REVIEW

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The effects of Δ^9-tetrahydrocannabinol on the dopamine system

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The effects of Δ^9-tetrahydrocannabinol (THC), the main psychoactive ingredient in cannabis, are a pressing concern for global mental health. Patterns of cannabis use are changing drastically owing to legalization, the availability of synthetic analogues (commonly termed spice), cannavaping and an emphasis on the purported therapeutic effects of cannabis. Many of the reinforcing effects of THC are mediated by the dopamine system. Owing to the complexity of the cannabinoid–dopamine interactions that take place, there is conflicting evidence from human and animal studies concerning the effects of THC on the dopamine system. Acute THC administration causes increased dopamine release and neuron activity, whereas long-term use is associated with blunting of the dopamine system. Future research must examine the long-term and developmental dopaminergic effects of THC.

Cannabis is a widely used recreational drug. Over half of young Americans have used the drug[1], whereas in Europe, cannabis has now overtaken heroin as the most widely reported illegal drug used amongst people entering specialist addiction services[2]. This provides the background to political debates worldwide concerning changes to the legal status of the drug. Although causality has not been conclusively demonstrated, heavy cannabis use is associated with increased risk of mental disorders[3] including psychosis[4], addiction[5], depression[6], suicidality[7], cognitive impairment[8] and amotivation[9].

THC, the main psychoactive component of cannabis[10], elicits its acute psychoactive effects through the endocannabinoid type 1 receptor (CB_1R)[11]. THC has been linked to the rewarding aspects of cannabis and the induction of symptoms of mental illnesses and cognitive impairment. Recently, the THC content of cannabis has been increasing[12] and synthetic THC analogues (potent cannabinoid receptor agonists; termed 'spice') are now widely used[13]. The likelihood of a future increase in consumption of cannabinoids through electronic cigarettes (cannavaping) and edible products[14] changes the landscape further[15]. Given the widespread use of cannabinoids, and the links between THC exposure and adverse outcomes, it is imperative that the neurobiological effects of THC are understood. Recently, we and others have found that heavy cannabis use is associated with reductions in dopaminergic function. Since both the rewarding and psychotogenic effects of THC and its analogues are thought to be mediated by the dopaminergic system, demonstrating dopaminergic alterations in human users *in vivo* is of clinical relevance for the prevention and treatment of cannabis-use-associated disorders and psychoses. Therefore, we present a review of the animal and human literature on the complex effects of acute and longer-term THC administration on dopamine synthesis and release and on its receptors, providing an account of the current understanding of the field. We critically analyse the factors that contribute to the effects of THC, and to variations between studies, before providing a framework for future research including a pharmacological dissection of these effects, especially in the developing brain.

THC receptor binding in the brain

THC is a CB_1R and endocannabinoid type 2 receptor (CB_2R) partial agonist[11]. The psychoactive effects of THC are blocked by the CB_1R antagonist rimonabant[16,17], indicating that these effects are mediated through the activation of G-protein-coupled CB_1R receptors, which reduce cAMP levels by inhibiting adenylate cyclase[18]. THC disrupts finely tuned endocannabinoid retrograde signalling systems owing to the temporal and neuronal specificity of endocannabinoids over THC. Under conditions of low CB_1R density, THC antagonizes endogenous agonists that possess greater receptor affinity than THC[19]. THC also allosterically modulates opioid receptors[20], which may provide additional indirect routes for altering dopamine transmission[21]. Furthermore, THC has psychoactive metabolites with CB_1R affinity, further complicating the analysis of receptor-binding studies[22].

CB_1 receptors and dopamine

Early animal studies described the interactions of amphetamine, which increases dopamine release, and THC[23]. These studies reported that the behavioural effects of amphetamine were potentiated or antagonized, depending on the dose of THC, leading researchers to propose that dopamine was "a prime candidate for…the mode of action of Δ^9-tetrahydrocannabinol"[24]. Indeed, THC produces complex effects on the dopamine system, contributing to the drug's recreational and harmful effects. However, there are inconsistencies between the preclinical and clinical findings, which have proven challenging for researchers in the field. It is, therefore, timely to review the evidence and provide a framework for understanding the inconsistencies between the preclinical and clinical findings.

Dopaminergic neurons are modulated by the endocannabinoid system[25]. CB_1Rs and the endocannabinoid ligands anandamide and 2-arachidonoylglycerol (2-AG) are abundant in dopaminergic pathways, including in the striatum[26], where they act as a retrograde feedback system on presynaptic glutamatergic and γ-aminobutyric acid (GABA) nerve terminals (Fig. 1), modulating dopamine transmission. Anandamide[27] and 2-AG[28] stimulate dopamine release in the nucleus accumbens (NAc) shell. Again, this effect is blocked by the CB_1 antagonist rimonabant, indicating that the dopaminergic effects of endocannabinoids involve CB_1 receptors. The rewarding properties of THC, which are mediated through increased dopamine release and dopaminergic neuron firing, are underpinned by biased signal transduction mechanisms from

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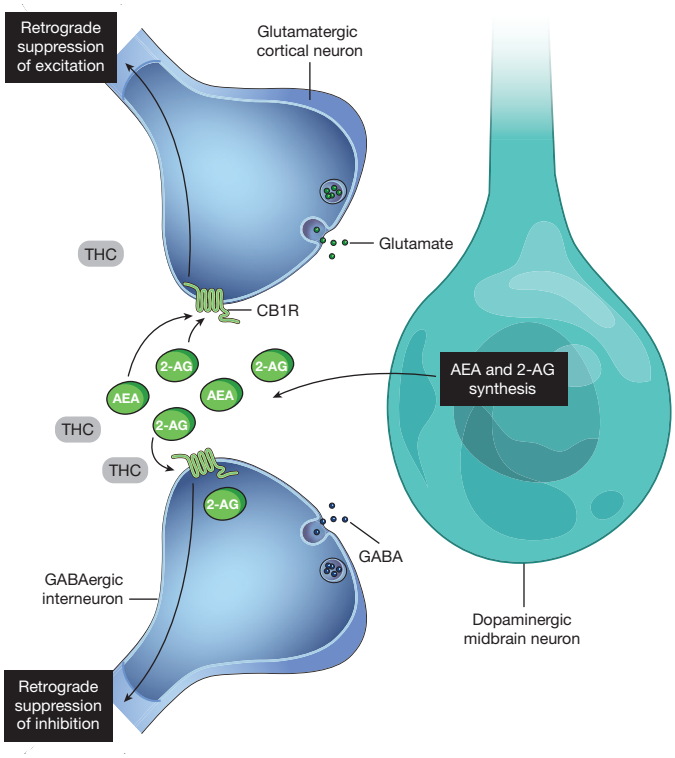

Figure 1 | THC binds to CB1 receptors on glutamatergic and GABAergic neurons, disrupting normal endocannabinoid retrograde signalling from dopaminergic neurons[135]. Endocannabinoids influence synaptic signalling in the midbrain. 2-AG is synthesized by diacylglycerol lipase in dopaminergic midbrain neurons and, once released, acts retroactively on CB_1Rs on nearby glutamatergic and GABAergic terminals. CB_1Rs mediate robust inhibition of GABA inputs onto midbrain dopamine cells[136], termed retrograde suppression of inhibition. CB_1Rs are also localized on glutamatergic terminals that synapse onto midbrain dopamine neurons[137], where endocannabinoids mediate retrograde suppression of excitation. Thus, endocannabinoids fine-tune the activity of the mesolimbic dopamine projections through the modulation of both excitatory and inhibitory signalling and THC disrupts this system.

