Time-Dependent Effects of Repeated THC Treatment on Dopamine D2/3R-mediated signalling in midbrain and striatum

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Abstract

This study examined the time-course of alterations in levels and functional sensitivities of dopamine D_{2/3} receptors (D_{2/3}R) during the course and up to 6 weeks following cessation of chronic treatment with Delta(9)-Tetrahydrocannabinol (THC) in rats. THC treatment led to an increase in D_{2/3}R levels in striatum, as assessed using [³H]-(+)-PHNO, that was readily observable after one week of treatment, remained stably elevated during the subsequent 2 weeks of treatment, but fully reversed within 2 weeks of THC discontinuation. THC-induced D_{2/3}R alterations were more pronounced and longer lasting in the dopamine cell body regions of the midbrain, wherein [³H]-(+)-PHNO binding was still elevated at 2 weeks but back to control values at 6 weeks after THC cessation. Parallel analyses of the psychomotor effects of pre- and post-synaptic doses of quinpirole also showed a pattern of D_{2/3}R functional supersensitivity indicative of more rapid subsidence in striatum than in midbrain following drug cessation. These results indicate that chronic THC is associated with a biochemical and functional sensitization of D_{2/3}R signaling, that these responses show a region-specific temporal pattern and are fully reversible following drug discontinuation. These results suggest that an increased post-synaptic D_{2/3}R function and a decreased DA presynaptic signaling, mediated by increased D_{2/3}R autoinhibition, may predominate during distinct phases of withdrawal and may contribute both to the mechanisms leading to relapse and to cannabinoid withdrawal symptoms. The different rates of normalization of D_{2/3}R function in striatum and midbrain may be critical information for the development of new pharmacotherapies for cannabis dependence.

Keywords.

Dopamine, THC, D_{2/3} receptors, behavioral sensitization, quinpirole

1. Introduction.

The reinforcing and addictive effects of virtually all drugs of abuse are thought to be mediated through activation of mesolimbic dopamine (DA) projections to the nucleus accumbens (NAcc), a major component of the ventral striatum. While the NAcc is important in the initial acquisition of drug-seeking, the transition from occasional to compulsive drug use, which is a core characteristic of addiction, may be associated with the progression of DA neuroadaptive changes from the ventral to the dorsal, nigrostriatal part, of the striatum, a region implicated in habit learning [1]. Chronic exposure to drugs of abuse can lead to persistent alterations in dopaminergic function that could be critical to the development and persistence of drug abuse. For example, chronic administration of cocaine or amphetamine in laboratory animals can decrease the density of dopamine D₂/D₃ receptors (D_{2/3}R) in striatum [2-6]. Positron emission tomography (PET) imaging studies in humans have repeatedly shown that subjects addicted to a variety of drugs of abuse, including cocaine, methamphetamine, heroin and alcohol exhibit subnormal levels of striatal D_{2/3}R receptors [7-14]. These reductions are observed both in the dorsal and ventral parts of the striatum and appear to persist for months after detoxification [15], indicating long-lasting drug-induced neuroadaptive changes in these regions.

Contrasting with what has been repeatedly observed with other drugs of abuse, neuroimaging studies investigating D_{2/3}R in subjects with cannabis abuse or dependence failed to reveal any alteration in striatal D_{2/3}R availability when compared to non-user controls [16-19]. Unlike addiction to other drugs, cannabis addiction does not seem to involve a decrease in striatal D_{2/3}R, thus suggesting that downregulated striatal dopamine receptor signaling does not play a prominent role in the neurochemical adaptations involved in the perpetuation of continued use of the drug. Although informative, all aforementioned imaging studies were performed using antagonist radiotracers such as [¹¹C]raclopride and [¹⁸F]fallypride, which bind with similar affinity to both D₂ (D₂R) and D₃ receptors (D₃R) [20, 21] and hence cannot inform us on the relative adaptive response of each receptor subtype to chronic drug abuse in certain regions. Moreover, as antagonist radiotracers do not discriminate between the high affinity (i.e., the D₃R and the active, G-protein coupled, form of the D₂R) and the low affinity (i.e., the non-active, G-protein uncoupled, form of the D₂R) D_{2/3}R [22, 23], information on the potential involvement of the high vs. low affinity state of the D₂R in drug abuse was limited. To overcome these limitations inherent to antagonists, agonist D_{2/3}R radioligands such as [¹¹C]-propyl-9-hydroxynaphthoxazine ([¹¹C]-(+)-PHNO) have been developed [24]. [¹¹C]-(+)-PHNO has a 20- to 50-fold selectivity for D₃R over D₂R in vivo [25] and allows the concurrent measurement of somatodendritic D₃R in the region of substantia nigra and ventral tegmental area (SN/VTA), where D₃R is predominant, and of D₂R in the dorsal striatum where this receptor subtype predominates [26-28].

