Δ^9 -Tetrahydrocannabinol Self-Administration Induces Cell-Type Specific Adaptations and cFOS Expression in the Nucleus Accumbens Core

Daniela Neuhofer (✉ neuhofer@musc.edu)

Medical University of South Carolina <https://orcid.org/0000-0001-5726-7855>

Constanza Garcia-Keller

Medical University of South Carolina <https://orcid.org/0000-0002-3141-9571>

Madeline Hohmeister

Medical University of South Carolina

Kailyn Seidling

Medical University of South Carolina

Lauren Beloate

Medical University of South Carolina

Vivian Chioma

Medical University of South Carolina

Sade Spencer

University of Minnesota

Peter Kalivas

Medical University of South Carolina <https://orcid.org/0000-0001-9487-0119>

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Abstract

Given that 30% of chronic cannabis users develop cannabis use disorder (CUD), it is critical to identify neuroadaptations that contribute to this disease. The nucleus accumbens core (NAcore) is important for drug seeking and ~ 90% of all NAcore neurons are divided into D1- and D2-medium spiny neurons (MSNs) that serve opposing roles in drug seeking. Drugs of abuse induce D1- and D2-MSN specific adaptations but whether Δ^9 -tetrahydrocannabinol (THC) use initiates similar neuroadaptations is unknown. D1- and D2-Cre transgenic rats were transfected with Cre-dependent reporters and trained to self-administer THC + cannabidiol (THC + CBD). After extinction training dendritic spine morphology, glutamate transmission, CB1R function and cFOS expression were quantified. We found that extinction from THC + CBD induced a loss of large spine heads in D1- but not D2-MSNs and a commensurate reduction in glutamate synaptic transmission. Also, CB1R function was impaired on glutamatergic synapses onto D1-MSNs and this was paralleled by an augmented capacity to potentiate glutamate transmission in D1-MSNs. CB1R function and glutamate synaptic transmission on D2-MSN synapses were unaffected by THC + CBD use. Using cFOS expression as an activity marker, we found that D1-MSNs activity remained unchanged after extinction from THC + CBD but significantly increased after 60 minutes cue-induced drug seeking. Surprisingly, the percentage of D2-MSNs expressing cFOS decreased after extinction from THC + CBD and this decrease was restored by drug cues. Thus, glutamatergic adaptations in D1-MSNs partially predict activity changes and pose modulating CB1R function that is down-regulated selectively at D1-MSN synapses as a potential treatment strategy for CUD.

Introduction

Cannabis is used for therapeutic and recreational purposes¹ and is rapidly being legalized around the globe with more than 190 million users². Approximately 30% of chronic cannabis users will develop cannabis use disorder (CUD)³, which is characterized by escalated use, failure to quit, craving, withdrawal, negative affect and high risk for relapse⁴.

Δ^9 -tetrahydrocannabinol (THC) is the major psychoactive component of cannabis and its primary pharmacological effect is as a partial agonist at the cannabinoid receptor 1 (CB1R)⁵. The activation of presynaptic CB1R inhibits neurotransmitter release, and can induce presynaptic long-term depression (LTD)⁶ and metaplasticity^{7,8}. Chronic THC exposure causes functional tolerance of CB1R in the striatum and nucleus accumbens⁹⁻¹⁴. Chronic THC also induces adaptations beyond CB1 and the endocannabinoid system⁴, including adaptations at glutamatergic synapses in the prefrontal cortex (PFC)¹⁵ and nucleus accumbens (NAc)^{9,16,17}. Whether these adaptations contribute to transient withdrawal-induced changes in network activity or are enduring allostatic responses to drug use is not well understood¹⁸.

The NAc core is an essential component of the mesocorticolimbic system and plays a prominent role in mood, motivation and reward¹⁹. Within the nucleus accumbens adaptations that disrupt glutamate

homeostasis are cardinal changes that drive drug seeking after the chronic use of drugs such as cocaine, nicotine and heroin^{20,21}. For example chronic heroin and cocaine decrease the function of mGluR2/3^{22,23}, a presynaptic metabotropic glutamate autoreceptor that regulates corticostriatal glutamate release²⁴. The restoration of mGluR2/3 signaling reverses heroin induced metaplasticity²⁵ and inhibits reinstated drug seeking^{26,27}. Similar to mGluR2/3, activation of presynaptic CB1 causes synaptic inhibition²⁸. Hence dysfunction of CB1R in the nucleus accumbens could promote glutamate release, cause presynaptic disinhibition in response to drug cues and thereby facilitate drug seeking.

GABAergic D1- and D2-medium spiny neurons (MSNs) together comprise 90–95% of all neurons in the NAc²⁹, express the D1 or the D2 dopamine receptor respectively and have opposing roles in and reward behaviors^{30–34}. Studies using cell type specific expression of activity indicators such as cFos immunoreactivity or cell selective opto- or chemogenetic stimulation in the nucleus accumbens demonstrate that D1 MSNs promote and D2 MSNs inhibit reward behaviors^{30,31}. Distinct MSN-type specific adaptations after chronic cocaine and morphine have been described that bias towards D1-MSN signaling after drug exposure^{32,35,36}. For example, withdrawal from non-contingent cocaine and morphine generates silent synapses in the nucleus accumbens through opposing cellular mechanisms. Cocaine induces silent synapses in D1 MSNs via synaptogenesis, whereas morphine induces silent synapses in D2 MSNs via internalization of AMPARs from established synapses. that mature into new synapses on D1-MSNs after cocaine but are pruned on D2-MSN after morphine³⁶. Although mechanistically distinct, both of these neuroadaptations are thought to bias network activity towards D1-MSNs signaling and hence augment reward seeking behavior. Using an intravenous self-administration (SA) model of THC in combination with another phytocannabinoid cannabis cannabidiol (CBD), which has been shown to be anxiolytic and to neutralize some of the aversive effects of THC⁴, we recently found that THC + CBD self-administration (SA) and extinction causes a loss of spines and a desensitization of CB1R that causes a loss of long term synaptic plasticity in NAc neurons^{16,17}. Here we investigate whether these THC + CBD induced neuroadaptations are cell-type specific and drive changes in neuronal activity.

To investigate these questions, we used our model of THC + CBD SA¹⁶ in D1- and D2-Cre transgenic rats that were transfected with Cre-dependent reporters to specifically label D1- and D2-MSNs. Rats underwent THC + CBD SA, followed by extinction training and cue-induced reinstatement, and were evaluated for changes in D1- and D2-MSN specific morphology, CB1 function, glutamatergic transmission, glutamatergic plasticity and cFOS expression as a proximate marker of cellular activity.

Methods

Catheter Surgery:

LE-Tg(Drd1a-iCre)30ttc and *LE-Tg(Drd2-iCre)10ttc* rats (cre recombinase under control of the dopamine receptor D1 or D2 promoter) were kindly provided by NIDA and previously validated³⁷. Animals were bred at our animal facility, kept in a 12–12 hour reverse light-dark cycle and experiments were performed

during the dark cycle. Before surgery, animals were anesthetized with ketamine (100 mg/kg) and xylazine (7 mg/kg), together with ketorolac analgesic (0.28–0.32 mg/kg) and prophylactic antibiotic (Cefazolin, 200 mg/ml, subcutaneous; West-Ward Pharmaceuticals, NJ). Over the course of the project, we switched to isoflurane anesthesia (induction 5%, maintenance 1.5–2.5%). Rats were implanted with indwelling jugular catheters as described previously¹⁶.