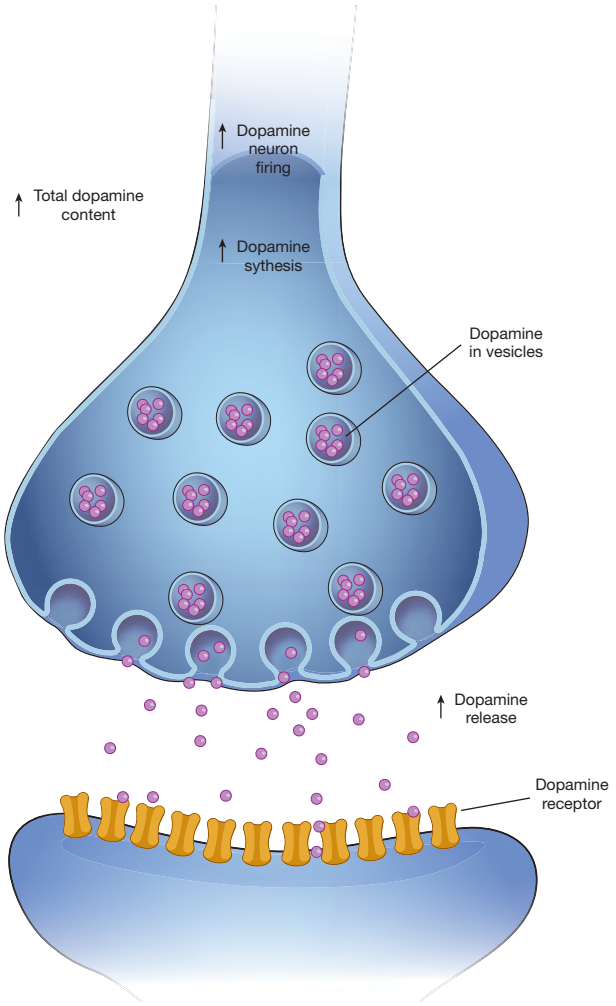

Figure 2 | Summary of the acute effects of THC on dopaminergic function. In animal models, acute THC challenge is associated with increased dopaminergic cell firing, and increased dopamine synthesis and release.

the CB_1R[16]. There is evidence that acute and chronic THC exposure have differing effects on the dopaminergic system. We will, therefore, deal with these separately and describe their effects on different neurobiological components of the dopaminergic system, including neuron firing, synthesis, release, reuptake and receptors.

Acute THC and presynaptic dopamine in animals

It has long been clear that THC exerts complex effects on the dopamine system. Early *in vitro* studies in rodent cells, using radiolabelled dopamine in synaptosomes, found that treatment with THC caused increased dopamine synthesis[29] and release[24] (Fig. 2 and Box 1). However, the effects on dopamine uptake yielded conflicting results, with evidence of both increases[30] and dose-dependent decreases[24]. Subsequently, biphasic and triphasic effects of THC were discovered, whereby low doses of THC produced increases in the conversion of tyrosine to dopamine, but high doses of THC resulted in decreased dopamine synthesis[31]. Similarly complicated temporal relationships between THC administration and changes in dopamine levels were observed[32], with repeated dosing resulting in behavioural and neurochemical tolerance—highly pertinent to the mechanisms of dependence to the drug. The complex dose-specific effects of THC in rodents were thought to be due to dose-related decreases in precursor uptake[30] and dopamine–opioid interactions via μ-opioid receptors[29].

Subsequent work investigated THC-induced increases in dopamine synthesis *in vivo*. THC increased ^{3}H-dopamine synthesis[33,34], tyrosine hydroxylase protein levels[35] and mRNA expression[36], the rate-limiting step in the dopamine synthesis pathway. Similarly, increases in dopamine metabolism, measured through the dihydroxphenylacetic acid/dopamine (DOPAC/DA) ratio, were reported in most[37], but not all[35], rodent studies. However, the majority of early studies using spectrophotofluorimetry were inconsistent due to technical limitations in detecting the rapid changes in extracellular dopamine concentration, deviations that are now detectable using contemporary microdialysis techniques[38].

In vivo microdialysis shows that acute THC administration increases dopamine efflux in the prefrontal cortex (PFC)[39], striatum[40] and NAc[41]. Only one study did not find that THC induced increases in dopamine efflux[42], an anomaly that may have been related to the route of administration as that study used a THC gavage—the other studies used intravenous injection, which produces a rapid increase in THC which reaches the brain promptly, whereas gavage favours sequestration in lipid compartments owing to the very high lipid solubility of THC[43]. Electrophysiological studies in rats have categorically demonstrated that THC dose-dependently increases firing rates in ascending midbrain dopaminergic projections via CB_1R agonism[45,46]. Together, these findings suggest that THC increases the firing rates of dopamine neurons, leading to increased dopamine synthesis and release in terminal fields.

Acute THC and postsynaptic dopamine in animals

Acute THC administration did not alter dopamine receptor protein levels in rhesus monkeys[47]. In the rat limbic forebrain, one study reported

BOX 1

Glossary of methods used to assess dopaminergic activity after THC exposure

- High-performance liquid chromatography (HPLC): a technique to separate, identify and quantify a particular chemical from a mixture
- *In vivo* electrophysiology: a method of studying single-neuron responses, using microelectrodes to record from cells, including in the living animal brain
- Microdialysis: a technique used for the continuous measurement of neurochemicals and metabolites through the insertion of a probe into a specific area of the brain
- Positron emission tomography (PET): an imaging technique that uses a positron-emitting radiotracer that binds to specific proteins including receptors, enzymes and transporters. The gamma rays produced by the radionuclide allow quantification of proteins in living tissue non-invasively with very high chemical specificity
- Single-photon emission computed tomography (SPECT): an imaging technique similar to positron emission tomography, but with lower spatial resolution and sensitivity
- Synaptosome: a homogenized mixture of isolated synaptic terminals

increased dopamine type 1 receptor (D_1R) availability[48] whereas other studies reported decreases[49]. In the striatum, the density of dopamine type 2 receptors (D_2Rs) either decreased[49] or did not significantly change, with decreases in D_1R also reported[48]. That there is no change in receptor protein levels, together with the tendency towards reduced receptor availability, almost certainly reflects THC-induced changes in synaptic dopamine levels.

Acute THC and dopamine in humans

Studies of brain activity in humans, using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), provide indirect measures of dopaminergic function through changes in cerebral blood flow and glucose metabolism. These serve as surrogate markers of brain activity in areas with dopaminergic projections. In humans, acute THC administration is associated with increased activity in frontal and sub-cortical regions[50]. However, as the CB_1R has the highest concentrations in these regions[51] these findings may be due to direct effects on the endocannabinoid system rather than representing dopamine-mediated processes. When studies investigating the effects of acute THC administration on resting brain activity have focused on regions with dense dopaminergic innervation, such as the striatum, there have been inconsistent findings, with reports of both increased and reduced activity[50]. However, certain cognitive tasks are modulated by dopaminergic signalling and may provide a more robust proxy for THC-induced changes in dopaminergic transmission. For example, motor response inhibition is associated with cortical dopamine release; an fMRI study of healthy humans with previous exposure to cannabis found that THC attenuates activation in the right inferior frontal cortex and the anterior cingulate cortex (ACC) during suppression of motor inhibition[52]. Further indirect evidence of blunted dopaminergic processing comes from a study of healthy humans with previous exposure to cannabis using a verbal working memory task in which it was found that THC attenuated striatal activation[53]. Additionally, a study of reward function in occasional cannabis users found that THC induced widespread attenuation of the brain response to feedback in reward trials[54] but not under reward-anticipation conditions[54].

It is also possible to directly measure activity of the dopamine system using molecular imaging. These studies have examined the effect of acute THC administration on dopamine release in humans with previous exposure to cannabis *in vivo*. Using PET, a combined analysis[55] of two previous studies has shown that THC does indeed cause dopamine release in the ventral striatum in the human brain[55]. Likewise, acute THC challenge elicited dopamine release in fronto-temporal cortical brain regions[56], although this finding requires replication with radiotracers that show higher affinity for cortical dopamine receptors. However, in a separate study using single-photon emission-computed tomography (SPECT), no significant THC-induced dopamine release was observed[57], which may be due to the relatively small sample size ($n = 9$) and the lower sensitivity of SPECT than PET to dopamine measures. This is likely to be because THC-induced dopamine release in the striatum appears to be of a lower magnitude than that caused by other drugs such as amphetamine and methylphenidate[58], combined with difficulties in imaging dopamine changes that are comparatively small[59].

