use cannabis and progression from opportunity to dependence: are influences consistent across transitions? *Drug Alcohol Depend.* **160**, 57–64 (2016).
130. Hindocha, C. *et al.* Associations between cigarette smoking and cannabis dependence: a longitudinal study of young cannabis users in the United Kingdom. *Drug Alcohol Depend.* **148**, 165–171 (2015).
131. Chen, C.-Y., O'Brien, M. S. & Anthony, J. C. Who becomes cannabis dependent soon after onset of use? Epidemiological evidence from the United States: 2000–2001. *Drug Alcohol Depend.* **79**, 11–22 (2005).
132. Verweij, K. J. *et al.* Genetic and environmental influences on cannabis use initiation and problematic use: a meta-analysis of twin studies. *Addiction* **105**, 417–430 (2010).
133. Agrawal, A. & Lynskey, M. T. Candidate genes for cannabis use disorders: findings, challenges and directions. *Addiction* **104**, 518–532 (2009).
134. Uhl, G. R. *et al.* "Higher order" addiction molecular genetics: convergent data from genome-wide association in humans and mice. *Biochem. Pharmacol.* **75**, 98–111 (2008).
135. Cooper, K., Chatters, R., Kaltenthaler, E. & Wong, R. Psychological and psychosocial interventions for cannabis cessation in adults: a systematic review short report. *Health Technol. Assess.* **19**, 1–130 (2015).
136. Marshall, K., Gowing, L., Ali, R. & Le Foll, B. Pharmacotherapies for cannabis dependence. *Cochrane Database Syst. Rev.* **12**, CD008940 (2014).
137. Mason, B. J. *et al.* A proof-of-concept randomized controlled study of gabapentin: effects on cannabis use, withdrawal and executive function deficits in cannabis-dependent adults. *Neuropsychopharmacology* **37**, 1689–1698 (2012).
138. Gray, K. M. *et al.* A double-blind randomized controlled trial of N-acetylcysteine in cannabis-dependent adolescents. *Am. J. Psychiatry* **169**, 805–812 (2012).
139. Levin, F. R. *et al.* Dronabinol for the treatment of cannabis dependence: a randomized, double-blind, placebo-controlled trial. *Drug Alcohol Depend.* **116**, 142–150 (2011).
140. Allsop, D. J. *et al.* Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial. *JAMA Psychiatry* **71**, 281–291 (2014).
141. Lutz, B., Marsicano, G., Maldonado, R. & Hillard, C. J. The endocannabinoid system in guarding against fear, anxiety and stress. *Nat. Rev. Neurosci.* **16**, 705–718 (2015).
142. Murillo-Rodriguez, E. *et al.* The emerging role of the endocannabinoid system in the sleep-wake cycle modulation. *Cent. Nerv. Syst. Agents Med. Chem.* **11**, 189–196 (2011).
143. Weinstein, A. M. & Gorelick, D. A. Pharmacological treatment of cannabis dependence. *Curr. Pharm. Des.* **17**, 1351–1358 (2011).
144. Clapper, J. R., Mangieri, R. A. & Piomelli, D. The endocannabinoid system as a target for the treatment of cannabis dependence. *Neuropharmacology* **56** (Suppl. 1), 235–243 (2009).
145. van der Pol, P. *et al.* Mental health differences between frequent cannabis users with and without dependence and the general population. *Addiction* **108**, 1459–1469 (2013).
146. Degenhardt, L., Hall, W. & Lynskey, M. Testing hypotheses about the relationship between cannabis use and psychosis. *Drug Alcohol Depend.* **71**, 37–48 (2003).
147. Flórez-Salamanca, L. *et al.* Probability and predictors of cannabis use disorders relapse: results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug Alcohol Depend.* **132**, 127–133 (2013).
148. Christensen, R., Kristensen, P. K., Bartels, E. M., Bliddal, H. & Astrup, A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *Lancet* **370**, 1706–1713 (2007).
149. Nissen, S. E. *et al.* Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA* **299**, 1547–1560 (2008).
150. Valverde, O. & Torres, M. CB₁ receptor-deficient mice as a model for depression. *Neuroscience* **204**, 193–206 (2012).
151. Zanelati, T. V., Biojone, C., Moreira, F. A., Guimarães, F. S. & Joca, S. R. Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT_{1A} receptors. *Br. J. Pharmacol.* **159**, 122–128 (2010).
152. Moreira, F. A. & Wotjak, C. T. in *Behavioral Neurobiology of Anxiety and Its Treatment* (eds Stein, M. B. & Steckler, T.) 429–450 (Springer, 2010).
153. Sidhura, N. & Parsons, L. H. Endocannabinoid-mediated synaptic plasticity and addiction-related behavior. *Neuropharmacology* **61**, 1070–1087 (2011).
154. Morgan, C. *et al.* Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. *Psychol. Med.* **42**, 391–400 (2012).
155. Buckner, J. D. & Carroll, K. M. Effect of anxiety on treatment presentation and outcome: results from the Marijuana Treatment Project. *Psychiatry Res.* **178**, 493–500 (2010).
156. Van Dam, N. T., Bedi, G. & Earleywine, M. Characteristics of clinically anxious versus non-anxious regular, heavy marijuana users. *Addict. Behav.* **37**, 1217–1223 (2012).