A total of 82 out of 97 (60 THC + CBD SA, 22 Vehicle SA) rats were used for this study. Seven animals were excluded from the study due to failing catheters or sickness during SA, eight animals were excluded during or after extinction training because of sickness or missed virus placement. Animals overlapped partly between different patch-clamp experiments, (for instance, different slices from the same animal were used for WIN-55,212 – 22 and LFS- plasticity experiment).

Virus injections:

After catheter surgery, AAV2.EF1a.DIO.eYFP.WPRE.hGH (Addgene27056, for patch-clamp experiments) or pAAV.CAG.Flex.Ruby2sm-Flag.WPRE.SV40 (Addgene 98928, for spine analysis) was infused bilaterally into the NAc (AP + 1.5mm, ML ± 1.7mm, V-7.2mm) with a rate of (0.75µl of, 0.15 µl/min to induce Cre-dependent expression of the reporter eYFP.

THC + CBD SA and reinstatement:

Rats had one week of recovery from surgery before starting behavioral training. The SA procedure has been described in detail in our previous publications¹⁶. Briefly, following 5 days of THC + CBD vapor pre-exposure, male and female rats began daily 90 min SA sessions with fixed ratio 1 delivery of intravenous THC + CBD in a 10:1 dose ratio (60 µg THC/infusion on d1-5, 30 µg THC/infusion on all subsequent days), paired with tone and light cues over a 2-week period. THC + CBD (NIDA, Bethesda, MD, USA) was dissolved in vehicle containing 0.28% ethanol, an equivalent concentration of Tween 80, and saline to volume. The vehicle solution for the vehicle control group was made up identically but without THC + CBD. The animals were extinguished without access to drug or cues for at least 10 days. Tissue was obtained 24hr after the last extinction session or after 30–60 min of cued reinstatement (Fig. 1A). The 30 minutes time point was chosen for whole cell patch clamp and spine imaging as it has been previously shown that morphological and physiological synaptic changes are transient and peak at 15–45 min after initiating cocaine reinstatement³⁸. The 60 minutes time point was chosen because the cFOS protein starts to peak around this time point³⁹.

In vitro whole cell patch clamp recordings:

Fresh NAc slices (250µm; VT1200S Leica vibratome) were collected into a vial containing aCSF as follows (in mM: 126 NaCl, 1.4 NaH₂PO₄, 25 NaHCO₃, 11 glucose, 1.2 MgCl₂, 2.4 CaCl₂, 2.5 KCl, 2.0 sodium pyruvate, 0.4 ascorbic acid, bubbled with 95% O₂ and 5% CO₂) and a mixture of 5 mM kynurenic acid and 50 µM D-APV. Slices were kept at 22°C–24°C until they were used for recordings, and were constantly perfused with oxygenated aCSF heated to 32°C (TC-344B, Warner Instruments). GABAA

synaptic transmission was blocked with 50 μ M picrotoxin (Tocris). Neurons were visualized with a Zeiss Axioscope 2 FS plus microscope with a 40x objective and voltage clamp recordings (Multiclamp 700B, Molecular Devices) performed from visualized MSNs in the medial NAc core near the anterior commissure. Glass microelectrodes (1.5–2.5 Ω M) were prepared using a PC-10 vertical puller (Narishige) and filled with internal solution as follows (in mM: 124 cesium methanesulfonate, 10 HEPES potassium, 1 EGTA, 1 MgCl₂, 10 NaCl, 2.0 MgATP, and 0.3 NaGTP, 1 QX-314, pH 7.2–7.3, 280 mOsm). To evoke postsynaptic currents, a bipolar stimulating electrode was placed 300 μ m dorso-medial of the recorded cell. Data was acquired at 10 kHz and filtered at 2 kHz using AxographX software (Axograph Scientific).

LTD-Protocol

MSNs were voltage clamped at -80 mV and a stable AMPA baseline response was recorded for at least 10 minutes. For LTD induction, cells were clamped to -50 mV for 3 minutes during which afferents were stimulated at 5 Hz. This sequence was repeated 3X with intermittent 5 min baseline recordings at -80 mV^{17,40,41}.

Perfusion and tissue preparation:

24hr after the last extinction session or immediately after the 30–60 min reinstatement session, the rats were anesthetized and perfused transcardially. Brains were removed, incubated for 2hr in 4% PFA and coronally sectioned at 100 μ m on a vibrating-blade microtome (Leica).

Spines:

We delivered pAAV.CAG.Flex.Ruby2sm-Flag.WPRE.SV40 during catheter surgery to induce Cre-dependent expression of the epitope tag Ruby2sm-Flag, in either D1- or D2-MSNs.

Immunohistochemistry and Spine Imaging:

Slices were incubated in 3% BSA and 0.03% Triton in PBS for 1-2hr and incubated in primary antibody 1:500 (rabbit anti-flag, Sigma) diluted in blocking solution overnight at 4°C. Sections were be rinsed and incubated with secondary antibody 1:1000 (goat anti-rabbit Alexa 488, Life Technology), for 3hr at room temperature. After rinsing with 0.1% Triton, tissue was mounted onto glass slides and coverslipped with ProLong Gold antifade (Thermo Fisher)⁴². Labeled sections in the NAc core were imaged using a confocal microscope (Leica SP5, Wetzlar, Germany) (Supplementary Fig. 1A). Images of labeled dendritic segments were acquired via optical sectioning using a 63X oil immersion objective with a numerical aperture of 1.4 using a 3.5x digital zoom. Acquisition settings were as follows: 1024 $\times$ 256 frame size, frame average of 4, and a 0.21 μ m z step size. Following acquisition, Z-stacks were exported to BitPlane Autoquant (Media Cybernetics, Rockville, MD) for deconvolution. Only spines on dendrites beginning at > 75 μ m and ending at $\leq$ 200 μ m distal to the soma and after the first branch point were quantified (Supplementary Fig. 1B). Individual spines were analyzed using the Imaris software version 8 (Bitplane Inc, Concord, MA) filament module (Supplementary Fig. 1C). At least four dendritic segments were

analyzed per animal. Spine head diameter was calculated, with the minimum diameter for identification at 0.14 μm . Spine density was normalized to the length of each segment. Treatment groups were unknown to the experimenter.

Immunohistochemistry and cFOS count:

For cFOS experiments the IHC for Flag was combined with IHC for cFOS. Free-floating sections were rinsed in PBS-Triton (0.25%) and incubated in normal goat serum and primary antibodies (mouse anti-flag, Sigma, 1:500, rabbit anti-phospho-cFos, Cell Signaling Technology, 1:1000, MA, USA) overnight at 4°C. After multiple PBS-Triton rinses, sections were incubated in Alexa-Fluor conjugated secondary antibodies (goat anti-mouse Alexa 488 for FLAG, goat anti-rabbit cFOS, Life Technologies, 1:1000) overnight. Labeled sections in the NAc core were imaged using a confocal microscope (Zeiss) (Fig. 5A + B) with 20x magnification. Acquisition settings were as follows: 299×299 frame size, frame average of 4, and a 0.15 μm z step size. Following acquisition, 15 μm thick Z-stacks were exported to BitPlane Autoquant (Media Cybernetics, Rockville, MD) for deconvolution. The number of cFOS + cells, Flag + cells and overlap between cFOS and Flag were quantified using the Imaris software version 8 (Bitplane Inc, Concord, MA) software by an observer blinded to the group allocations to avoid any biasing effects. Only images with more than 50 Flag positive cells were used for analysis. 4 Images had to be discarded for that reason. 2–5 images were analyzed per animal in the medial NAc Core, sampling Bregma 2.52 to 1.68 (see supplementary Fig. 2.)