Repeated THC treatment and presynaptic dopamine

Studies of repeated THC dosing have yielded complex, region-specific effects, such as increased total striatal dopamine levels in rats[60] but reduced hippocampal dopamine levels in mice[61]. Reduced dopamine metabolism was found in the medial PFC in two rat studies[60], an effect that was not observed in the striatum or the NAc[62]. Administration of THC for 21 days downregulated tyrosine hydroxylase mRNA expression in the substantia nigra and ventral tegmental area (VTA) midbrain nuclei, but not in the cortex or striatum[63]. Several studies have reported that multiple THC doses do not significantly change dopamine release in the NAc in rats[64]. However, this may be due to differing dopaminergic responses within the NAc; in one rat study, repeated THC doses led to increased dopamine release in the NAc core but decreased release in the NAc shell[65]. The picture is further complicated by genetic strain effects, with increased dopamine release in the NAc observed in Lewis, but not Fischer 344, rat strains[66]. Together, these studies indicate that repeated THC dosing produces region-specific effects on dopamine function.

Studies in human cannabis users

Several molecular imaging studies of dopaminergic function have been conducted in human cannabis users. Using PET, the capacity for dopamine synthesis was observed to be reduced in cannabis users[67]. Notably, this reduction was driven by users meeting clinical criteria for abuse or dependence and was related to the degree of cannabis use (Fig. 3). Similarly, in two separate studies, cannabis users exhibited reduced dopamine release to a stimulant challenge, which was related to degree of cannabis use[68], and cognitive deficits that included poor working memory[69]. Since no alteration in amphetamine-induced dopamine release was seen in recently abstinent cannabis users[70], this effect is likely to be related to active use of the drug. Although chronic use was not associated with alterations in stress-induced dopamine release[71], there was evidence of a positive relationship between the duration of cannabis use and the level of stress-induced dopamine release in the limbic striatum[71]. Likewise, recent data show that cannabis users have an attenuated metabolic response to methylphenidate challenge in the striatum, with a negative relationship between methylphenidate-induced metabolic increases and severity of cannabis use[72]. There is also evidence of reduced dopamine transporter (DAT) density in chronic cannabis users[73]. Although the interpretation of some of these studies is complicated by the fact that cannabis users also frequently smoke tobacco, a recent experiment has addressed this by studying cannabis users without comorbid substance dependence, showing that cannabis users do indeed exhibit reduced dopamine release[69]. Overall there is converging evidence that presynaptic dopaminergic function is reduced in cannabis users.

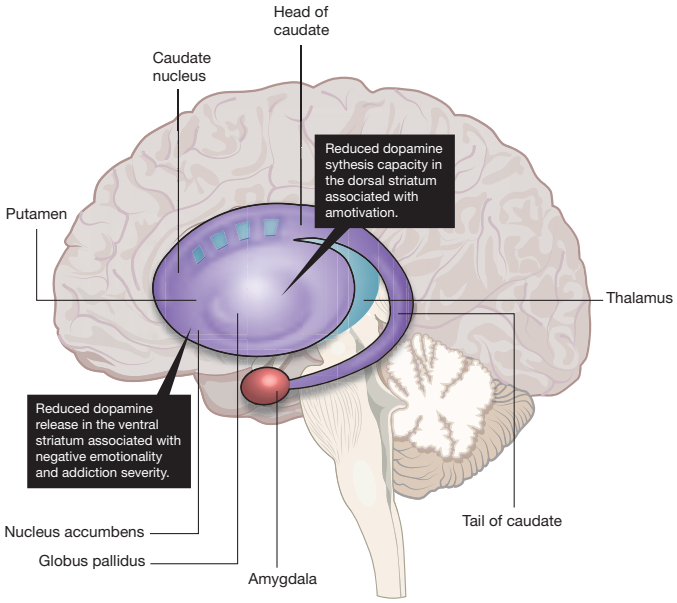

Figure 3 | Cannabis use in humans is associated with reduced dopamine in the striatum. PET studies have shown lower striatal dopamine synthesis and release capacity in cannabis users. Lower dopamine synthesis capacity in the dorsal striatum is directly associated with reduced motivation levels[102], whereas reduced dopamine release in the ventral striatum is directly associated with negative emotion levels and severity of addiction[68].

Repeated THC administration and postsynaptic dopamine

Studies in rats have reported that multiple THC dosing results in increased D_2R availability in the midbrain, striatum and PFC[63,74] and that this is associated with dopamine receptor sensitization. There is further evidence of downstream dopaminergic effects of THC; one study in rats[75] reported upregulated postsynaptic dopamine receptor signalling in the NAc through an increase in adenylyl cyclase activity, which was proposed to underlie THC-induced changes in amphetamine-induced locomotive behaviour. These findings suggest that repeated THC dosing results in altered dopamine receptor signal transduction.

Dose-dependent increases in burst-firing in the VTA in response to multiple THC administrations have been reported in nearly all murine electrophysiological studies[46], with only one exception of which we are aware[76]. In the substantia nigra pars compacta (SNpc), multiple THC dosing was associated with increased neuronal firing, although this increase was smaller in magnitude than in the VTA[77,78]. This suggests that VTA and SNpc dopamine neurons develop differing responses to repeated THC exposures. There also appears to be an effect of withdrawal from multiple THC doses, whereby decreased firing is elicited by the abrupt cessation of repeated THC or administration of the CB_1R antagonist SR141716A (ref. 79).

Recent studies in current cannabis users and abstinent former users have found no significant difference in striatal D_2R availability compared to individuals with no history of chronic cannabis use[68–70,80–82]. However, there was an inverse relationship between the age of first cannabis use and D_2R availability in one study[70] and an inverse relationship between current cannabis use and receptor availability in another study[80], suggestive of possible dose effects or susceptibility to drug use.

THC exposure and dopamine cell morphology

In addition to changes in cell function, there is evidence from studies in rodents that THC exposure causes abnormalities in the structure of dopamine neurons. These morphological effects are region-specific and include reductions across a range of measures of neuronal cell size in the VTA[83,84] and increased neuronal arborization in the NAc shell and frontal cortex[85].

Developmental THC exposure and dopamine

Adolescence is an important time for brain development and adolescent cannabinoid exposure has consequences in adulthood[86]. The endocannabinoid system plays an important role in brain development[87]; developmental THC exposure produces complex alterations in the dopamine system that are apparent from an early stage. For example, gestational THC exposure is associated with increased fetal brain tyrosine hydroxylase mRNA expression in rats[88] and changes in dopamine receptor gene expression. THC exposure in early development is associated with increased cortical D_2R availability[89]. In rats, exposure to THC from gestational day 5 to post-natal day 21 resulted in decreased levels of *DRD2* mRNA (encoding D_2R) in the NAc. Likewise, human maternal cannabis use was associated with decreased *DRD2* mRNA in the fetal NAc[90]. Exposure to THC in early life also blunts the dopaminergic response to dopamine-releasing stimuli, such as stress and amphetamine, later in life. This was seen in the hypothalamus, striatum and limbic forebrain[91], and frontal cortex[92]. In a study of CB_1R agonist self-administration in rats[93], THC exposure during adolescence enhanced the reinforcing effects of cannabinoids in adulthood, suggesting that exposure to THC during adolescence increased addiction potential during a critical period of development. Importantly, THC-exposed rodents had a reduced capacity for cannabinoid-induced increases in firing of dopaminergic neurons, consistent with a blunting of the dopamine response in adulthood.

Inconsistencies between animal and human studies

Preclinical evidence shows that acute THC administration increases nerve-firing rates and the results of animal studies using microdialysis indicate that acute THC challenge causes dopamine release, yet the results of experiments from human studies have not been consistent[94,95]. A number of factors may underlie the inconsistencies in the results of the studies presented above.