157. Schafer, G. *et al.* Investigating the interaction between schizotypy, divergent thinking and cannabis use. *Conscious. Cogn.* **21**, 292–298 (2012).

158. Weiland, B. J. *et al.* Daily marijuana use is not associated with brain morphometric measures in adolescents or adults. *J. Neurosci.* **35**, 1505–1512 (2015).

159. Yücel, M. *et al.* The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. *Schizophr. Bull.* **38**, 316–330 (2012).

160. Hindocha, C., Freeman, T. P., Winstock, A. R. & Lynskey, M. T. Vaping cannabis (marijuana) has the potential to reduce tobacco smoking in cannabis users. *Addiction* **111**, 375 (2015).

161. Hasin, D. S. *et al.* Prevalence of marijuana use disorders in the United States between 2001–2002 and 2012–2013. *JAMA Psychiatry* **72**, 1235–1242 (2015).

162. Nutt, D. J., King, L. A. & Nichols, D. E. Effects of Schedule I drug laws on neuroscience research and treatment innovation. *Nat. Rev. Neurosci.* **14**, 577–585 (2013).

163. Mokrysz, C. *et al.* Are IQ and educational outcomes in teenagers related to their cannabis use? A prospective cohort study. *J. Psychopharmacol.* **30**, 159–168 (2016).

164. Jackson, N. J. *et al.* Impact of adolescent marijuana use on intelligence: results from two longitudinal twin studies. *Proc. Natl Acad. Sci. USA* **113**, E500–E508 (2016).

References 163 and 164 both examined associations between adolescent cannabis use and IQ by using data from large longitudinal cohorts in the United Kingdom and United States; both papers suggest that there are non-causal explanations for the observed associations between adolescent cannabis use and lower IQ.

165. Temple, E. C., Brown, R. F., Hine, D. W. The 'grass ceiling': limitations in the literature hinder our understanding of cannabis use and its consequences. *Addiction* **106**, 238–244 (2011).

An insightful review covering methodological inconsistencies in cannabis research, offering useful guidance for future research.

166. Fergusson, D. M., Horwood, L. J. & Beautrais, A. L. Cannabis and educational achievement. *Addiction* **98**, 1681–1692 (2003).

167. Silins, E. *et al.* Young adult sequelae of adolescent cannabis use: an integrative analysis. *Lancet Psychiatry* **1**, 286–293 (2014).

168. Lynskey, M. T. & Hall, W. The effects of adolescent cannabis use on educational attainment: a review. *Addiction* **95**, 1621–1630 (2000).

169. Townsend, L., Flisher, A. J. & King, G. A systematic review of the relationship between high school dropout and substance use. *Clin. Child Fam. Psychol. Rev.* **10**, 295–317 (2007).

170. Verweij, K. J., Huizink, A. C., Agrawal, A., Martin, N. G. & Lynskey, M. T. Is the relationship between early-onset cannabis use and educational attainment causal or due to common liability? *Drug Alcohol Depend.* **133**, 580–586 (2013).

171. Bloomfield, M. A., Morgan, C. J., Kapur, S., Curran, H. V. & Howes, O. D. The link between dopamine function and apathy in cannabis users: an [¹⁸F]-DOPA PET imaging study. *Psychopharmacology* **231**, 2251–2259 (2014).

172. McCaffrey, D. F., Liccardo Pacula, R., Han, B. & Ellickson, P. Marijuana use and high school dropout: the influence of unobservables. *Health Econ.* **19**, 1281–1299 (2010).

173. Hooper, S. R., Woolley, D. & De Bellis, M. D. Intellectual, neurocognitive, and academic achievement in abstinent adolescents with cannabis use disorder. *Psychopharmacology* **231**, 1467–1477 (2014).

174. Stiby, A. I. *et al.* Adolescent cannabis and tobacco use and educational outcomes at age 16: birth cohort study. *Addiction* **110**, 658–668 (2015).