Statistics:

All data were analyzed using Prism, version 8.0 (GraphPad Software, La Jolla, CA, USA). A Shapiro-Wilk test for Normality was performed and the subsequent statistical tests were chosen accordingly. T-tests, 1-way, 2way and nested ANOVAs + Fisher LSD post-hoc tests were used for normally distributed data sets. Mann-Whitney test and Kruskal-Wallis test with Dunn's post-hoc test were used for not normally distributed data sets.

Results

D1 and D2 BAC-transgenic rats display similar THC + CBD SA behavior.

D1 and D2-Cre rats were trained to self-administer THC + CBD and then extinguished. Figure 1A shows the treatment groups used: Vehicle + extinction (Ctrl), THC + CBD + extinction (Ext), THC + CBD + extinction + cued reinstatement (Rst). Figure 1B summarizes the comparable THC acquisition and extinction behavior for the two transgenic lines used. There were no differences in average daily infusion rate (Fig. 1C), and D1 and D2 Cre rats showed comparable lever presses during cue-induced reinstatement (Fig. 1D). No sex differences were found in any behavioral parameter quantified (Supplementary Fig. 3A-C). Animals in the Vehicle control group lost lever discrimination by day 8 of vehicle SA (Supplementary Fig. 3D), consistent with a lack of maintained reinforcement. In accordance with previous studies^{13,14} we found a comparable

number of average infusion between vehicle controls and THC + CBD self-administering animals, but a higher discrimination index in THC + CBD compared with vehicle controls (Supplementary Fig. 3G-F).

The loss of large spine heads and decrease in basal spontaneous glutamate transmission indicated pruning of D1-MSNs, but not D2-MSN synapses after THC + CBD

Previous reports show that chronic THC leads to a loss of spines in the PFC¹⁵ and NAc¹⁶. To test whether THC + CBD differentially affects synapses onto the two major NAc cell populations, we made three-dimensional (3D) renderings of confocal images from identified D1-MSNs and D2-MSNs in the NAc core that were Alexa-488 labeled with the epitope tag Ruby2sm-Flag⁴². Figure 2A shows representative D1-MSN dendrite segments for the 3 treatment groups. The average spine head diameter (dh) was decreased in THC + CBD extinguished but not cue-reinstated rats compared to vehicle controls (Figs. 2B left panel). The average spine density was not different between treatment groups (Figs. 2B right panel). The dh frequency distribution in Fig. 2C demonstrates a shift from bigger to smaller spines after extinction from THC + CBD SA with intermediate changes after cue-induced reinstatement.

To determine whether the THC + CBD induced loss of large dendritic spines in D1-MSNs was accompanied by a reduction of functional synapses, slices containing the NAc core were prepared after completing the behavioral procedures and whole cell patch clamp recordings made of basal spontaneous glutamate transmission in visualized D1-MSNs. Figure 2D shows representative traces of spontaneous activity from NAc core D1-MSNs for each treatment group. We found no difference in the average amplitude of spontaneous events (Fig. 2E). However, the average inter-event-interval in THC + CBD extinguished rats was increased, and this increase remained after cue-induced reinstatement (Fig. 2F). The paired-pulse ratio was not different between Ctrl (1.12 ± 0.08), THC + CBD extinguished (1.18 ± 0.11) and reinstated (1.06 ± 0.04) rats ($p = 0.829$, Kruskal-Wallis test), supporting that the reduced spontaneous activity is more likely due to a loss of AMPAR containing synapses rather than a decrease in release probability.

Equivalent to Fig. 2, we quantified the spine morphology and basal AMPAR transmission in D2-MSNs in Fig. 3. Figure 3A shows representative D2-MSN dendrite segments for the 3 treatment groups. The average spine head diameter (dh) and average spine density were not different between the three treatment groups (Figs. 3B). However, while the dh frequency distribution (Fig. 3C) was not different between Ctrl and Ext animals there was a shift from larger to smaller spines in rats that underwent cued reinstatement. Figure 3D shows representative traces of spontaneous activity from NAc core D2-MSNs for each treatment group. The shift from larger to smaller dendritic spines in reinstated animals was not reflected in changes in sEPSC amplitude (Fig. 3E) or frequency (Fig. 3F).

THC + CBD use decreases CB1R function and was accompanied by potentiation of D1-MSNs after a low-frequency-pairing protocol

The desensitization of CB1R by chronic THC is region and activity dependent¹¹. Hence, we probed if THC + CBD intake induced changes in CB1R function of glutamatergic synapses onto D1-MSN and D2-MSNs.

To this goal, whole cell patch-clamp recordings of visualized D1-MSNs from THC + CBD extinguished and vehicle control animals were performed and the inhibition of glutamate transmission after CB1R activation measured. We found that the capacity of the CB1R agonist WIN55,212 – 22 (5 μ M dissolved in 0.5% DMSO and saline, Sigma) to inhibit glutamatergic transmission was reduced in NAc core slices from THC + CBD-extinguished animals. (Fig. 4A).

We recently demonstrated that chronic THC + CBD induced a loss of NMDAR dependent long-term depression (LTD) of MSNs in the NAc core is mediated by a loss of CB1R function¹⁷. Here we examined THC + CBD induced metaplasticity on D1-MSNs synapses using a low frequency pairing protocol. Although we did not observe NMDAR-LTD in vehicle controls, we found significant differences between the 3 treatment groups. D1-MSNs in slices from THC + CBD extinguished rats exhibited significant long-term potentiation (LTP) (Fig. 4B). Measurements of changes in sEPSC amplitude and event number indicate a presynaptic locus of the LTP (Supplementary Fig. 4). Surprisingly, D1-MSNs from reinstated animals showed a reversal of the extinction associated LTP and responded similarly to vehicle control animals.

Next we probed the CB1R function of glutamatergic synapses onto D2-MSNs by measuring the inhibition of glutamate transmission with the CB1R agonist WIN55,212 – 22 (Fig. 4C). Contrary to D1-MSN glutamatergic synapses, we found that the ability of WIN55,212 – 22 to inhibit glutamatergic transmission was not different between THC + CBD extinguished animals and vehicle controls (Fig. 4E). Also, in contrast to D1-MSNs, the low frequency pairing protocol did not induce any plasticity in D2-MSNs (Fig. 4D).

cFOS in D2-MSNs was decreased 24h after extinction from THC + CBD and cFOS in D1-MSNs was increased after 1 hour of cue-induced reinstatement

Next we wanted to test whether the identified D1 MSN specific neuroadaptations were associated with cell type specific changes in neuronal activity by quantifying the expression of cFOS, an immediate early gene used as a marker of neuronal activation (Fig. 5A,B)⁴³. Counting the total number of cFOS expressing cells in tissue sections of the NAc core, we found an increase of cFOS expression after 30 minutes of cue-induced reinstatement that was further increased after 60 minutes cue-induced reinstatement (Fig. 5C). Next we looked at the overlap of cells expressing the cre-dependent marker with cells that expressed cFOS to compute the percentage of D1- or D2-MSNs expressing cFOS. In contrast to the adaptations that we found in glutamate transmission for this cell population after extinction from THC + CBD, the percentage of D1 MSNs neurons expressing cFos was not altered by extinction from THC + CBD. While after 30 minutes of cue-induced reinstatement the overlap was still indistinguishable from the vehicle and extinguished group, we found an increase in cFos expression in D1 MSNs after 60 minutes reinstatement (Fig. 5D). Interestingly, the percentage of D2 MSNs expressing cFOS was significantly decreased after extinction from THC + CBD and this change in activity was partly restored by 30 and 60 minutes cue-induced reinstatement (Fig. 5E).