The animal evidence indicates that differences in dopamine activity following THC administration are highly region-specific and include differing dopaminergic responses in the shell and core of the NAc[65]. Although human PET imaging provides a reliable measure of dopaminergic function in the striatum[59], the spatial resolution of most PET cameras is approximately 4 mm, which limits accuracy when measuring activity in small brain volumes (such as in the core and shell of the NAc) owing to partial-volume and spill-over effects[96]. For the foreseeable future, therefore, human *in vivo* imaging techniques lack the spatial resolution to detect these complex effects on different parts of the dopamine pathway, whereas research on living human brain tissue *in vitro* of course raises substantial ethical challenges[97].

A further possibility underlying the lack of consistent dopamine release in the human studies is that the dose of THC used in human imaging studies has not been sufficient to consistently elicit measurable dopamine release. Human studies all involved the administration of 10 mg or less of THC because older pharmacological studies indicated that the 'standard joint' (or cannabis cigarette; defined by the US National Institute on Drug Abuse) delivered an approximate THC dose of 8–15 mg, equivalent to about 170 $\mu g\ kg^{-1}$ (ref. 98). These doses are considerably less than those used in animal microdialysis studies, which are typically around 1 $mg\ kg^{-1}$. Correspondingly, though, the THC content of cannabis has increased considerably since early clinical studies[99], with current THC doses possibly exceeding 40 mg per joint[100], meaning that the doses that were used in the published imaging studies no longer reflect typical THC exposure in cannabis users. The picture is further complicated by animal data indicating that THC exerts complex dose-dependent effects on the dopamine system[31]; the dose–response profile for THC-induced dopamine release has not been investigated in humans so it is possible that human imaging studies are missing peak dopamine changes. Likewise, heterogeneity in animal data may be a reflection of time of sampling in

relation to THC treatment. For example, repeated daily THC administration led to an initial decrease in dopamine levels, followed by a gradual return to baseline in brains that were studied 1 h after THC dosing, but increases were subsequently observed 2 h after dosing[32]. This time period is consistent with acute release and diffusion away, followed by an upregulation of synthesis, increasing levels at 2 h. Other factors that may contribute to the complex temporal course of dopaminergic effects include intricate changes in dopamine synthesis and metabolism. These comprise both CB_1R-mediated increases in tyrosine hydroxylase activity[36] and acute increases and longer-term decreases in monoamine oxidase activity[101], indicating that THC may exert differential effects on the time courses of dopamine synthesis and degradation. Equally, the partial agonist properties of THC on the CB_1R serve only to obfuscate its dopaminergic effects, since THC can both activate and block cannabinoid receptors in a region-specific and species-dependent manner, based on the density and efficiency of populations of receptors and the concentrations of endogenous agonists[19].

There are a number of challenges involved in assessing the long-term effects of exposure to THC. First, it is plausible that discrepant findings may be attributable to the duration of THC treatment. Animal experiments are typically conducted following administration of THC for no longer than three weeks[63,78,84], whereas human studies are conducted in participants who have taken cannabis over years (the duration of regular cannabis use is typically in excess of three years[102]). Furthermore, it remains unclear to what degree homeostatic mechanisms are able to compensate for these alterations over time. Additionally, in humans there are a number of psychosocial and genetic risk factors that may predispose an individual to develop a cannabis use disorder. Given the evidence of interactions between early life psychological deprivation and THC-induced effects on dopamine in animals[74], it is possible that the human data relating to cannabis-dependent participants is confounded by a range of environmental factors, including parental loss and other psychosocial factors[103]. Other environmental and physiological factors have been found to influence the dopaminergic effects of THC. Of particular relevance to humans is sleep deprivation, which decreases dopamine turnover in response to THC[104], and stress, which increases THC-induced dopamine synthesis[105]. Cannabis also contains a multitude of other compounds, called phytocannabinoids, which probably exert different complex actions on the dopamine system. These compounds could be behind the varied response observed in human cannabis users when compared to the more consistent animal data that uses only THC. Owing to the co-morbidity of addictions, it is particularly challenging for human imaging researchers to recruit participants with cannabis dependence who do not use other psychoactive compounds, including nicotine and alcohol, in more naturalistic studies.

Finally, whether THC is delivered in a contingent or non-contingent way is likely to be important, given evidence that anticipation leads to dopamine release. In general, animal studies include a variety of contingencies around the dosing of THC. By contrast, in human acute studies THC was given to participants with a prior exposure to the drug but was not delivered in the habitual manner in which the drug is consumed, potentially reducing the degree of dopamine release.

Dopamine and the behavioural effects of THC

THC is associated with a number of behavioural effects that are likely to involve alterations in dopaminergic function. The first evidence of this came from early animal studies, which reported similarities between the behavioural effects of THC, including catalepsy and hypothermia[106], and dopamine antagonists[107]. However, THC also antagonizes the locomotor and hyperthermic effects of amphetamine, which causes dopamine release[108]. These seemingly paradoxical effects may be due to the high doses used in the early research together with the partial agonist effects of THC and/or non-specific receptor-independent effects.

THC promotes increased food intake in animals[109] and humans[110]. Since appetite is modulated by the dopamine system[111], studies have investigated dopaminergic involvement in THC-induced feeding. The dopamine D_1 receptor antagonist SCH23390 attenuated THC-induced feeding at a dose that did not affect feeding on its own[112]. Recent research has identified CB_1R-mediated changes in hypothalamic pro-opiomelanocortin (POMC) neurons[113] as the potential underlying cause of this process, through mitochondrial uncoupling protein 2, which is involved in ghrelin-mediated dopaminergic function[114].

Heavy cannabis use is associated with impaired educational and occupational outcomes[115]. Factors that may underlie this association include cognitive impairment, which includes executive dysfunction[116], working memory impairments[117] and amotivation[118] (defined as reduced motivation for goal-directed behaviour[119]). These functions are susceptible to mesocortical dopaminergic manipulation[120] (for example, prefrontal D_1 receptor blockade[121]). Although a preclinical study reported that D_2 receptor antagonism blocks THC-induced working memory deficits[122], this was not replicated in humans[123]. Nonetheless, there is recent evidence that THC-induced working-memory deficits are moderated by catechol-*O*-methyltransferase, a key enzyme in the dopamine metabolic pathway[124], which also modulates the effects of THC use on dopaminergic cell size in adolescents[84]. Chronic heavy cannabis use produces apathetic behaviour in rhesus monkeys[125], while a study in humans[102] found that the reduced dopamine synthesis capacity observed in heavy cannabis users was related to amotivation. Overlapping features of the amotivational syndrome associated with cannabis use disorders, such as reduced reward sensitivity and negative emotionality[126], were also found to be inversely related to methylphenidate-induced dopamine ventral striatal dopamine release[68].

The dopamine system is involved in risk of psychosis[127]. An early case report described increased striatal dopamine release following cannabis intoxication, associated with the exacerbation of psychotic symptoms in a patient with schizophrenia[128]. Furthermore, cannabis users with a diagnosis of schizophrenia, and those at clinical high risk for schizophrenia, displayed blunted striatal stress-induced dopamine release[129]. Although dopamine release was blunted in cannabis users with schizophrenia, it was nevertheless directly related to the induction of psychotic symptoms[130]. Supersensivity of postsynaptic D_2Rs[63] could explain this apparent paradox; alternatively it could be due to impaired endocannabinoid regulation of dopamine signal transduction. Patients with schizophrenia using high levels of cannabis show reduced anandamide levels, supporting the latter explanation[131]. Accordingly, cannabidiol, a phytocannabinoid compound that elevates anandamide levels, has been shown to reduce psychotic symptoms[132]. Alternative postsynaptic mechanisms include CB_1R–D_2R heterodimerization[133] and downstream intracellular mechanisms such as the neuregulin 1–erbB2 receptor tyrosine kinase 4–phosphoinositide 3 kinase–protein kinase B (*NRG1–ERBB4–PI3K–AKT1*) pathway[134].