175. Grant, J. D. *et al.* Associations of alcohol, nicotine, cannabis, and drug use/dependence with educational attainment: evidence from cotwin-control analyses. *Alcohol. Clin. Exp. Res.* **36**, 1412–1420 (2012).

176. Large, M., Sharma, S., Compton, M. T., Slade, T. & Nielsen, O. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. *Arch. Gen. Psychiatry* **68**, 555–561 (2011).

177. Morgan, C. J. A., Freeman, T. P., Powell, J. & Curran, H. V. *AKT1* genotype moderates the acute psychotomimetic effects of naturally smoked cannabis in young cannabis smokers. *Transl. Psychiatry* **6**, e738 (2016).

This is the largest study to date conducted on the acute response to cannabis and shows that psychotic-like symptoms in young healthy cannabis users are predicted by their *AKT1* genotype, replicating the same effect observed in schizophrenia (see references 178 and 179).

178. Di Forti, M. *et al.* Confirmation that the *AKT1* (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biol. Psychiatry* **72**, 811–816 (2012).

179. van Winkel, R., van Beveren, N. J., Simons, C. & Genetic Risk and Outcome of Psychosis (GROUP) Investigators. *AKT1* moderation of cannabis-induced cognitive alterations in psychotic disorder. *Neuropsychopharmacology* **36**, 2529–2537 (2011).

180. Di Forti, M. *et al.* Proportion of patients in south London with first-episode psychosis attributable to use of high potency cannabis: a case-control study. *Lancet Psychiatry* **2**, 235–238 (2015).

The risk of individuals having a psychotic disorder was roughly threefold higher in those saying they used skunk (which contains high concentrations of Δ^9 -THC and negligible CBD) and fivefold higher in those reporting daily skunk use, compared with non-users; hash (which has a lower Δ^9 -THC content and more CBD) did not affect risk of a psychotic disorder.

181. Morgan, C. J. & Curran, H. V. Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *Br. J. Psychiatry* **192**, 306–307 (2008).

The first study to show that psychosis-like symptoms are greater in those whose hair contains only Δ^9 -THC than in those whose hair contains both Δ^9 -THC and CBD or no cannabinoids — suggesting a protective effect of CBD.

182. Leweke, F. M., Giuffrida, A., Wurster, U., Emrich, H. M. & Piomelli, D. Elevated endogenous cannabinoids in schizophrenia. *Neuroreport* **10**, 1665–1669 (1999).

183. Koethe, D. *et al.* Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. *Br. J. Psychiatry* **194**, 371–372 (2009).

184. Di Marzo, V. Targeting the endocannabinoid system: to enhance or reduce? *Nat. Rev. Drug Discov.* **7**, 438–455 (2008).

185. Volk, D. W. & Lewis, D. A. The role of endocannabinoid signaling in cortical inhibitory neuron dysfunction in schizophrenia. *Biol. Psychiatry* <http://dx.doi.org/10.1016/j.biopsych.2015.06.015> (2015).

186. American Psychiatric Association. (eds) *Diagnostic and Statistical Manual of Mental Disorders* 5th edn (American Psychiatric Publishing, 2013).

187. American Psychiatric Association. (eds) *Diagnostic and Statistical Manual of Mental Disorders* 4th edn text revision (American Psychiatric Publishing, 2000).

188. Kandel, D. B. *et al.* *Stages and Pathways of Drug Involvement: Examining the Gateway Hypothesis*. (Cambridge Univ. Press, 2002).

189. Fergusson, D. M., Boden, J. M. & Horwood, L. J. Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. *Addiction* **101**, 556–569 (2006).

190. Lynskey, M. T. *et al.* Escalation of drug use in early-onset cannabis users versus co-twin controls. *JAMA* **289**, 427–433 (2003).

191. Cadoni, C., Simola, N., Espa, E., Fenu, S. & Di Chiara, G. Strain dependence of adolescent cannabis influence on heroin reward and mesolimbic dopamine transmission in adult Lewis and Fischer 344 rats. *Addict. Biol.* **20**, 132–142 (2015).

192. Tomasiwicz, H. C. *et al.* Proenkephalin mediates the enduring effects of adolescent cannabis exposure associated with adult opiate vulnerability. *Biol. Psychiatry* **72**, 803–810 (2012).

193. Higuera-Matas, A. *et al.* Augmented acquisition of cocaine self-administration and altered brain glucose metabolism in adult female but not male rats exposed to a cannabinoid agonist during adolescence. *Neuropsychopharmacology* **33**, 806–813 (2008).

194. Sztutorisz, H. *et al.* Parental THC exposure leads to compulsive heroin-seeking and altered striatal synaptic plasticity in the subsequent generation. *Neuropsychopharmacology* **39**, 1315–1323 (2014).