Discussion

The neuroadaptations found during withdrawal from drugs of abuse often seem to counteract the acute effect of the drug⁴⁴ and are interpreted as a change towards homeostatic adaptations to balance acute network perturbation⁴⁴. However, a direct connection between cellular adaptations and changes in cellular activity has not often been established, leaving it unclear whether distinct neuroadaptations are homeostatic or driving drug induced activity changes. Using THC + CBD SA we evaluated how daily exposure to two of the main constituents of cannabis affects glutamatergic transmission onto D1- and D2-MSNs in the NAc. We chose this brain region because it is an essential component of the mesocorticolimbic system and plays a prominent role in mood, motivation and many addiction related behaviors^{19,45}. Although earlier studies have described THC induced adaptations using either non-contingent models of THC administration^{9,46,47} or SA^{16,17}, this is the first study to investigate distinct cell-specific adaptations in the two main NAc cell populations. We found that the adaptations caused by THC + CBD were specific for D1 MSNs, distinct from adaptations described after heroin and cocaine use and only partially predicted the overall activity levels of these two cell types in vehicle control, extinguished and reinstated animals.

Synaptic pruning decreased basal glutamate transmission in D1-MSNs but not D2-MSNs

Dendritic spines contain the postsynaptic components of glutamatergic synapses and changes in spine density and spine head diameter are often paralleled by changes in glutamate transmission⁴⁸. Changes in spine morphology are a hallmark of drug-induced adaptations, with morphological potentiation produced following cocaine or nicotine self-administration^{49–51}, and depotentiation induced by opioids and THC^{16,36,52}. Similar to morphine³⁶, we expected a loss of dendritic spines selectively on D2-MSNs. Strikingly we found that THC + CBD leads to a reduction of larger spines (0.45-0.60µm) and an increase of spines with a head diameter smaller than 0.15 µm in D1- but not on D2-MSNs. Since this shift from large to small spines was accompanied by a loss of spontaneous events rather than a change in the amplitude of spontaneous events, we conclude that THC + CBD induced not simply a depotentiation but rather a pruning of functional synapses, suggesting that chronic THC + CBD reduces baseline glutamatergic transmission onto D1-MSNs (Fig. 2). Indeed, the density of AMPA receptors in the postsynapse is tightly correlated with spine geometry, and small spines and filopodia only sparsely express AMPAR⁵³.

In D2-MSNs we found no spine changes after extinction from THC + CBD, but a shift from larger to smaller spines after reinstatement was observed that was not reflected by changes in sEPSC amplitude or inter-event interval. This could indicate that the morphological depotentiation had no consequence for the basal glutamate transmission or that the recording of sEPSCs was not sufficiently sensitive to detect a change in a subset of synapses. Indeed, a recent study found that chronic noncontingent THC weakens PFC glutamate input and strengthens inputs from basolateral amygdala and ventral hippocampus to unidentified MSNs in the NAc shell⁴⁶. Also D1- and D2-MSNs in the NAc receive differential input from

prefrontal and limbic structures, and differ in the mode by which eCB signaling modulates these inputs⁵⁴. It is therefore likely that THC induced adaptations are not only cell-type specific, but also input specific.

D1-MSN specific desensitization of CB1 and loss of feedback inhibition increases glutamate transmission after LFS-pairing Stimulation

CB1R are the most abundant Gi/o-protein-coupled receptors in the brain and are key modulators of neurotransmission and synaptic function^{7,55}. A dampening of endocannabinoid transmission contributes to mood disorders⁵⁶. Here, we demonstrated that CB1R desensitization after extinction from THC + CBD SA occurs specifically on glutamatergic afferents to NAc D1-MSNs. Since we did not detect a change in basal glutamate release probability, we conclude that this adaptation only affects glutamate transmission during increased network activity when CB1R acts to presynaptically inhibit glutamate transmission⁵⁵. CB1R dysfunction after THC + CBD was paralleled by LTP selectively in D1-MSNs after low frequency pairing stimulation, indicating that the CB1 dependent control of glutamate transmission is activity dependent. This result is in accordance with our previous publication, where we showed that THC + CBD induced metaplasticity could be reversed by strengthening CB1R signaling¹⁷. However, in contrast with this publication¹⁷, neither D1-MSNs nor D2-MSNs from drug naïve Cre rats expressed NMDAR-LTD, which is typically induced using this stimulus protocol. To test whether the lack of NMDAR-LTD was caused by the transgenes or reflects differences between the Sprague Dawley rats (used in previous publication) and Long Evans rats (background strain for D1- and D2-Cre rats), we conducted control experiments on untreated animals of both strains. Long Evans rats did not express LTD as compared to untreated Sprague Dawley rats (Supplementary Fig. 5). Future studies will investigate whether strain dependent differences in cannabinoid signaling could have caused the differences in plasticity thresholds between Long-Evans and Sprague Dawley rats.

Akin to our previous publication we found that cue-induced reinstatement rectified D1 MSN associated metaplasticity after extinction from THC + CBD¹⁷. Since extinguished and reinstated animals show similar functional tolerance of CB1R¹⁷, a transient restoration of CB1R does not likely explain a reversal of plasticity. Cue-induced reinstatement causes a transient molecular reorganization at glutamatergic synapse in the NAc as well as associated astrocytes⁵⁷. After extinction from heroin and cocaine SA the synaptic proximity of the astroglial surface and glutamate transporter GLT-1, the glutamate transporter preferentially expressed on astroglial perisynaptic processes, is reduced, resulting in a reduced uptake of glutamate synaptic metaplasticity⁵⁸. Interestingly, heroin associated cues lead to a transient re-association of the astroglial surface with NAc core synapses which is thought to re-establish glutamate homeostasis and could rectify synaptic plasticity changes⁵⁹. Future studies will investigate whether similar transient changes occur in response to THC + CBD associated cues and whether these changes affect metaplasticity.

Change in cell type specific cFOS expression was only partially predicted by THC + CBD induced Neuroadaptations

Here we compared the neuroadaptations identified after 10 days of chronic THC exposure to changes in cFOS expression in D1 and D2 MSNs. cFOS protein synthesis requires consistent high levels of Ca^{2+} influx, and is therefore commonly used as a proxy for neuronal activity⁶⁰. We predicted that the loss of basal glutamate transmission after extinction from THC + CBD in D1-MSNs would lead to a decrease of baseline activity in this cell type; while the activity of D2-MSNs would remain unchanged.

Interestingly we found the opposite. The percentage of D1 MSNs expressing cFOS remained unchanged and there was a decrease of D2-MSNs expressing cFOS 24 hours after extinction from THC + CBD SA that was partially restored after cue-induced reinstatement. We therefore conclude that the D1-MSN specific loss of functional synapses is not driving D1-MSN activity levels but is rather an allostatic response to acute THC + CBD induced network perturbations. Indeed, it has been recently shown that acute (24h) withdrawal from non-contingent THC leads to increases in cFOS expression selectively in accumbens D1-MSNs⁶¹, indicating the D1-MSN specific loss of basal glutamate transmission may be a response to enhanced D1-MSNs activity after acute withdrawal.

Withdrawal induced D2-MSN hypoactivity has also been demonstrated after cocaine conditioned place preference where the authors used fiber photometry to demonstrate that cocaine context induced decrease in D2-MSN activity³¹. This finding was interpreted as driving the motivation to remain in cocaine associated contexts³¹. Given that we could not find any adaptations in glutamate transmission that were associated with this D2-MSN hypoactivity or its rectification after reinstatement, we assume that the activity change is driven by changes upstream in the circuit or by changes in intrinsic excitability that we did not investigate in this study. Interestingly, we recently found hypofunction in D2-MSN GABAergic inputs to the ventral pallidum from the nucleus accumbens after extinction from cocaine SA and the inhibition of these terminals contributed to reinstated cue-induced cocaine seeking⁶² highlighting the role of imbalanced D1/D2 MSN signaling for drug craving.