Recent PET studies using [¹¹C]-(+)-PHNO revealed elevated levels of D₃R availability in the SN/VTA of cocaine [29, 30] and methamphetamine polydrug [31] users, suggesting that a supersensitivity of the somatodendritic D₃ autoreceptor system could be involved in addiction and addiction-related behaviors. Moreover, and in contrast to studies performed using D_{2/3}R antagonists, [9, 12], no deficit in D_{2/3}R were found in the striatum using [¹¹C]-(+)-PHNO [29-31]. This failure of detecting striatal D_{2/3}R deficits with [¹¹C]-(+)-PHNO suggests that while the overall expression of D₂R may decrease in striatum, either the

expression of D₃R or the high affinity (and hence, the sensitivity) of D₂R may increase following chronic psychostimulant exposure. Very few studies have examined [¹¹C]-(+)-PHNO binding in cannabis abuse or dependence but a study performed in chronic cannabis users reported elevated D_{2/3}R availabilities in striatum as compared to non-user controls [32]. However, as this study used a sensorimotor control task during PET scanning, D_{2/3}R measurements might have been confounded by potential group differences in task-induced changes in endogenous DA. Similarly, evidence in rodents indicate that chronic exposure to Delta(9)-tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis, is associated with increased densities of D_{2/3}R both in SN/VTA and striatum as measured with [³H]-(+)-PHNO [33], indicating an upregulation of D₃R and/or increase expression of the high affinity D₂R.

To date, thus, available data support the hypothesis that chronic use of drugs including psychostimulants and cannabis is associated with elevated levels of D₃R availability in the SN/VTA together with an increase expression of the high affinity D₂R in striatum despite no change or even a reduction in the overall expression of D₂R in this region. Such subtle alterations in D₂R and/or D₃R functioning within the mesolimbic circuitry may contribute to the development of behavioral sensitization upon repeated exposure to these drugs, an effect that is thought to play an important role in the incentive motivational properties of drugs and persistence of drug seeking [34]. Consistent with this view, rats chronically exposed to drugs such as cocaine [35], heroin [36] or THC [33] are hypersensitive to the psychomotor effect of direct acting D_{2/3}R agonists such as quinpirole. With respect to drugs such as cocaine or even heroin, this D_{2/3}R hypersensitivity declines over time following drug withdrawal with a different temporal profile depending on the drug. While D_{2/3}R hypersensitivity is still observed, albeit attenuated, at 3 weeks withdrawal from cocaine, it is no longer apparent at 3 weeks withdrawal for heroin [36]. These time-dependent changes in D_{2/3}R sensitivity moreover parallels temporal changes in D_{2/3}R-mediated drug-seeking behaviors [36, 37], thus supporting a different time-dependent role of D_{2/3}R mechanisms in relapse to drugs such as heroin and cocaine. To date though, no studies have investigated the temporal profile of the effects of chronic THC exposure on D_{2/3}R availability and functional sensitivity. The length of time needed for recovery of D_{2/3}R-mediated signaling, and even whether recovery takes place at all, may be critical information for tailoring more effective pharmacotherapies for patients with cannabis dependence.

The present study investigated brain region-specific changes in the pattern and magnitude of D_{2/3}R levels during the course and up to 6 weeks following cessation of chronic THC exposure. D_{2/3}R availabilities were measured in the DA cell body regions of the midbrain, the ventral tegmental area (VTA) and substantia nigra (SN), and in their respective striatal terminal fields, the NAcc and caudate putamen (CPu), using the D_{2/3}R agonist radiotracer [³H]-(+)-PHNO. The effect of THC treatment on the psychomotor effects of the D_{2/3}R agonist quinpirole, used at pre- and post-synaptic doses, was also examined in parallel to determine whether regional changes in [³H]-(+)-PHNO binding are associated with temporal changes in behavioral sensitivity to D_{2/3}R-mediated events. The present study thus provides an examination of the D_{2/3}R neuroadaptive changes that occur over time and across distinct brain regions during and following a three-weeks treatment with THC.