Outlook

There is now substantial evidence that THC exerts effects on the dopamine system in animals. The key challenges for the field must be to understand the complexity of these effects, identify how they translate to humans and appreciate how they relate to the potential negative effects of the drug in humans (Box 2). Animal studies demonstrate that the acute administration of THC causes region-specific increases in dopamine release and nerve activity—we must understand the functional significance of these increases. The available preclinical evidence suggests that chronic THC administration causes long-term changes to the dopamine system, but these models are limited as they do not reflect typical patterns of human use. Future studies should, therefore, be of longer duration to reflect human use and should also include co-administration with other drugs, such as nicotine and alcohol, which are commonly used with cannabis. Related to this is a need to understand how the dopaminergic effects of THC are modulated by the other phytocannabinoids. Human PET studies have demonstrated that dopamine synthesis and dopamine release are blunted in cannabis

BOX 2

Strategy for future work

Preclinical and clinical research
- Identify the relationships between cannabis-induced alterations in the dopamine system and behavioural phenomena in humans and animal models
- Use translational techniques that can be applied in human and animal studies alike and employ study designs that better reflect patterns of human use, including modelling contingency in acute THC challenge studies
- Achieve consensus on dose equivalence across species
- Determine whether THC-induced dopaminergic changes during key developmental phases persist into later life and whether this is linked to behavioural changes
- Investigate how gene variants that modulate the endocannabinoid and dopamine systems influence the sensitivity to the rewarding effects of THC and vulnerability to addiction, amotivation and psychosis following chronic exposure
- Investigate the effects of sex in THC response. This will be useful to understand the differences in susceptibility to cannabis effects that have been reported between males and females[72].

Preclinical research
- Identify the effects on the dopamine system of long-term THC exposure alongside co-administration with nicotine to reflect typical patterns of human use
- Determine the mechanisms underlying the complex dose–response effects of THC on dopaminergic function
- Elucidate the mechanisms of regional differences in dopaminergic effects and the functional significance of these on behaviour
- Determine if the long-term effects of THC are reversible with abstinence

Clinical research
- Determine if the blunted dopamine release and synthesis seen in chronic users is a pre-existing vulnerability factor or a direct result of repeated THC exposure
- Determine the dopaminergic changes over the course of repeated THC exposures and dose–response effects
- Achieve consensus on how to report previous exposure to cannabis use in the human literature
- Determine whether there are regional differences in dopamine release in response to THC administration in humans
- Determine the dose and context dependency of THC effects on the dopamine system

users relative to non-users, yet we still need to understand the precise mechanisms through which this occurs.

It is necessary to identify whether the effects of THC on the dopamine system are reversible and, if they are not, to recognize when they become so. Likewise, further molecular imaging studies in humans are needed to determine the dose–response and timing of the acute effects of THC on dopamine release, and to determine whether there are regional differences in dopamine release—particularly between cortical and sub-cortical regions.

Ultimately, one key challenge in understanding the effects of THC on the dopamine system is translating results from animal studies, many of which cannot be carried out clinically, into humans. We must also reach consensus on THC dose equivalence across species. It is therefore difficult to know what the implications of some preclinical animal work are for human research and how to back-translate human findings into preclinical models. There must be movement away from untranslatable, standalone research, towards the use of translational techniques, such as PET and MRI approaches that can be conducted in both humans and preclinical models that can capture human use patterns in combination with traditional preclinical techniques. We must also understand how psychoactive metabolites of THC and endocannabinoids modulate THC-induced changes in dopaminergic function.

Given the changing patterns in cannabis use across the world, particularly in young people, and the increase in the THC content of cannabis, there is clearly a pressing need to understand how THC alters dopaminergic function. This is especially important given that emerging evidence that links dopaminergic alterations to a number of the adverse cognitive and behavioural consequences of THC, and that there is a dearth of current effective biological interventions for many of the psychiatric sequelae of cannabis use. Dopaminergic dysfunction may thus represent an area for future treatment targets.

There is evidence that gestational exposure to THC is associated with dysregulated dopamine synthesis in later life. This has major potential public health implications, given the prevalence of cannabis use in women of child-bearing age and that the liberalization of cannabis laws around the world may be associated with increased use of the drug amongst gravid and nursing mothers. However, there remain gaps in our knowledge of the developmental effects of THC. In particular, we do not know how long the dopaminergic effects persist for and what the behavioural consequences are. Perhaps most critically, we do not yet know what the effects of THC exposure are on the adolescent dopamine system.

The available evidence indicates that THC exposure produces complex, diverse and potentially long-term effects on the dopamine system. These include increased nerve firing and dopamine release in response to acute THC, and dopaminergic blunting associated with long-term use. Future research should focus on probing the relationships between cannabis-induced alterations in the dopamine system and behavioural effects in humans and animal models.

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1. Substance Abuse and Mental Health Services Administration. *Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings* Vol. HHS Publication No. (SMA) 14–4863 (Substance Abuse and Mental Health Services Administration, 2014).
2. EMCDDA. *European Drug Report 2015: Trends and Developments* (European Monitoring Centre for Drugs and Drug Addiction, Lisbon, 2015).
3. Volkow, N. D. *et al.* Effects of cannabis use on human behavior, including cognition, motivation, and psychosis: a review. *JAMA Psychiatry* **73,** 292–297 (2016).
4. Di Forti, M. *et al.* Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophr. Bull.* **40,** 1509–1517 (2014).
5. 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).
6. Horwood, L. J. *et al.* Cannabis and depression: an integrative data analysis of four Australasian cohorts. *Drug Alcohol Depend.* **126,** 369–378 (2012).
7. Silins, E. *et al.* Young adult sequelae of adolescent cannabis use: an integrative analysis. *Lancet Psychiatry* **1,** 286–293 (2014).
8. 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).
9. Cherek, D. R., Lane, S. D. & Dougherty, D. M. Possible amotivational effects following marijuana smoking under laboratory conditions. *Exp. Clin. Psychopharmacol.* **10,** 26–38 (2002).
10. Wachtel, S. R., ElSohly, M. A., Ross, S. A., Ambre, J. & de Wit, H. Comparison of the subjective effects of Δ^9-tetrahydrocannabinol and marijuana in humans. *Psychopharmacology (Berl.)* **161,** 331–339 (2002).
11. Felder, C. C., Veluz, J. S., Williams, H. L., Briley, E. M. & Matsuda, L. A. Cannabinoid agonists stimulate both receptor- and non-receptor-mediated signal transduction pathways in cells transfected with and expressing cannabinoid receptor clones. *Mol. Pharmacol.* **42,** 838–845 (1992).
12. Mehmedic, Z. *et al.* Potency trends of Δ^9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *J. Forensic Sci.* **55,** 1209–1217 (2010).
13. Spaderna, M., Addy, P. H. & D'Souza, D. C. Spicing things up: synthetic cannabinoids. *Psychopharmacology (Berl.)* **228,** 525–540 (2013).
14. Benjamin, D. M. & Fossler, M. J. Edible cannabis products: it is time for FDA oversight. *J. Clin. Pharmacol.* (2016).