In accordance with our previous study we did not find that the downregulation of CB1R changed basal release probability¹⁴. It has however been shown that the autoinhibition via CB1R can provide protection from excess presynaptic activity⁵⁵. Previous studies show that cue-induced drug seeking increases cFOS expression in D1-MSNs⁶³. For drugs like cocaine, nicotine and heroin this cell-type specific activity increase is thought to be caused by a transient synaptic potentiation of glutamate transmission⁶⁴ but distinct-drug specific mechanisms are possible. Cue induced reinstatement is driven by increased activity in cortical inputs to the NAc⁶⁵ and the deregulation of glutamate release disinhibits drug seeking behaviors²¹. Thus, selective desensitization of CB1 on glutamatergic afferents onto NAc core D1-MSNs but not D2-MSNs after THC + CBD

Conclusions And Clinical Relevance

In this study we characterized D1 and D2 MSN selective neuroadaptations and cFOS expression in a rodent model of voluntary chronic cannabis use. We discovered a reduction in basal glutamate

transmission and desensitization of CB1R in the nucleus accumbens, that was selective for glutamatergic synapses onto D1-MSNs. The pathway specific desensitization of CB1R is highly interesting because it poses new therapeutic avenues for the treatment of cannabis use disorder via the targeted enhancement of endogenous CB1R activity. For example, CB1R stimulation can be increased by using positive allosteric modulators (PAM) of CB1R⁶⁶, or inhibitors of the endocannabinoid degrading enzymes MAGL or FAAH^{67,68}. The PAM GAT-211 and the MAGL and FAAH inhibitors like PF-3845 and JZL-184 are currently under preclinical investigation for their potential analgesic and anxiolytic properties, respectively^{66,69}.

The D1-MSN specific neuroadaptations were paralleled by an increased cFOS expression during cue-induced reinstatement. Despite the lack of significant adaptations on glutamatergic synapses onto D2-MSNs, our measures of cFOS expression indicated a D2-hypoactivity 24h after the last extinction session that was partially rectified during cue-induced reinstatement. Overall our results suggest that albeit derived by distinct drug specific neuroadaptations, cannabis-induced hyperactivity of D1-MSNs and hypoactivity of D2-MSNs is a shared adaptation of withdrawal of all addictive drugs that contributes to drug craving.

Declarations

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Conflict of Interest

The authors report no biomedical financial interests or potential conflicts of interest.

References

1. Russo EB. History of cannabis and its preparations in saga, science, and sobriquet. *Chem Biodivers*. 2007;4(8):1614–1648. doi:10.1002/cbdv.200790144
2. *World Drug Report* 2020.; 2020. https://wdr.unodc.org/wdr2020/field/WDR20_Booklet_2.pdf
3. Hasin DS, Saha TD, Kerridge BT, et al. Prevalence of marijuana use disorders in the United States between 2001–2002 and 2012–2013. *JAMA Psychiatry*. 2015;72(12):1235–1242. doi:10.1001/jamapsychiatry.2015.1858
4. Ferland J-MN, Hurd YL. Deconstructing the neurobiology of cannabis use disorder. *Nat Neurosci*. Published online 2020. doi:10.1038/s41593-020-0611-0

5. Pertwee RG. The diverse CB 1 and CB 2 receptor pharmacology of three plant cannabinoids: D 9 - tetrahydrocannabinol, cannabidiol and D 9 -tetrahydrocannabivarin. Published online 2008:199–215. doi:10.1038/sj.bjp.0707442
6. Chevalleyre V, Takahashi K a, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu Rev Neurosci.* 2006;29:37–76. doi:10.1146/annurev.neuro.29.051605.112834
7. Melis M, Greco B, Tonini R. Interplay between synaptic endocannabinoid signaling and metaplasticity in neuronal circuit function and dysfunction. *Eur J Neurosci.* 2014;39(7):1189–1201. doi:10.1111/ejn.12501
8. Neuhofer D, Kalivas P. Metaplasticity at the addicted tetrapartite synapse: A common denominator of drug induced adaptations and potential treatment target for addiction. *Neurobiol Learn Mem.* Published online February 9, 2018. doi:10.1016/j.nlm.2018.02.007
9. Hoffman AF, Oz M, Caulder T, Lupica CR. Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *J Neurosci.* 2003;23(12):4815–4820. doi:23/12/4815 [pii]
10. Mato S, Robbe D, Puente N, Grandes P, Manzoni OJ. Presynaptic homeostatic plasticity rescues long-term depression after chronic Delta 9-tetrahydrocannabinol exposure. *J Neurosci.* 2005;25(50):11619–11627. doi:10.1523/JNEUROSCI.2294-05.2005
11. Lazenka MF, Selley DE, Sim-Selley LJ. dFosB induction correlates inversely with CB 1 receptor desensitization in a brain region-dependent manner following repeated D 9 -THC administration. *Neuropharmacology.* 2014;77:224–233. doi:10.1016/j.neuropharm.2013.09.019
12. Sim-Selley LJ, Schechter NS, Rorrer WK, et al. Prolonged recovery rate of CB1 receptor adaptation after cessation of long-term cannabinoid administration. *Mol Pharmacol.* 2006;70(3):986–996. doi:10.1124/mol.105.019612
13. Spencer S, Neuhofer D, Chioma VC, et al. A Model of Δ 9-Tetrahydrocannabinol Self-administration and Reinstatement That Alters Synaptic Plasticity in Nucleus Accumbens. *Biol Psychiatry.* 2018;84(8):601–610. doi:10.1016/j.biopsych.2018.04.016
14. Neuhofer D, Spencer SM, Chioma VC, Beloate LN, Schwartz D, Kalivas PW. The loss of NMDAR-dependent LTD following cannabinoid self-administration is restored by positive allosteric modulation of CB1 receptors. *Addict Biol.* Published online 2019. doi:10.1111/adb.12843
15. Miller ML, Chadwick B, Dickstein DL, et al. Adolescent exposure to Δ 9-tetrahydrocannabinol alters the transcriptional trajectory and dendritic architecture of prefrontal pyramidal neurons. *Mol Psychiatry.* Published online 2018. doi:10.1038/s41380-018-0243-x
16. Spencer S, Neuhofer D, Chioma VC, et al. A Model of Δ 9-Tetrahydrocannabinol Self-administration and Reinstatement That Alters Synaptic Plasticity in Nucleus Accumbens. *Biol Psychiatry.* Published online May 3, 2018. doi:10.1016/j.biopsych.2018.04.016
17. Neuhofer D, Spencer SM, Chioma VC, Beloate LN, Schwartz D, Kalivas PW. The loss of NMDAR-dependent LTD following cannabinoid self-administration is restored by positive allosteric modulation of CB1 receptors. *Addict Biol.* 2019;(January):1–10. doi:10.1111/adb.12843