2. Materials and Methods.

2.1. Animals and treatments

All animal experiments were performed in accordance with the Swiss Federal Law on animal care and received formal approval from the Cantonal Veterinary Office of Geneva. Young adult male Sprague-Dawley weighing 250–275 g (Janvier, Le-Genest-Saint-Isle, France) were pair-housed in a temperature-controlled environment under a 12-hour light/dark cycle with *ad libitum* access to food and water. THC (Tocris Biosciences, UK) was dissolved in 0.9% saline/ethanol/cremophor (18:1:1) and administered intraperitoneally (i.p.) at 1 mg/kg/day. Control rats received the vehicle (VEH) solution. The effects of THC were measured at 24h following cessation of a 1-week, 2-weeks or 3-weeks daily treatment with THC or VEH (designated as 1WT, 2WT and 3WT, respectively). Measurements were also performed at 1-week, 2-weeks or 6-weeks after cessation of a 3-weeks daily treatment with THC (designated as 1WC, 2WC and 6WC, respectively).

Quinpirole hydrochloride (Tocris Biosciences, UK) was administered i.p. at 0.05 or 0.5 mg/kg.

2.2. *Ex vivo* autoradiography of [³H]-(+)-PHNO binding

Measurements of [³H]-(+)-PHNO binding were performed at 1WT, 2WT and 3WT and at 1WC, 2WC and 6WC in THC- and VEH-treated rats (n=4 per group and per time point). Rats were injected intravenously with 3 MBq of [³H]-(+)-PHNO (1.7 GBq/μmol; Moravek, USA) and killed by decapitation at 60 minutes after radiotracer injection. Two series of 30μm adjacent coronal sections covering the rostrocaudal extent of CPu, NAcc, SN and VTA were cut on a cryostat. The first section was stained for acetylcholinesterase and used as histological reference. The second section was exposed for 55-days onto β-sensitive phosphor imaging plates (Fuji Film BAS-TR 2025) along with tritium activity standards (³H-microscale; Amersham). Autoradiograms were analyzed with the Fujifilm BAS-1800II phosphorimager using Aida Software V4.06 (Raytest Isotopenmessgeräte GmbH). Regions-of-interest (ROIs) were delineated for CPu, NAcc, SN, VTA and cerebellum on histological references and transferred onto adjacent autoradiographic sections of [³H]-(+)-PHNO binding. Background film signal values were subtracted from the ROI signal and a mean radioactivity value was calculated for each ROI. The densitometric measurements fell within the linear range of the standard curves generated from the ³H-radioactive scale in all ROIs examined. *Ex vivo* binding in target ROIs was quantified using the specific binding ratio (SBR), which is defined as $SBR = ([^3H]\text{-}(+)\text{-PHNO binding in target ROI} - [^3H]\text{-}(+)\text{-PHNO binding in cerebellum}) / [^3H]\text{-}(+)\text{-PHNO binding in cerebellum}$, and where cerebellar cortex is used as reference to estimate free and nonspecifically bound radiotracer.

2.3. Effects of THC on locomotor response to quinpirole

Locomotor activity was measured using transparent 48x48x40 cm open field chambers (ActiMot, TSE Systems, Bad Homburg, Germany), each one containing three sets of 16 beams each: two sets in the x–y axis to measure horizontal activity and one set in the z-axis to monitor rears.

Low doses of DA agonists such as the D₂R agonist quinpirole are known to inhibit locomotion [38], presumably through the stimulation of presynaptic D₂-autoreceptors, notably those present on midbrain DA neurons [39, 40]. Conversely, high doses of quinpirole are known to activate locomotion, presumably through the stimulation of postsynaptic D₂R in NAcc [41, 42].

To determine the effects of chronic THC on the locomotor response to a pre-synaptic dose of quinpirole [0.05 mg/kg; 38], rats were tested on two consecutive days. On the day of their last scheduled injection of chronic THC or VEH treatment, and prior to their injection, rats were given an i.p. administration of saline and immediately placed in the open field for 90 min to measure their baseline locomotor activity. The following day, rats received quinpirole (0.05mg/kg; i.p.) instead of saline and were immediately placed in the open field for 90 min. The locomotor response to the pre-synaptic dose of quinpirole was measured at 1WT, 3WT, 2WC and at 6WC in THC- and VEH-treated rats (n=8 per group and per time point).

To determine the effects of chronic THC on the locomotor response to a post-synaptic dose of quinpirole [0.5 mg/kg; 38], rats were tested on two consecutive days. On the day of their last scheduled injection of chronic THC or VEH treatment, and prior to their injection, rats were placed in the open-field for 45min of habituation. Animals then received an i.p. injection of saline as a mild stressor and were monitored for an additional 90min to measure their baseline locomotor activity. The following day, the same recording procedure was repeated using a quinpirole injection (0.5mg/kg; i.p.) instead of saline. The locomotor response to the post-synaptic dose of quinpirole was measured at 1WT, 3WT, 1WC and at 2WC in THC- and VEH-treated rats (n=8 per group and per time point).