15. Varlet, V. *et al.* Drug vaping applied to cannabis: Is "Cannavaping" a therapeutic alternative to marijuana? *Sci. Rep.* **6,** 25599 (2016).
16. Vallee, M. *et al.* Pregnenolone can protect the brain from cannabis intoxication. *Science* **343,** 94–98 (2014).
17. Huestis, M. A. *et al.* Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch. Gen. Psychiatry* **58,** 322–328 (2001).
The psychoactive effects of cannabis are mediated via the CB_1 receptor.
18. Elphick, M. R. & Egertova, M. The neurobiology and evolution of cannabinoid signalling. Phil. *Trans. R. Soc. Lond. B* **356,** 381–408 (2001).
19. Pertwee, R. G. The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. *Br. J. Pharmacol.* **153,** 199–215 (2008).
20. Kathmann, M., Flau, K., Redmer, A., Trankle, C. & Schlicker, E. Cannabidiol is an allosteric modulator at μ- and δ-opioid receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **372,** 354–361 (2006).
21. Chartoff, E. H. & Connery, H. S. It's MORe exciting than mu: crosstalk between mu opioid receptors and glutamatergic transmission in the mesolimbic dopamine system. *Front. Pharmacol.* **5,** 116 (2014).
22. Hollister, L. E. & Gillespie, H. K. Action of Δ^9-tetrahydrocannabinol. An approach to the active metabolite hypothesis. *Clin. Pharmacol. Ther.* **18,** 714–719 (1975).
23. Garriott, J. C., King, L. J., Forney, R. B. & Hughes, F. W. Effects of some tetrahydrocannabinols on hexobarbital sleeping time and amphetamine induced hyperactivity in mice. *Life Sci.* **6,** 2119–2128 (1967).
24. Howes, J. & Osgood, P. The effect of Δ^9-tetrahydrocannabinol on the uptake and release of ^{14}C-dopamine from crude striatal synaptosoma; preparations. *Neuropharmacology* **13,** 1109–1114 (1974).
25. Fernandez-Ruiz, J., Hernandez, M. & Ramos, J. A. Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. *CNS Neurosci. Ther.* **16,** e72–e91 (2010).
26. Herkenham, M., Lynn, A. B., Decosta, B. R. & Richfield, E. K. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res.* **547,** 267–274 (1991).
27. Solinas, M., Justinova, Z., Goldberg, S. R. & Tanda, G. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J. Neurochem.* **98,** 408–419 (2006).
28. De Luca, M. A. *et al.* Endocannabinoid 2-arachidonoylglycerol self-administration by Sprague–Dawley rats and stimulation of *in vivo* dopamine transmission in the nucleus accumbens shell. *Front. Psychiatry* **5,** 140 (2014).
29. Bloom, A. S. & Dewey, W. L. A comparison of some pharmacological actions of morphine and Δ^9-tetrahydrocannabinol in the mouse. *Psychopharmacology (Berl.)* **57,** 243–248 (1978).
30. Hershkowitz, M. & Szechtman, H. Pretreatment with Δ^1-tetrahydrocannabinol and psychoactive drugs: effects on uptake of biogenic amines and on behavior. *Eur. J. Pharmacol.* **59,** 267–276 (1979).
31. Poddar, M. K. & Dewey, W. L. Effects of cannabinoids on catecholamine uptake and release in hypothalamic and striatal synaptosomes. *J. Pharmacol. Exp. Ther.* **214,** 63–67 (1980).
32. Aulakh, C. S., Bhattacharyya, A. K., Hossain, M. A. & Pradhan, S. N. Behavioral and neurochemical effects of repeated administration of Δ^9-tetrahydrocannabinol in rats. *Neuropharmacology* **19,** 97–102 (1980).
33. Maitre, L., Staehelin, M. & Bein, H. J. Effect of an extract of cannabis and of some cannabinols on catecholamine metabolism in rat brain and heart. *Agents Actions* **1,** 136–143 (1970).
34. Bloom, A. S., Johnson, K. M. & Dewey, W. L. The effects of cannabinoids on body temperature and brain catecholamine synthesis. *Res. Commun. Chem. Pathol. Pharmacol.* **20,** 51–57 (1978).
35. Romero, J., Demiguel, R., Garciapalomero, E., Fernandezruiz, J. J. & Ramos, J. A. Time-course of the effects of anandamide, the putative endogenous cannabinoid receptor-ligand, on extrapyramidal function. *Brain Res.* **694,** 223–232 (1995).
36. Bosier, B. *et al.* Differential modulations of striatal tyrosine hydroxylase and dopamine metabolism by cannabinoid agonists as evidence for functional selectivity *in vivo*. *Neuropharmacology* **62,** 2328–2336 (2012).
37. Navarro, M. *et al.* An acute dose of Δ^9-tetrahydrocannabinol affects behavioral and neurochemical indices of mesolimbic dopaminergic activity. *Behav. Brain Res.* **57,** 37–46 (1993).
38. Heien, M. L. *et al.* Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. *Proc. Natl Acad. Sci. USA* **102,** 10023–10028 (2005).
39. 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).
40. Ng Cheong Ton, J. M. *et al.* The effects of Δ^9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an *in vivo* electrochemical and *in vivo* microdialysis study. *Brain Res.* **451,** 59–68 (1988).
41. Chen, J. P. *et al.* Δ^9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. *Psychopharmacology (Berl.)* **102,** 156–162 (1990).
42. Castaneda, E., Moss, D., Oddie, S. & Whishaw, I. THC does not affect striatal dopamine release: Microdialysis in freely moving rats. *Pharmacol. Biochem. Behav.* **40,** 587–591 (1991).
43. Nahas, G. G. in *Medical Aspects of Drug Abuse* (ed. R. W. Richter) 16–36 (Harper & Row, 1975).
44. Chen, J., Paredes, W., Lowinson, J. & Gardner, E. Strain-specific facilitation of dopamine efflux by Δ^9-tetrahydrocannabinol in the nucleus accumbens of rat: An *in vivo* microdialysis study. *Neurosci. Lett.* **129,** 136–140 (1991).
45. Oleson, E. B. & Cheer, J. F. A brain on cannabinoids: the role of dopamine release in reward seeking. *Cold Spring Harb. Perspect. Med.* **2,** a012229 (2012).
46. French, E. Δ^9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci. Lett.* **226,** 159–162 (1997).
47. Ali, S. F. *et al.* Chronic marijuana smoke exposure in the rhesus monkey. IV: Neurochemical effects and comparison to acute and chronic exposure to Δ^9-tetrahydrocannabinol (THC) in rats. *Pharmacol. Biochem. Behav.* **40,** 677–682 (1991).
48. Navarro, M. *et al.* Motor disturbances induced by an acute dose of Δ^9-tetrahydrocannabinol: Possible involvement of nigrostriatal dopaminergic alterations. *Pharmacol. Biochem. Behav.* **45,** 291–298 (1993).
49. Rodríguez De Fonseca, F. *et al.* Acute effects of Δ^9-tetrahydrocannabinol on dopaminergic activity in several rat-brain areas. *Pharmacol. Biochem. Behav.* **42,** 269–275 (1992).
50. Volkow, N. D. *et al.* Brain glucose metabolism in chronic marijuana users at baseline and during marijuana intoxication. *Psychiatry Res.* **67,** 29–38 (1996).
51. Pertwee, R. G. & Ross, R. A. Cannabinoid receptors and their ligands. *Prostaglandins Leukot. Essent. Fatty Acids* **66,** 101–121 (2002).
52. Borgwardt, S. J. *et al.* Neural basis of Δ^9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biol. Psychiatry* **64,** 966–973 (2008).
53. Bhattacharyya, S. *et al.* Opposite effects of Δ^9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* **35,** 764–774 (2010).
54. van Hell, H. H. *et al.* Involvement of the endocannabinoid system in reward processing in the human brain. *Psychopharmacology (Berl.)* **219,** 981–990 (2012).
55. 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 (Berl.)* (2015).
56. Stokes, P. R. *et al.* Significant decreases in frontal and temporal [11C]-raclopride binding after THC challenge. *Neuroimage* **52,** 1521–1527 (2010).
57. Barkus, E. *et al.* Does intravenous Δ^9-tetrahydrocannabinol increase dopamine release? A SPET study. *J. Psychopharmacol.* **25,** 1462–1468 (2011).
58. Volkow, N. D. *et al.* Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D_2 receptors. *J. Pharmacol. Exp. Ther.* **291,** 409–415 (1999).
59. Egerton, A., Demjaha, A., McGuire, P., Mehta, M. A. & Howes, O. D. The test-retest reliability of 18F-DOPA PET in assessing striatal and extrastriatal presynaptic dopaminergic function. *Neuroimage* **50,** 524–531 (2010).
60. Jentsch, J., Verrico, C., Le, D. & Roth, R. Repeated exposure to Δ^9-tetrahydrocannabinol reduces prefrontal cortical dopamine metabolism in the rat. *Neurosci. Lett.* **246,** 169–172 (1998).
61. Avraham, Y. *et al.* Very low doses of Δ^8-THC increase food consumption and alter neurotransmitter levels following weight loss. *Pharmacol. Biochem. Behav.* **77,** 675–684 (2004).
62. Jentsch, J., Andrusiak, E., Tran, A., Bowers, Jr. & Roth, R. 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).
63. Ginovart, N. *et al.* Chronic Δ^9-tetrahydrocannabinol exposure induces a sensitization of dopamine $D_{2/3}$ receptors in the mesoaccumbens and nigrostriatal systems. *Neuropsychopharmacology* **37,** 2355–2367 (2012).
64. 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).
THC increased extracellular dopamine concentrations in the nucleus accumbens shell.
65. Cadoni, C., Valentini, V. & Di Chiara, G. Behavioral sensitization to Δ^9-tetrahydrocannabinol and cross-sensitization with morphine: differential changes in accumbal shell and core dopamine transmission. *J. Neurochem.* **106,** 1586–1593 (2008).
66. 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).
67. Bloomfield, M. A. P. *et al.* Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biol. Psychiatry* **75,** 470–478 (2014).
Dopamine synthesis capacity is reduced in long-term human cannabis users.
68. Volkow, N. D. *et al.* Decreased dopamine brain reactivity in marijuana abusers is associated with negative emotionality and addiction severity. *Proc. Natl Acad. Sci. USA* **111,** E3149–E3156 (2014).
Dopamine release is blunted in chronic human cannabis users.
69. van de Giessen, E. *et al.* Deficits in striatal dopamine release in cannabis dependence. *Mol. Psychiatry* http://dx.doi.org/10.1038/mp.2016.21 (2016).