18. George FK, Michel Le M. Drug Addiction, Dysregulation of Reward, and Allostasis. *Neuropsychopharmacology*. 2001;24(2):97. <http://proquest.umi.com/pqdweb?did=998365851&Fmt=7&clientId=39490&RQT=309&VName=PQD>
19. Floresco SB. The Nucleus Accumbens: An Interface Between Cognition, Emotion, and Action. *Annu Rev Psychol*. 2015;66(1):25–52. doi:10.1146/annurev-psych-010213-115159
20. Neuhofer D, Kalivas P. Metaplasticity at the addicted tetrapartite synapse: A common denominator of drug induced adaptations and potential treatment target for addiction. *Neurobiol Learn Mem*. 2018;154. doi:10.1016/j.nlm.2018.02.007
21. Kalivas PW. The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci*. 2009;10(8):561–572. doi:10.1038/nrn2515
22. Xi Z-X, Ramamoorthy S, Baker DA, Shen H, Samuvel DJ, Kalivas PW. Modulation of Group II Metabotropic Glutamate Receptor Signaling by Chronic Cocaine. *J Pharmacol Exp Ther*. 2002;303(2):608–615. <http://jpet.aspetjournals.org/content/303/2/608.abstract>
23. Bowers MS, McFarland K, Lake RW, et al. Activator of G protein signaling 3: a gatekeeper of cocaine sensitization and drug seeking. *Neuron*. 2004;42(2):269–281. <http://doi.wiley.com/10.1002/ana.22528>
24. Moran MM, McFarland K, Melendez RI, Kalivas PW, Seamans JK. Cystine/Glutamate Exchange Regulates Metabotropic Glutamate Receptor Presynaptic Inhibition of Excitatory Transmission and Vulnerability to Cocaine Seeking. *J Neurosci*. 2005;25(27):6389–6393. doi:10.1523/JNEUROSCI.1007-05.2005
25. Moussawi K, Pacchioni A, Moran M, et al. N -Acetylcysteine reverses cocaine-induced metaplasticity. *Nat Neurosci*. 2009;12(2):182–189. doi:10.1038/nn.2250
26. Justinova Z, Panlilio L V, Secci ME, et al. The novel metabotropic glutamate receptor 2 positive allosteric modulator, AZD8529, decreases nicotine self-administration and relapse in squirrel monkeys. *Biol Psychiatry*. 2016;78(7):452–462. doi:10.1016/j.biopsych.2015.01.014.
27. Peters J, Kalivas PW. The group II metabotropic glutamate receptor agonist, LY379268, inhibits both cocaine- and food-seeking behavior in rats. *Psychopharmacology (Berl)*. 2006;186(2):143–149. doi:10.1007/s00213-006-0372-9
28. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med*. 2008;14(9):923–930. doi:http://www.nature.com/nm/journal/v14/n9/supinfo/nm.f.1869_S1.html
29. Smith RJ, Lobo MK, Spencer S, Kalivas PW. Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). *Curr Opin Neurobiol*. 2013;23(4):546–552. doi:10.1016/j.conb.2013.01.026
30. Lobo MK, Covington HE, Chaudhury D, et al. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science (80-)*. 2010;330(6002):385–390. doi:10.1126/science.1188472

31. Calipari ES, Bagot RC, Purushothaman I, et al. In vivo imaging identifies temporal signature of D1 and D2 medium spiny neurons in cocaine reward. *Proc Natl Acad Sci U S A*. 2016;113(10):2726–2731. doi:10.1073/pnas.1521238113
32. Heinsbroek JA, Neuhofer D, Griffin WC, et al. Loss of Plasticity in the D2-Accumbens Pallidal Pathway Promotes Cocaine Seeking. *J Neurosci*. 2017;37(4):757–767. doi:10.1523/JNEUROSCI.2659-16.2016
33. Roberts-Wolfe D, Bobadilla A-C, Heinsbroek JA, Neuhofer D, Kalivas PW. Drug Refraining and Seeking Potentiate Synapses on Distinct Populations of Accumbens Medium Spiny Neurons. *J Neurosci*. 2018;38(32):7100–7107. doi:10.1523/jneurosci.0791-18.2018
34. Roberts-Wolfe DJ, Heinsbroek JA, Spencer SM, et al. Transient synaptic potentiation in nucleus accumbens shell during refraining from cocaine seeking. *Addict Biol*. 2019;(March). doi:10.1111/adb.12759
35. Pascoli V, Terrier J, Espallergues J, Valjent E, O'Connor EC, Lüscher C. Contrasting forms of cocaine-evoked plasticity control components of relapse. *Nature*. 2014;509(7501):459–464. doi:10.1038/nature13257
36. Graziane NM, Sun S, Wright WJ, et al. Opposing mechanisms mediate morphine- and cocaine-induced generation of silent synapses. *Nat Neurosci*. 2016;19(7):915–925. doi:10.1038/nn.4313
37. Pardo-garcia TR, Garcia-keller XC, Penalzoa T, et al. Ventral Pallidum Is the Primary Target for Accumbens D1 Projections Driving Cocaine Seeking. 2019;39(11):2041–2051.
38. Gipson CD, Kupchik YM, Shen H-W, Reissner KJ, Thomas C a, Kalivas PW. Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. *Neuron*. 2013;77(5):867–872. doi:10.1016/j.neuron.2013.01.005
39. Barros VN, Mundim M, Galindo LT, Bittencourt S, Porcionatto M, Mello LE. The pattern of c-Fos expression and its refractory period in the brain of rats and monkeys. *Front Cell Neurosci*. 2015;9(March):1–8. doi:10.3389/fncel.2015.00072
40. Martin M, Chen BT, Hopf FW, Bowers MS, Bonci A. Cocaine self-administration selectively abolishes LTD in the core of the nucleus accumbens. *Nat Neurosci*. 2006;9(7):868–869. doi:10.1038/nn1713
41. Kasanetz F, Deroche-Gamonet V, Berson N, et al. Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science (80-)*. 2010;328(5986):1709–1712. doi:10.1126/science.1187801
42. Viswanathan S, Williams ME, Bloss EB, et al. High-performance probes for light and electron microscopy. 2015;12(6):568–576.
43. Johnson AR, Thibeault KC, Lopez AJ, et al. Cues play a critical role in estrous cycle-dependent enhancement of cocaine reinforcement. *Neuropsychopharmacology*. 2019;(January). doi:10.1038/s41386-019-0320-0
44. Muntean BS, Dao MT, Martemyanov KA, Muntean BS, Dao MT, Martemyanov KA. Allostatic Changes in the cAMP System Drive Opioid- Induced Adaptation in Striatal Dopamine Signaling Article

- Allostatic Changes in the cAMP System Drive Opioid-Induced Adaptation in Striatal Dopamine Signaling. *CellReports*. 2019;29(4):946–960.e2. doi:10.1016/j.celrep.2019.09.034
45. Scofield MD, Heinsbroek JA, Gipson CD, et al. The Nucleus Accumbens: Mechanisms of Addiction across Drug Classes Reflect the Importance of Glutamate Homeostasis. *Pharmacol Rev*. 2016;68(3):816–871. doi:10.1124/pr.116.012484
 46. Hwang EK, Lupica CR. Altered Corticolimbic Control of the Nucleus Accumbens by Long-term $\Delta 9$ -Tetrahydrocannabinol Exposure. *Biol Psychiatry*. Published online 2019:1–13. doi:10.1016/j.biopsych.2019.07.024
 47. Mato S. Presynaptic Homeostatic Plasticity Rescues Long-Term Depression after Chronic 9-Tetrahydrocannabinol Exposure. *J Neurosci*. 2005;25(50):11619–11627. doi:10.1523/JNEUROSCI.2294-05.2005
 48. Bosch M, Castro J, Saneyoshi T, Matsuno H, Sur M, Hayashi Y. Structural and Molecular Remodeling of Dendritic Spine Substructures during Long-Term Potentiation. *Neuron*. 2014;82(2):444–459. doi:10.1016/j.neuron.2014.03.021
 49. Gipson CD, Kupchik YM, Shen H-W, Reissner KJ, Thomas A, Kalivas PW. NIH Public Access. 2014;77(5):867–872. doi:10.1016/j.neuron.2013.01.005.Relapse
 50. Garcia-Keller C, Neuhofer D, Bobadilla A-C, et al. Extracellular Matrix Signaling Through $\beta 3$ Integrin Mediates Cocaine Cue-induced Transient Synaptic Plasticity and Relapse. *Biol Psychiatry*. 2019; (6):1–11. doi:10.1016/j.biopsych.2019.03.982
 51. Gipson CD, Reissner KJ, Kupchik YM, et al. Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. *Proc Natl Acad Sci U S A*. 2013;110(22):9124–0120. doi:10.1073
 52. Shen H-W, Moussawi K, Zhou W, Toda S, Kalivas PW. Heroin relapse requires long-term potentiation-like plasticity mediated by NMDA2b-containing receptors. *Proc Natl Acad Sci U S A*. 2011;108(48):19407–19412. doi:10.1073/pnas.1112052108
 53. Matsuzaki M, Ellis-Davies GCR, Nemoto T, Miyashita Y, Iino M, Kasai H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci*. 2001;4(11):1086–1092. doi:10.1038/nn736
 54. Deroche MA, Lassalle O, Castell L, Valjent E, Manzoni OJ. Cell-type- And endocannabinoid-specific synapse connectivity in the adult nucleus accumbens core. *J Neurosci*. 2020;40(5):1028–1041. doi:10.1523/JNEUROSCI.1100-19.2019
 55. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med*. 2008;14(9):923–930. doi:http://www.nature.com/nm/journal/v14/n9/supinfo/nm.f.1869_S1.html
 56. Hillard C, Liu Q. *Endocannabinoid Signaling in the Etiology and Treatment of Major Depressive Illness*. Vol 20.; 2014. doi:10.2174/13816128113196660735
 57. Kruger A, Kalivas PW. Astrocytes as cellular mediators of cue reactivity in addiction. *Curr Opin Pharmacol*. 2021;56:1–6. doi:10.1016/j.coph.2020.07.009