Horizontal locomotor activity, expressed as distance travelled (in meters), and exploratory vertical activity (ie. number of rearings) were measured for the entire post-injection time period. As a complementary measure, the behavioural pattern of the animal activity in the open-field was also examined by measuring the time spent in the center of the open-field. This latter measure can be related to locomotor exploration but can also be associated with some aspect of emotionality, including anxiety [43, 44].

2.4. Statistics

[³H]-(+)-PHNO binding in VEH-treated rats was analyzed using a 2-factor mixed ANOVA with *test day* (1WT, 2WT, 3WT, 1WC, 2WC and 6WC) as between-subject factor and brain region as within-subject factor. Differences in [³H]-(+)-PHNO binding between VEH- and THC-treated rats were analyzed using a 3-factor mixed ANOVA with treatment (VEH or THC) and *test day* as between-subject factors and brain region as within-subject factor. Differences in percent changes in [³H]-(+)-PHNO binding induced by THC as compared to VEH were assessed among brain regions with a 2-factor mixed ANOVA with duration of treatment (1WT, 2WT and 3WT) as between-subject factor and brain region as within-subject factor. Behavioral data were analyzed with a 3-way ANOVA with chronic treatment (THC or VEH), acute challenge (saline or quinpirole) and *test day* as between-subject factors. When significant differences were detected, LSD *post hoc* tests were used to compare groups. Significance was defined as p<0.05. Data are presented as mean ± SEM.

3. Results

3.1. THC increased D_{2/3}R availabilities

Regional brain differences in [³H]-(+)-PHNO binding were observed in VEH-treated rats ($F_{3,51}=1149$; $p<0.001$). [³H]-(+)-PHNO binding, which is used as an index of D_{2/3}R availability, was highest in CPu (SBR=5.33 ±0.12), intermediate in NAcc (SBR=2.63 ±0.06) and lowest in both SN (SBR=0.44 ±0.02) and VTA (SBR=0.43 ±0.02; Fig. 1). Importantly, [³H]-(+)-PHNO binding measured in each of the different brain regions was stable across the different test days over the course of VEH-treatment (treatment duration: $F_{5,51}=0.03$; $p>0.05$; treatment duration x brain region: $F_{15,51}=0.29$; $p>0.05$).

THC treatment had a significant effect ($F_{1,96}=17.46$; $p<0.001$) on [³H]-(+)-PHNO binding. This effect was dependent both on the brain region (brain regions*THC effect: $F_{3,96}=2.73$; $p<0.05$) and day of testing (day of testing*THC effect: $F_{5,96}=2.63$; $p<0.05$). Importantly, ANOVA revealed a significant interaction of treatment x test day x brain region ($F_{15,96}=1.86$; $p<0.05$). When compared with VEH, a 1-week treatment (1WT) with THC was sufficient to increase D_{2/3}R availabilities in CPu (+18%; $p<0.05$; Fig. 1A), NAcc (+20%; $p<0.01$; Fig. 1B) and VTA (+37%; $p<0.01$; Fig. 1D). In SN, increase D_{2/3}R availabilities were only apparent at 2WT (+40%; $p<0.01$; Fig. 1C). Furthermore, there was an overall brain region effect on the magnitude of THC effect on [³H]-(+)-PHNO binding during the 3-weeks of chronic treatment ($F_{3,27}=6.74$; $p<0.01$), independently of the test day (test day: $F_{2,27}=1.25$; $p>0.05$; interaction : $F_{6,27}=0.85$; $p>0.05$). Overall, during treatment, THC-induced increases in [³H]-(+)-PHNO binding were higher in VTA than in NAcc ($p<0.01$) or CPu ($p<0.001$) on one hand, and in SN as compared to CPu ($p<0.05$) on the other hand, thus indicating a more pronounced effect of the drug on D_{2/3}R availabilities in the cell bodies of DA neurons than in their terminal areas (Fig. 1).

Following cessation of a 3-week chronic THC treatment, D_{2/3}R levels were back to vehicle control values in CPu and NAcc at 1WC and 2WC, respectively (Fig. 1A, B). In marked contrast, recovery of D_{2/3}R availabilities was only observed at 6WC in both SN and VTA (Fig. 1C, D).

3.2. THC transitorily increased the functional activity of pre-synaptic D_{2/3}R.

To investigate the relationship between the availability and functional activity of presynaptic D_{2/3}-autoreceptors, the locomotor response to a presynaptic dose of quinpirole (0.05 mg/kg) was measured at four key stages of THC-induced alterations in midbrain D_{2/3}R availability: at the onset of changes (1WT), after chronic treatment (3WT), and after cessation of drug exposure, when changes were persistent (2WC) or abolished (6WC).