70. Urban, N. B. L. *et al.* Dopamine release in chronic cannabis users: a [c-11] raclopride positron emission tomography study. *Biol. Psychiatry* **71,** 677–683 (2012).
71. Mizrahi, R. *et al.* Dopamine response to psychosocial stress in chronic cannabis users: a PET study with [11C]-(+)-PHNO. *Neuropsychopharmacology* **38,** 673–682 (2013).
72. Wiers, C. E. *et al.* Cannabis abusers show hypofrontality and blunted brain responses to a stimulant challenge in females but not in males. *Neuropsychopharmacology* **41,** 2596–2605 (2016).
73. Leroy, C. *et al.* Striatal and extrastriatal dopamine transporter in cannabis and tobacco addiction: a high-resolution PET study. *Addict. Biol*. **17,** 981–990 (2012).
74. Zamberletti, E. *et al.* Gender-dependent behavioral and biochemical effects of adolescent Δ^9-tetrahydrocannabinol in adult maternally deprived rats. *Neuroscience* **204,** 245–257 (2012).
75. Cortright, J. J., Lorrain, D. S., Beeler, J. A., Tang, W. J. & Vezina, P. Previous exposure to Δ^9-tetrahydrocannibinol enhances locomotor responding to but not self-administration of amphetamine. *J. Pharmacol. Exp. Ther.* **337,** 724–733 (2011).
76. Gifford, A., Gardner, E. & Ashby Jr., C. R. The effect of intravenous administration of Δ^9-tetrahydrocannabinol on the activity of A10 dopamine neurons recorded in vivo in anesthetized rats. *Neuropsychobiology* **36,** 96–99 (1997).
77. French, E. D., Dillon, K. & Wu, X. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport* **8,** 649–652 (1997).
78. Wu, X. & French, E. Effects of chronic Δ^9-tetrahydrocannabinol on rat midbrain dopamine neurons: An electrophysiological assessment. *Neuropharmacology* **39,** 391–398 (2000).
79. 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).
Chronic THC exposure is associated with reduced dopamine neuron activity in the meso-accumbens.
80. Albrecht, D. S. *et al.* Striatal D_2/D_3 receptor availability is inversely correlated with cannabis consumption in chronic marijuana users. *Drug Alcohol Depend*. **128,** 52–57 (2013).
81. Sevy, S. *et al.* Cerebral glucose metabolism and D_2/D_3 receptor availability in young adults with cannabis dependence measured with positron emission tomography. *Psychopharmacology (Berl.)* **197,** 549–556 (2008).
82. Stokes, P. R. A. *et al.* History of cannabis use is not associated with alterations in striatal dopamine D_2/D_3 receptor availability. *J Psychopharmacol.* **26,** 144–149 (2012).
83. Spiga, S., Lintas, A., Migliore, M. & Diana, M. Altered architecture and functional consequences of the mesolimbic dopamine system in cannabis dependence. *Addict. Biol*. **15,** 266–276 (2010).
84. Behan, A. *et al.* Chronic adolescent exposure to Δ^9-tetrahydrocannabinol in COMT mutant mice: Impact on indices of dopaminergic, endocannabinoid and GABAergic pathways. *Neuropsychopharmacology* **37,** 1773–1783 (2012).
85. Kolb, B., Gorny, G., Limebeer, C. L. & Parker, L. A. Chronic treatment with Δ^9-tetrahydrocannabinol alters the structure of neurons in the nucleus accumbens shell and medial prefrontal cortex of rats. *Synapse* **60,** 429–436 (2006).
86. Renard, J., Krebs, M. O., Le Pen, G. & Jay, T. M. Long-term consequences of adolescent cannabinoid exposure in adult psychopathology. *Front. Neurosci.* **8,** 361 (2014).
87. Berghuis, P. *et al.* Hardwiring the brain: endocannabinoids shape neuronal connectivity. *Science* **316,** 1212–1216 (2007).
88. Bonnin, A., de Miguel, R., Hernandez, M. L., Ramos, J. A. & Fernandez-Ruiz, J. J. The prenatal exposure to Δ^9-tetrahydrocannabinol affects the gene expression and the activity of tyrosine hydroxylase during early brain development. *Life Sci.* **56,** 2177–2184 (1995).
89. Walters, D. E. & Carr, L. A. Perinatal exposure to cannabinoids alters neurochemical development in rat brain. *Pharmacol. Biochem. Behav.* **29,** 213–216 (1988).
90. DiNieri, J. A. *et al.* Maternal cannabis use alters ventral striatal dopamine D_2 gene regulation in the offspring. *Biol. Psychiatry* **70,** 763–769 (2011).
Prenatal cannabis exposure decreases dopamine receptor D_2 messenger RNA expression in the ventral striatum of offspring in humans.
91. Garcia-Gil, L. *et al.* Perinatal Δ^9-tetrahydrocannabinol exposure alters the responsiveness of hypothalamic dopaminergic neurons to dopamine-acting drugs in adult rats. *Neurotoxicol. Teratol.* **19,** 477–487 (1997).
92. Mokler, D. J., Robinson, S. E., Johnson, J. H., Hong, J. S. & Rosecrans, J. A. Neonatal administration of Δ^9-tetrahydrocannabinol (THC) alters the neurochemical response to stress in the adult Fischer-344 rat. *Neurotoxicol. Teratol.* **9,** 321–327 (1987).
93. Scherma, M. *et al.* Adolescent Δ^9-tetrahydrocannabinol exposure alters WIN55,212–2 self-administration in adult rats. *Neuropsychopharmacology* **41,** 1416–1426 (2016).
94. Bossong, M. G. *et al.* Δ^9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology* **34,** 759–766 (2009).
Combined analysis of two previous PET studies showing that acute THC administration causes dopamine release in humans.
95. Stokes, P., Mehta, M., Curran, H., Breen, G. & Grasby, P. Can recreational doses of THC produce significant dopamine release in the human striatum? *Neuroimage* **48,** 186–190 (2009).
96. Mawlawi, O. *et al.* Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D_2 receptor parameter measurements in ventral striatum. *J. Cereb. Blood Flow Metab.* **21,** 1034–1057 (2001).
97. Do we need an ethics of self-organizing tissue? *Nat. Methods* **12,** 895 (2015).
98. Lindgren, J. E., Ohlsson, A., Agurell, S., Hollister, L. & Gillespie, H. Clinical effects and plasma levels of Δ^9-tetrahydrocannabinol (Δ^9-THC) in heavy and light users of cannabis. *Psychopharmacology (Berl.)* **74,** 208–212 (1981).
99. Hardwick, S. K. L. *Home Office Cannabis Potency Study* (Home Office, 2008).
100. Hunault, C. C. *et al.* Disposition of smoked cannabis with high Δ^9-tetrahydrocannabinol content: a kinetic model. *Toxicol. Appl. Pharmacol.* **246,** 148–153 (2010).
101. Banerjee, S. P., Snyder, S. H. & Mechoulam, R. Cannabinoids: influence on neurotransmitter uptake in rat brain synaptosomes. *J. Pharmacol. Exp. Ther.* **194,** 74–81 (1975).
102. 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 (Berl.)* **231,** 2251–2259 (2014).
103. von Sydow, K., Lieb, R., Pfister, H., Hofler, M. & Wittchen, H. U. What predicts incident use of cannabis and progression to abuse and dependence? A 4-year prospective examination of risk factors in a community sample of adolescents and young adults. *Drug Alcohol Depend*. **68,** 49–64 (2002).
104. Carlini, E. A., Lindsey, C. J. & Tufik, S. Cannabis, catecholamines, rapid eye movement sleep and aggressive behaviour. *Br. J. Pharmacol*. **61,** 371–379 (1977).
105. MacLean, K. I. & Littleton, J. M. Environmental stress as a factor in the response of rat brain catecholamine metabolism to Δ^8-tetrahydrocannabinol. *Eur. J. Pharmacol.* **41,** 171–182 (1977).
106. Lomax, P. Acute tolerance to the hypothermic effect of marihuana in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* **2,** 159–167 (1971).
107. Andén, N. E. Dopamine turnover in the corpus striatum and the lumbic system after treatment with neuroleptic and anti-acetylcholine drugs. *J. Pharm. Pharmacol*. **24,** 905–906 (1972).
108. Hattendorf, C., Hattendorf, M., Coper, H. & Fernandes, M. Interaction between Δ^9-tetrahydrocannabinol and d-amphetamine. *Psychopharmacology (Berl.)* **54,** 177–182 (1977).
109. Williams, C. M., Rogers, P. J. & Kirkham, T. C. Hyperphagia in pre-fed rats following oral Δ^9-THC. *Physiol. Behav.* **65,** 343–346 (1998).
110. Foltin, R. W., Brady, J. V. & Fischman, M. W. Behavioral analysis of marijuana effects on food intake in humans. *Pharmacol. Biochem. Behav.* **25,** 577–582 (1986).
111. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand. Suppl*. **367,** 95–122 (1971).
112. Verty, A., McGregor, I. & Mallet, P. The dopamine receptor antagonist SCH 23390 attenuates feeding induced by Δ^9-tetrahydrocannabinol. *Brain Res.* **1020,** 188–195 (2004).
113. Koch, M. *et al.* Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature* **519,** 45–50 (2015).
114. Andrews, Z. B. *et al.* Ghrelin promotes and protects nigrostriatal dopamine function via a UCP2-dependent mitochondrial mechanism. *J. Neurosci.* **29,** 14057–14065 (2009).
115. Fergusson, D. M., Horwood, L. J. & Beautrais, A. L. Cannabis and educational achievement. *Addiction* **98,** 1681–1692 (2003).
Longitudinal birth cohort study indicating that cannabis use in adolescents and early adults is associated with reduced educational achievement.
116. Hooker, W. D. & Jones, R. T. Increased susceptibility to memory intrusions and the Stroop interference effect during acute marijuana intoxication. *Psychopharmacology (Berl.)* **91,** 20–24 (1987).
117. Ranganathan, M. & D'Souza, D. C. The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl.)* **188,** 425–444 (2006).
118. McGlothlin, W. H. W. L. The marihuana problem: an overview. *Am. J. Psychiatry* **125,** 126–134 (1968).
119. Levy, R. & Dubois, B. Apathy and the functional anatomy of the prefrontal cortex–basal ganglia circuits. *Cereb. Cortex* **16,** 916–928 (2005).
120. Goldman-Rakic, P. S. Regional and cellular fractionation of working memory. *Proc. Natl Acad. Sci. USA* **93,** 13473–13480 (1996).
121. Sawaguchi, T. & Goldman-Rakic, P. S. D_1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* **251,** 947–950 (1991).
122. Nava, F., Carta, G. & Gessa, G. Permissive role of dopamine D_2 receptors in the hypothermia induced by Δ^9-tetrahydrocannabinol in rats. *Pharmacol. Biochem. Behav.* **66,** 183–187 (2000).
123. D'Souza, D. C. *et al.* Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Δ^9-tetrahydrocannabinol in humans. *Psychopharmacology (Berl.)* **198,** 587–603 (2008).
124. Tunbridge, E. M. *et al.* Genetic moderation of the effects of cannabis: catechol-*O*-methyltransferase (COMT) affects the impact of Δ^9-tetrahydrocannabinol (THC) on working memory performance but not on the occurrence of psychotic experiences. *J. Psychopharmacol.* **29,** 1146–1151 (2015).
125. Paule, M. G. *et al.* Chronic marijuana smoke exposure in the rhesus monkey. II: Effects on progressive ratio and conditioned position responding. *J. Pharmacol. Exp. Ther.* **260,** 210–222 (1992).
126. Campbell, I. The amotivational syndrome and cannabis use with emphasis on the Canadian scene. *Ann. NY Acad. Sci.* **282,** 33–36 (1976).
127. Howes, O. D. *et al.* The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch. Gen. Psychiatry* **69,** 776–786 (2012).