58. Gipson CD, Kupchik YM, Kalivas PW. Rapid, transient synaptic plasticity in addiction. *Neuropharmacology*. 2014;76 Pt B:276–286. doi:10.1016/j.neuropharm.2013.04.032
59. Kruger A, Scofield MD, Wood D, Reissner KJ, Kalivas PW. Heroin Cue–Evoked Astrocytic Structural Plasticity at Nucleus Accumbens Synapses Inhibits Heroin Seeking. *Biol Psychiatry*. 2019;86(11):811–819. doi:10.1016/j.biopsych.2019.06.026
60. Cruz FC, Koya E, Guez-Barber DH, et al. New technologies for examining the role of neuronal ensembles in drug addiction and fear. *Nat Rev Neurosci*. 2013;14(11):743–754. doi:10.1038/nrn3597
61. Lobo MK, Zaman S, Damez-werno DM, et al. FosB Induction in Striatal Medium Spiny Neuron Subtypes in Response to Chronic Pharmacological, Emotional, and Optogenetic Stimuli. 2013;33(47):18381–18395. doi:10.1523/JNEUROSCI.1875-13.2013
62. Heinsbroek JA, Neuhofer DN, Griffin WC, et al. Loss of plasticity in the D2-accumbens pallidal pathway promotes cocaine seeking. *J Neurosci*. 2017;37(4). doi:10.1523/JNEUROSCI.2659-16.2016
63. Bobadilla AC, Dereschewitz E, Vaccaro L, Heinsbroek JA, Scofield MD, Kalivas PW. Cocaine and sucrose rewards recruit different seeking ensembles in the nucleus accumbens core. *Mol Psychiatry*. 2020;25(12):3150–3163. doi:10.1038/s41380-020-00888-z
64. Bobadilla A-C, Heinsbroek JA, Gipson CD, et al. Corticostriatal plasticity, neuronal ensembles, and regulation of drug-seeking behavior. In: *Brain Research in Addiction*.; 2017. doi:10.1016/bs.pbr.2017.07.013
65. Stefanik MT, Kupchik YM, Kalivas PW. Optogenetic inhibition of cortical afferents in the nucleus accumbens simultaneously prevents cue-induced transient synaptic potentiation and cocaine-seeking behavior. *Brain Struct Funct*. 2016;221(3):1681–1689. doi:10.1007/s00429-015-0997-8
66. Slivicki RA, Xu Z, Kulkarni PM, et al. Positive Allosteric Modulation of Cannabinoid Receptor Type 1 Suppresses Pathological Pain Without Producing Tolerance or Dependence. *Biol Psychiatry*. 2017;011(26):1–12. doi:10.1016/j.biopsych.2017.06.032
67. Serrano AM, Parsons LH. Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol Ther*. 2011;132(3):215–241. doi:10.1016/j.pharmthera.2011.06.005
68. Schlienz NJ, Budney AJ, Lee DC, Vandrey R. Cannabis Withdrawal: a Review of Neurobiological Mechanisms and Sex Differences. *Curr Addict Reports*. 2017;4(2):75–81. doi:10.1007/s40429-017-0143-1
69. Bedse G, Bluett RJ, Patrick TA, et al. Therapeutic endocannabinoid augmentation for mood and anxiety disorders: Comparative profiling of FAAH, MAGL and dual inhibitors. *Transl Psychiatry*. 2018;8(1). doi:10.1038/s41398-018-0141-7

Figures

Figure 1

Treatment groups and THC+CBD SA behavior A) Timeline. 3 different treatment groups, vehicle control (Ctrl), extinguished (Ext), reinstated (Rst) were tested. B) Comparable THC+CBD SA behavior, reinstatement and drug intake between D1 and D2 Cre lines and treatment groups. SA of (N=34) and D2 Cre (N=26) rats followed by extinction training. 2-way ANOVA revealed no significant difference in active lever presses during THC+CBD SA and extinction (interaction: $F(19,11011)=0.9283$, $p=0.5471$, time:

$F(1.733,100.4)=5.616$, $p<0.01$, treatment group: $F(1,52)=0.006$, $p=0.941$). C) The average daily infusion rates over the last 5 days of SA revealed no difference in intake between D1 and D2 Cre rats ($p=0.633$, Mann-Whitney test). D) Active and inactive lever pressing during 30 min of cued-reinstatement compared to average lever pressing of the last 3 days of extinction training. 2-way ANOVA revealed significant reinstatement that was comparable between the two rat strains (interaction $F(3,51)=0.8893$, $p=0.4530$; Rst vs. Ext $F(1.720,29.25)=30.94$ $p<0.001$, D1 vs. D2 $F(1,17)=1.585$ $p=0.225$). # comparing active and inactive lever pressing; * comparing active lever pressing between Rst and Ext.

Figure 2

Extinction from THC+CBD SA induces a loss of large spines and reduces spontaneous glutamatergic transmission in D1 Medium Spiny Neurons. A) Representative dendritic segment from NAc core D1-MSNs for each treatment group. Bar = 5 μm . Average THC+CBD infusions were not significantly different between the THC+CBD extinguished and Reinstated animals (Supplementary Figure 2G). B) left bar chart: Significant reduction dendritic spine head diameter between vehicle (Veh) and THC+CBD extinguished rats ($F(2,12)=4.558$, $p=0.034$; Ctrl vs. Ext $p=0.009$, Ctrl vs. Rst $p=0.08$ Nested one-way ANOVA with Fisher's LSD Post-hoc). Right bar chart: No significant change in overall spine density between treatment groups ($F(2,12)=1.203$, $p=0.334$; Nested one-way ANOVA). Sample size (Neurons/Animals): Vehicle N=21/5;