Three-way ANOVA indicated main effects of quinpirole ($F_{1,112}=348.3$; $p<0.001$) and day of testing ($F_{3,112}=7.07$; $p<0.001$) on locomotion and a significant interaction between chronic treatment, acute challenge and test day ($F_{3,112}=2.83$; $p<0.05$). Post-hoc tests revealed that, when challenged with saline, the locomotor activity of THC-treated rats did not differ from that measured in the VEH-treated control group, irrespective of the day of testing ($p>0.05$; Fig. 2A-D). When compared with saline, challenge with a presynaptic dose of quinpirole significantly reduced locomotion ($p<0.001$) in both VEH- and THC-treated

rats, irrespective of the testing day. At 1WT, the locomotor suppressive effect of quinpirole was similar between both treatment groups (Fig. 2A). However, at 3WT (Fig. 2B), quinpirole had a significantly ($p<0.05$) higher effect in THC- (-64%) than in VEH-treated rats (-47%), suggesting a THC-induced functional supersensitivity of pre-synaptic $D_{2/3}R$. This enhanced behavioral responsiveness of THC-treated rats to quinpirole persisted at 2WC (Fig. 2C) but had resolved by 6WC (Fig. 2D).

Quinpirole also reduced the number of rearings ($F_{1,112}=208.9$; $p<0.001$; Supplemental Fig. 1A-D), but THC treatment did not affect the magnitude of this inhibition at any of test days during or following cessation of treatment (chronic treatment x acute challenge: $F_{1,112}=0.01$, $p>0.05$; chronic treatment x test day: $F_{1,112}=0.28$, $p>0.05$; chronic treatment x acute challenge x test day: $F_{3,112}=0.31$, $p>0.05$). Similarly, quinpirole reduced the time spent at the center of the open field ($F_{1,112}=12.47$; $p<0.001$; Supplemental Fig. 1E-H) but this effect was independent of both the chronic treatment group and the test day (chronic treatment x acute challenge: $F_{1,112}=0.01$, $p>0.05$; chronic treatment x test day: $F_{1,112}=0.77$, $p>0.05$; chronic treatment x acute challenge x duration of treatment interaction: $F_{3,112}=0.34$, $p>0.05$).

3.3. THC transitorily increased the functional activity of post-synaptic $D_{2/3}R$.

To investigate the relationship between the availability and functional activity of post-synaptic D_2R in NAcc, the locomotor response to a postsynaptic dose of quinpirole (0.5 mg/kg) was measured at four key stages of THC-induced alterations in striatal $D_{2/3}R$ availability: at the onset of changes (1WT), after chronic treatment (3WT), and after cessation of drug exposure, when changes were persistent (1WC) or abolished (2WC).

Three-way ANOVA indicated main effects of quinpirole ($F_{1,112}=115.4$; $p<0.001$) and chronic treatment ($F_{1,112}=13.31$; $p<0.001$). Importantly, the three-way interaction between chronic treatment, acute challenge and test day was significant ($F_{3,112}=3.17$; $p<0.05$). Post-hoc tests indicated that THC treatment did not alter the locomotor response to a challenge dose of saline, irrespective of the testing day ($p>0.05$; Fig. 3A-D). When compared with saline, challenge with a postsynaptic dose of quinpirole significantly increased locomotion ($p<0.001$) in both VEH- and THC-treated rats, irrespective of the testing day (Fig. 3A-D). At 1WT, the locomotor activating effect of quinpirole was similar between the THC and VEH groups ($p>0.05$; Fig. 3A). In contrast, at 3WT, THC-treated rats displayed a significant ($p<0.001$) higher locomotor response to quinpirole (+505%; Fig. 3B) than VEH-treated animals (+110%; $p<0.05$; Fig. 3B), suggesting a THC-induced functional supersensitivity of post-synaptic D_2R . This higher locomotor activating effect of quinpirole in THC- than in VEH-treated rats was still observed at 1WC ($p<0.001$; Fig. 3C) but had resolved by 2WC ($p>0.05$; Fig. 3C).

Analyses also indicated a significant main effect of quinpirole on the number of rearings ($F_{1,112}=74.63$; $p<0.001$), and a significant interaction between chronic treatment, acute challenge and test day ($F_{3,112}=2.71$; $p<0.05$). Post-hoc tests showed that the number of rearings was not different between the THC- and VEH-treated groups when rats were challenged with saline (Supplemental Fig. 2A-D). In contrast, when challenged with a postsynaptic dose of quinpirole, THC-treated rats displayed a significant higher locomotor response to the drug than VEH-treated animals both at 3WT ($p<0.001$; Supplemental Fig. 2B) and at 1WC

($p < 0.001$; Supplemental Fig. 2C) but not at 1WT ($p > 0.05$; Supplemental Fig. 2A) or at 2WC ($p > 0.05$; Supplemental Fig. 2D).