128. Voruganti, L. N., Slomka, P., Zabel, P., Mattar, A. & Awad, A. G. Cannabis induced dopamine release: an *in-vivo* SPECT study. *Psychiatry Res.* **107,** 173–177 (2001).
129. Mizrahi, R. *et al.* Stress-induced dopamine response in subjects at clinical high risk for schizophrenia with and without concurrent cannabis use. *Neuropsychopharmacology* **39,** 1479–1489 (2014).
130. Thompson, J. L. *et al.* Striatal dopamine release in schizophrenia comorbid with substance dependence. *Mol. Psychiatry* **18,** 909–915 (2013).
131. Leweke, F. M. *et al.* Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: Impact of cannabis use. *Schizophr. Res.* **94,** 29–36 (2007).
132. Leweke, F. M. *et al.* Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl. Psychiatry* **2,** e94 (2012).
133. Kearn, C. S., Blake-Palmer, K., Daniel, E., Mackie, K. & Glass, M. Concurrent stimulation of cannabinoid CB_1 and dopamine D_2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Mol. Pharmacol.* **67,** 1697–1704 (2005).
134. 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).
135. Parsons, L. H. & Hurd, Y. L. Endocannabinoid signalling in reward and addiction. *Nat. Rev. Neuroci.* **16,** 579–594 (2015).
Impaired endocannabinoid signalling dysregulates synaptic plasticity and increases stress responsivity, negative emotional states and cravings that propel addiction.
136. Lecca, S., Melis, M., Luchicchi, A., Muntoni, A. L. & Pistis, M. Inhibitory inputs from rostromedial tegmental neurons regulate spontaneous activity of midbrain dopamine cells and their responses to drugs of abuse. *Neuropsychopharmacology* **37,** 1164–1176 (2012).
137. Marinelli, S. *et al. N*-arachidonoyl-dopamine tunes synaptic transmission onto dopaminergic neurons by activating both cannabinoid and vanilloid receptors. *Neuropsychopharmacology* **32,** 298–308 (2007).

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