THC+CBD extinguished N=24/5; Reinstated N:23/5. (same N for C and D). C) Frequency distribution of Spine head sizes reveals significant changes in spine density between treatment groups that are dependent on spine head diameter. (interaction $F(16,520)=6.336$, $p<0.01$, frequency $F(2,304,149.8)=323.9$ $p<0.01$, treatment $F(2,65)=1.123$ $p=0.332$; 2-way ANOVA and with repeated measures over spine head diameter) * $p<0.05$ comparing Ctrl vs. Ext ; + $p<0.05$ comparing Ctrl. Vs. Rst using Fisher's LSD test. D) Representative traces of spontaneous activity from NAcCore D1-MSNs for each treatment group. Scale bar shows 50pA vs 2 sec. E) Cumulative probability and mean values of amplitude for sEPSCs indicate no difference between treatment groups. F) Cumulative probability and mean values of sEPSC interevent-intervals from D1-MSNs show a reduction in spontaneous glutamate transmission in THC+CBD extinguished and cue-reinstated rats. ($p=0.042$, Kruskal-Wallis test with Dunn's multiple comparison) All data are shown as mean $\pm$ SEM; * $p < 0.05$)

Figure 3

Cued-Reinstatement induces a pruning of large spines but does not affect spontaneous glutamatergic transmission in D2 Medium Spiny Neurons. A) Representative dendrites from NAcCore D2-MSNs for each treatment group. Bar = 5 μ m B) There was no significant change in spine head diameter (left bar chart, $F(2,8)=0.784$, $p=0.4887$; Nested one-way ANOVA) or spine density (right bar chart, $F(2,8)=1.275$,

$p=0.3306$; Nested one-way ANOVA)) between treatment groups. N as number of neurons over Number of animals: Vehicle N=22/4; THC+CBD extinguished N=23/4; Reinstated N:15/3. (same N for C). C) Frequency distribution reveals spine head dependent changes in spine density between treatment groups. (interaction $F(16,424)=2.096$, $p<0.01$, frequency $F(2.710,143.6)=176.7$ $p<0.01$, treatment $F(2,53)=2.129$ $p=0.129$; 2-way ANOVA with repeated measures over spine head diameter) * $p<0.05$ comparing Ctrl vs. Ext ; + $p<0.05$ comparing Ctrl. Vs. Rst using Fisher's LSD Post-Hoc test. D) Representative traces of spontaneous activity from NAc core D2-MSNs for each treatment group. Scale bar shows 50 pA vs 2 sec. E) Cumulative probability and mean values of amplitude for sEPSCs recordings from D2-MSNs show no difference between treatment groups. ($F(2,36)=0.0131$, $p=0.987$; 1-way ANOVA) F) Cumulative probability and mean values of sEPSC Inter-Event-Intervals show no difference between treatment groups. ($F(2,36)=0.9544$, $p=0.3046$; 1-way ANOVA). All data are shown as mean $\pm$ SEM. * $p < 0.05$

Figure 4

Extinction from THC+CBD SA impedes CB1R function and induces long-term potentiation of glutamate transmission in D1 but not D2 Medium Spiny Neurons. A) (Top) Representative D1 MSN current traces pre and post WIN55,212-2 application from a vehicle and a THC+CBD extinguished rat. Scale bar: 550 pA vs 25 ms. Left: Averaged EPSC shows that WIN55,212-2 (5 μ M) reduced EPSC amplitude to a larger extent in vehicle rats than in D1 MSNs of THC-CBD extinguished. (time $F(2.486, 29,84) = 34.51$, $P < .01$; treatment $F(1,12) = 3.994$, interaction $F(19, 228) = 2.519$, $P < .01$; $p = 0.0722$; 2-way ANOVA for time course of current response after WIN55,212-22 application). Right: Bar graph summarizing the average response 15 to 25 minutes into WIN55,212-2 application ($p = 0.0262$, Kruskal-Wallis test). B) (Top) Representative D1 MSN current traces pre and post plasticity induction. Left: Time course of D1 MSNs synaptic response to the induction protocol. Extinction of THC + CBD induced metaplasticity that was rectified by 30 minutes of cue-induced reinstatement (interaction $F(32, 528) = 0.3459$, $P < .99$; time, $F(3.859, 128.5) = 12.31$, $P < .01$; treatment $F(2,33) = 4.118$, $p = 0.0253$; 2-way ANOVA for time course of current response after plasticity induction). Right: Bar graph summarizing the average response 15 to 25 minutes after LTD induction. D1-MSNs in slices of THC extinguished animals exhibited significant LTP but not reinstated or control animals exhibited significant LTP ($t(8) = 2.566$, $p < 0.05$, $p = 0.1375$ and $p = 0.3521$ student's t-test). * $P < .05$, comparing baseline response to 15 to 25 post-induction. C) (Top) Sample D2 MSN current traces pre and post WIN55,212-2 application from the three treatment groups. Scale bar: 550pA vs 25 ms. Left: Average time course of CB1 agonist WIN55,212-2 dependent inhibition of EPSPs revealed no difference between treatment groups (time $F(1.995, 21.94) = 31.8$, $p < 0.01$; treatment $F(1,11) = 0.2507$, $p = 0.6264$; interaction $F(18, 198) = 1.361$, $p = 0.1547$; 2-way ANOVA for time course of current response after WIN55,212-22 application) Right: Bar graph summarizing the average response 15 to 25 minutes into WIN55,212-2 application ($t(12) = 2.478$, $p < 0.05$, $t(11) = 0.4045$; $p = 0.694$; unpaired t-test). D) (Top) Representative current traces pre and post plasticity induction. Left: Time course of D2-MSNs synaptic response to plasticity induction protocol revealed no difference between treatment groups (interaction $F(32, 368) = 0.5123$, $P < 0.9882$, time $F(2.219, 51.04) = 2.462$, $p = 0.0898$; treatment $F(2,23) = 0.00184$, $p = 0.9982$; 2-way ANOVA for time course of current response after plasticity induction). Right: Bar graph show averaged responses, comparing baseline to the average of 15-25 min after LTD induction protocol. D2-MSNs in slices of ctrl animals exhibited significant long-term potentiation (LTP) ($t(7) = 2.927$, $p < 0.05$, student's t-test). N is shown as cells/animals recorded. $p < 0.05$, # comparing pre- to post-stimulation; * comparing treatment groups.

Figure 5

Cell type specific effects of Extinction from THC+CBD and cue-induced reinstatement on cFOS expression. A) Representative images of cFOS (red) and D1 MSNs (green) labeling in the nucleus accumbens core in a vehicle (D1 Veh), THC+CBD extinguished (D1 Ext) and cue-reinstated (D1 Rst) rat. Scale bar = 20μm B) Representative images of cFOS (red) and D2 MSNs (green) labeling in the nucleus accumbens core in a vehicle (D2 Veh), THC+CBD extinguished (D2 Ext) and cue-reinstated (D2 Rst). C) Scatter dot plot illustrating the significant increase in cFOS expression after cue-induced reinstatement to THC. ($p < 0.001$, Kruskal Wallis test; Veh vs. Rst 60 mins $p < 0.0001$, Ext vs. Rst 30 mins $p = 0.019$, Ext vs. Rst 60 mins $p = 0.0048$; Dunn's post-hoc test) D) 60 minutes of Reinstatement did significantly increase the percentage of D1 MSNs expressing cFOS. $F_{3,13} = 3.436$, $p = 0.0495$, Nested one-way ANOVA; Veh vs Ext 60

p=0.4494, Veh vs Rst 60 min p=0.043, Ext vs Rst 60 min, p=0.015, Fisher's LSD) E) Extinction from THC+CBD decreased the overall activity of D2 MSNS which was rectified after cue-induced reinstatement. (F3,10 =3.61, p=0.05, Nested one-way ANOVA; Veh vs. Ext p=0.008, Veh vs Rst 60 min p=0.182, Ext vs Rst 60 min, p=0.127 Fisher's LSD).

Supplementary Files

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