Similarly, quinpirole increased the time spent at the center of the open field ($F_{1,112}=244.33$; $p < 0.001$), and this effect was dependent of the chronic treatment group (chronic treatment x acute challenge: $F_{1,112}=5.13$, $p < 0.05$). However, the three-way interaction between chronic treatment, acute challenge and test day was not significant ($F_{3,112}=2.20$; $p > 0.05$). Post-hoc tests indicated that response to the saline challenge was similar between THC- and VEH-groups (Supplemental Fig. 2E-H). In contrast, and when compared with the saline session, the time spent at the center of the open field after quinpirole was increased in both the THC- and VEH treated groups ($p < 0.001$), but the elevation was significantly higher ($p < 0.001$) in THC- than in VEH-treated animals (Supplemental Fig. 2E-H).

4. Discussion

The present study confirms and extends previous work from our group [33] on the effects of chronic THC treatment on $D_{2/3}R$ signaling to show that both a biochemical and functional sensitization of $D_{2/3}R$ signaling occurs with chronic THC and, further, that these responses show a region-specific temporal pattern in midbrain and striatum and are fully reversible following discontinuation from the drug. Indeed, increases in [3H]-(+)-PHNO binding were readily observable in the striatum after one week of treatment, remained stably elevated during the subsequent weeks of treatment, but fully reversed within 2 weeks of THC discontinuation. THC-induced $D_{2/3}R$ alterations were more pronounced and longer lasting in the DA cell body regions of the midbrain, wherein [3H]-(+)-PHNO binding was still elevated at 2 weeks but back to control values within 6 weeks after THC cessation. Parallel analyses of the psychomotor effects of quinpirole also showed a pattern of $D_{2/3}R$ functional supersensitivity indicative of more rapid subsidence in the striatal than in the midbrain area following discontinuation of the drug. These data provide novel insight into the potential region specific and time-dependent brain mechanisms that may underlie cannabis seeking-behaviors.

As aforementioned, previous work has indicated that [3H]-(+)-PHNO binding can be considered to be mainly specific for D_3R in the midbrain region of the SN/VTA, while binding in striatum mainly reflects binding to high affinity D_2R . Consequently, the observed elevated [3H]-(+)-PHNO signals suggest that chronic THC exposure leads to a region-specific up-regulation of the high affinity D_2R and of the D_3R in striatum and midbrain, respectively. Another plausible explanation is that the increased [3H]-(+)-PHNO signal results from decreased concentrations of endogenous dopamine leading following THC. Indeed, one well-described binding characteristic of agonist radiotracers, including the equivalent radiotracer used in PET, [^{11}C]-(+)-PHNO, is that it primarily binds to the high affinity, functionally active form of the $D_{2/3}R$. DA also primarily binds to this form of the receptor. With both ligands competing for binding to the same target, [3H]-(+)-PHNO is highly sensitive to competition with endogenous dopamine [24, 45]. Decreased basal levels of endogenous DA may thus be an explanation for the increased [3H]-(+)-PHNO binding observed at 24 hours withdrawal from a 1-week treatment with THC in spite of the absence of significant change in the locomotor response to quinpirole in THC- versus saline-treated rats at this time point. This hypothesis is supported by the finding that the spontaneous firing activity of midbrain DA neurons is reduced when measured at 24 hours withdrawal from a similar short-lasting schedule (ie. 1-week) of THC exposure [46]. At 3-weeks of THC treatment though, increases in [3H]-(+)-PHNO binding in both midbrain and striatum were associated with an increased responsiveness to both a presynaptic and a postsynaptic dose of quinpirole, indicating that at that time, repeated THC has resulted in a robust sensitization of both presynaptic and postsynaptic $D_{2/3}R$ -mediated effects. Remarkably, both processes followed a similar time course following discontinuation of the drug, with striking parallel changes being observed in the normalization of [3H]-(+)-PHNO binding in striatum and midbrain on the one hand, and in the evolution of the psychomotor response to pre- and postsynaptic doses of quinpirole during the same period on the other hand. The finding that chronic treatment with THC produced a similar time-course for recovery of $D_{2/3}R$

receptor densities and function suggests that this prolonged adaptive response is a fundamental property of D_{2/3}R receptor regulation. Moreover, the different time-course of D_{2/3}R changes observed in midbrain and striatum suggest that D_{2/3}R regulation by THC is regionally dependent.