195. MacCoun, R. & Reuter, P. Evaluating alternative cannabis regimes. *Br. J. Psychiatry* **178**, 123–128 (2001).

196. Panlilio, L. V., Zanetini, C., Barnes, C., Solinas, M. & Goldberg, S. R. Prior exposure to THC increases the addictive effects of nicotine in rats. *Neuropsychopharmacology* **38**, 1198–1208 (2013).

197. Solinas, M., Panlilio, L. & Goldberg, S. Exposure to Δ^9 -tetrahydrocannabinol (THC) increases subsequent heroin taking but not heroin's reinforcing efficacy: a self-administration study in rats. *Neuropsychopharmacology* **29**, 1301–1311 (2004).

198. Panlilio, L. V., Solinas, M., Matthews, S. A. & Goldberg, S. R. Previous exposure to THC alters the reinforcing efficacy and anxiety-related effects of cocaine in rats. *Neuropsychopharmacology* **32**, 646–657 (2007).

199. Patton, G. C., Coffey, C., Carlin, J. B., Sawyer, S. M. & Lynskey, M. Reverse gateways? Frequent cannabis use as a predictor of tobacco initiation and nicotine dependence. *Addiction* **100**, 1518–1525 (2005).

200. Rubino, T. *et al.* Chronic Δ^9 -tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. *Neuropsychopharmacology* **33**, 2760–2771 (2008).

201. Carlezon, W. A. & Thomas, M. J. Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. *Neuropharmacology* **56** (Suppl. 1), 122–132 (2009).

202. Glass, M., Dragunow, M. & Faull, R. L. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**, 299–318 (1997).

203. Wang, X., Dow-Edwards, D., Keller, E. & Hurd, Y. L. Preferential limbic expression of the cannabinoid receptor mRNA in the human fetal brain. *Neuroscience* **118**, 681–694 (2003).

204. Herkenham, M. *et al.* Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.* **11**, 563–583 (1991).

205. Egertova, M. & Elphick, M. R. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB₁. *J. Comp. Neurol.* **422**, 159–171 (2000).

206. Tsou, K., Brown, S., Sanudo-Pena, M. C., Mackie, K. & Walker, J. M. Immunohistochemical distribution of cannabinoid CB₁ receptors in the rat central nervous system. *Neuroscience* **83**, 393–411 (1998).

207. Hoffman, A. F. & Lupica, C. R. Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. *J. Neurophysiol.* **85**, 72–83 (2001).

208. Pistis, M., Munttoni, A. L., Pillolla, G. & Gessa, G. L. Cannabinoids inhibit excitatory inputs to neurons in the shell of the nucleus accumbens: an *in vivo* electrophysiological study. *Eur. J. Neurosci.* **15**, 1795–1802 (2002).

209. Tzavara, E. T., Wade, M. & Nomikos, G. G. Biphasic effects of cannabinoids on acetylcholine release in the hippocampus: site and mechanism of action. *J. Neurosci.* **23**, 9374–9384 (2003).

210. Pisanu, A., Acquas, E., Fenu, S. & Di Chiara, G. Modulation of Δ^9 -THC-induced increase of cortical and hippocampal acetylcholine release by μ opioid and D₂ dopamine receptors. *Neuropharmacology* **50**, 661–670 (2006).

211. Pistis, M. *et al.* Δ^9 -tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an *in vivo* microdialysis study. *Brain Res.* **948**, 155–158 (2002).

212. Higuera-Matas, A. *et al.* Periadolescent exposure to cannabinoids alters the striatal and hippocampal dopaminergic system in the adult rat brain. *Eur. Neuropsychopharmacol.* **20**, 895–906 (2010).

213. Ellgren, M., Spano, S. M. & Hurd, Y. L. Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. *Neuropsychopharmacology* **32**, 607–615 (2007).

214. Morel, L. J., Giros, B. & Dauge, V. Adolescent exposure to chronic Δ^9 -tetrahydrocannabinol blocks opiate dependence in maternally deprived rats. *Neuropsychopharmacology* **34**, 2469–2476 (2009).

215. van der Pol, P. *et al.* Predicting the transition from frequent cannabis use to cannabis dependence: a three-year prospective study. *Drug Alcohol Depend.* **133**, 352–359 (2013).

Acknowledgements

This work was supported by grants from the US National Institutes of Health to L.H.P. (AA020404, AA006420, AA022249 and AA017447) and by grants from the UK Medical Research Council to H.V.C. and C.J.A.M. (G0800268; MR/K015524/1).

Competing interests statement

The authors declare **competing interests**: see Web version for details.