Increased D_{2/3}R autoinhibition on midbrain DA neurons may have an important role in regulating the mesolimbic dopaminergic output during THC withdrawal. Indeed, THC-induced supersensitization of D_{2/3}R in midbrain was more sustained and outlasted that measured in striatum, likely leading to prolonged blunting of presynaptic DA function following THC cessation. Indeed, D_{2/3}R in midbrain act as autoreceptors, which function to retroinhibit impulse flow, synthesis and release rates of DA neurons [47]. Consistent with an increased D_{2/3}R-mediated autoinhibition of midbrain DA neurons, chronic THC treatment has been associated with a reduction of midbrain DA neuron activity [46], a decreased gene expression of tyrosine hydroxylase, the rate-limiting enzyme in DA biosynthesis, in midbrain and a blunted locomotor response to the presynaptic DA releaser amphetamine [33], thus suggesting a presynaptic hypodopaminergic functioning with chronic THC exposure. Further support for a THC-induced hypodopaminergic state comes from neuroimaging studies showing that cannabis-dependent users exhibit a reduced presynaptic capacity for DA synthesis in striatum [48] and a reduced release of striatal DA following amphetamine [49]. The observation here of a slower recovery of D_{2/3}R from supersensitization in midbrain than in striatum suggests that during the early phase of THC cessation, supersensitivity of post-synaptic D_{2/3}R in striatum may counteract a diminished presynaptic DA function. Thus although DA release in striatum, and likely also DA levels, are decreased, the enhanced sensitivity of striatal D_{2/3}R during the early phase of withdrawal could result in enhanced behavioral responses to drug-associated cues or stress and contribute to relapse of drug taking. However, with more extended period of withdrawal and the subsidence of striatal D_{2/3}R supersensitivity, a net depression of mesolimbic DA function could predominate and may underlie the anhedonia and dysphoric mood observed after discontinuation of chronic cannabis use [50, 51].

The mechanism by which D_{2/3}R-mediated signalling is sensitized after chronic THC exposure remains unknown but may be mediated by a functional interaction between the endocannabinoid CB₁ receptor (CB₁R), the main receptor target of THC in brain, and the D_{2/3}R systems. Within the striatum, CB₁R colocalize with D_{2/3}R on the medium spiny neurons [52] where they appear to antagonize D₂R signaling [53, 54] and to regulate dopamine D_{2/3}R availability. For instance, studies using genetic knock-out of the CB₁R or D₂R gene in mice have shown that the chronic deletion in either receptor resulted in up-regulated expression of the other receptor in striatum [55, 56]. Besides altering D_{2/3}R function, chronic exposure to THC has been shown to produce reversible desensitization and downregulation of striatal CB₁R that peaked at 24 hours after THC treatment and then normalized towards control value within 2 weeks after THC cessation in rodents [57, 58]. Similar to its effects in rodents, chronic cannabis exposure downregulates striatal CB₁R in daily cannabis users, and this downregulation is reversible within 4 weeks of abstinence [59]. Chronic THC thus induces a time-dependent, reversible, agonist-induced-CB₁R desensitization that, due to a CB₁R/D₂R antagonistic interaction, may lead to an inversely related D₂R hypersensitization in striatum.

Although multiple neuronal systems are involved in drug seeking and relapse, sensitization processes within the mesocorticolimbic DA reward circuitry play an important role in these phenomena [34]. Notably,

a supersensitive response to D₂R stimulation may be important for enabling drug-seeking behaviors [36, 37]. As observed here with THC, previous studies in rodents have shown increases in the number of functional striatal D_{2/3}R and/or increases in the behavioral response to direct D_{2/3}R stimulation following treatment with drugs such as cocaine [60] [36], amphetamine [61, 62], alcohol [63], and heroin [36, 37]. However, the temporal subsidence of striatal D_{2/3}R functional supersensitivity following drug cessation appears to be variable among different drugs, with psychostimulants such as cocaine and amphetamine [36, 62, 64] still showing supersensitivity even after a 1-month of abstinence whereas for other drugs such as heroin [36] and now THC, this supersensitivity does not last more than 2 weeks after abstinence. Thus, although the development of a D_{2/3}R functional supersensitivity in striatum seems to represent a common neuroadaptation to a number of drugs of abuse, recovery of these sensitized responses greatly differ among different classes of drugs. Due to the fast recovery of striatal D_{2/3}R function upon THC cessation, activation of D_{2/3}R in striatum may be an important molecular substrate mediating drug-seeking and relapse of cannabis use during early but not late withdrawal. Further studies are however needed to confirm this view.

One limitation of the present study is that THC was administered to the animals by the experimenter, and the possible stress induced by the repeated injections may have affected the rodents' behavior and potentially have confounded the results. However, to control for injection stress, rats in the control group received an equal volume of normal saline, thus allowing to assess the specific effects of chronic THC exposure on brain and behavioral measures. Another limitation of this study is its relatively short duration. Rats were exposed to THC for 21 days, which might seem short relative to months or years of cannabis use in dependent human individuals. Therefore, it is unclear whether the observed effects would be more sustained with longer term of THC exposure. Similarly, it should be noted that only one dose (1 mg/kg) of THC was tested. Although THC at this dose is psychoactive, as it enhances brain stimulation reward [65] and produces rewarding effects in the place preference paradigm [66, 67], it is possible that greater long-term effects may have occurred if a higher, or perhaps lower, dose of THC had been tested. Finally, one limitation of [³H]-(+)-PHNO is its lack of receptor selectivity for D₂R vs. D₃R, that can render interpretation of binding data in brain regions containing both D₂R and D₃R such as the NAcc difficult. Despite these limitations, our findings still provide important insights on the effects of THC exposure on D_{2/3}R function.

In conclusion, chronic exposure to THC resulted in time-dependent and regionally-specific supersensitization of D_{2/3}R-mediated signaling in the DA systems projecting from the ventral mesencephalon to the striatum. These neuroadaptations were reversible but persisted up to several weeks after treatment was discontinued, with D_{2/3}R function returning to vehicle control levels more rapidly in striatum than in midbrain. Taken together, these results suggest that increased post-synaptic D_{2/3}R function and decreased DA presynaptic signaling, mediated by increased D_{2/3}R autoinhibition, may predominate during distinct phases of withdrawal and may contribute, at least in part, both to the mechanisms leading to relapse and to cannabinoid withdrawal symptoms. The different rates of normalization of D_{2/3}R function in striatum and midbrain may be critical information for the development of new pharmacotherapies for cannabis dependence.

6. Conflict of interest

The authors declare no conflict of interest.

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7. Figure legends

Fig. 1. Time-course of the effects of chronic THC on D_{2/3}R levels.

Measurements of D_{2/3}R levels, using *ex vivo* binding of [³H]-(+)-PHNO, were performed at 24h after cessation of a 1-week, 2-weeks or 3-weeks treatment with THC or vehicle (indicated as 1WT, 2WT and 3WT, respectively), and at 1-week, 2-weeks and 6-weeks after cessation of a 3-weeks treatment with THC or vehicle (indicated as 1WC, 2WC and 6WC, respectively). Data are shown as mean $\pm$ SEM (n=4/group) in the caudate/putamen (CPu, A), nucleus accumbens (NAcc, B), substantia nigra (SN, C) and ventral tegmental area (VTA, D). Significantly different from the vehicle-treated group at *p < 0.05, **p < 0.01 and ***p < 0.001 using a repeated two-way ANOVA followed by a LSD *post hoc* test.

Fig. 2. Time-course of the effects of chronic THC on functional sensitivity of pre-synaptic D₂R.

Effects of a presynaptic dose of quinpirole (0.05mg/kg) on locomotor activity were measured at 24h after cessation of a 1-week (A) or 3-weeks (B) treatment with THC or VEH, and at 2-weeks after cessation (C) or 6-weeks after cessation (D) of a 3-weeks treatment with THC or VEH. Locomotor activity is illustrated as the total distance (in meters) travelled during the 120min post-drug time period. Data are presented as mean $\pm$ SEM (n=8). Significantly different from the VEH-treated group at *p < 0.05, and significantly different from the respective saline-treated group at †††p < 0.001 using a three-way ANOVA followed by a LSD *post hoc* test.

Fig. 3. Time-course of the effects of chronic THC on functional sensitivity of post-synaptic D₂R.

Effects of a postsynaptic dose of quinpirole (0.5mg/kg) on locomotor activity were measured at 24h after cessation of a 1-week (A) or 3-weeks (B) treatment with THC or VEH, and at 1-week after cessation (C) or 2-weeks after cessation (D) of a 3-weeks treatment with THC or VEH. Locomotor activity is illustrated as the total distance (in meters) travelled during the 90min post-drug time period. Data are presented as mean $\pm$ SEM (n=8). Significantly different from the VEH-treated group at ***p < 0.001 and significantly different from the respective saline-treated group at †p < 0.05, ††p < 0.01, †††p < 0.001 using a three-way ANOVA followed by a LSD *post hoc* test.

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Figure 1

Figure 2

Figure 3