Altered Corticolimbic Control of the Nucleus Accumbens by Chronic Δ^9- Tetrahydrocannabinol

Supplementary Information

Supplementary Figures

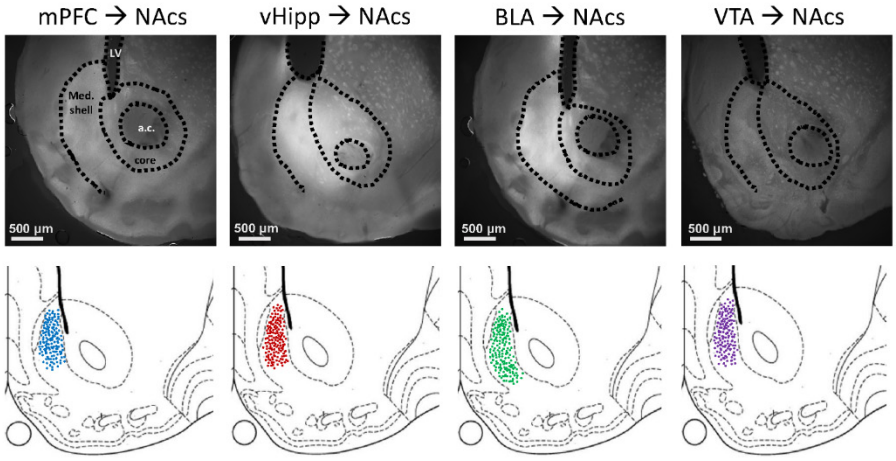

Figure S1. Illustration of ChR2-associated fluorescence and recordings sites in brain slices containing the NAc.
Six to 8 weeks after bilateral injection with ChR2-expressing virus into the mPFC, vHipp, BLA, or VTA, brain slices (250 µm) containing the NAcs, from approximately 2.0 mm to 1.2 mm anterior to Bregma, were cut in a coronal plane using a vibratome. Epifluorescence imaging with a fixed-stage microscope was used to verify eYFP expression and successful transfection with the virus in each of the projection areas. The lateral ventricle (LV), anterior commissure (a.c.), and dorsal striatum were used as primary landmarks to guide recordings. All recordings were made in an area of the NAcs that was medial to the a.c. and the NAc core, and ventral or medial to the lateral ventricle (blue, red, green, or purple dots indicate individual recordings). Medial is left, and lateral right in this diagram.

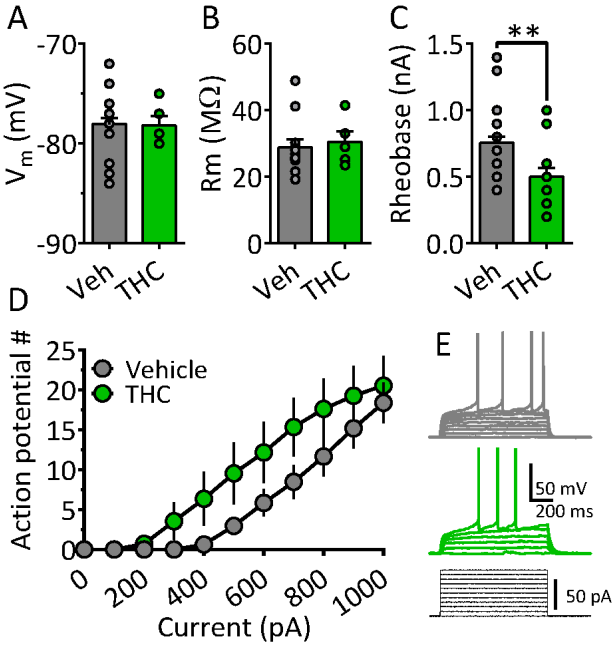

Figure S2. Effects of chronic Δ^9-THC on NAcs neuron membrane properties measured in current clamp. Treatment with Δ^9-THC did not alter the resting mean membrane potential (Vm, **A**) or whole-cell input resistance (Rm, **B**) of medium spiny neurons in the NAcs. **C.** Chronic treatment with Δ^9-THC significantly reduced the threshold current necessary to generate action potentials (rheobase), compared to chronic vehicle ($p = 0.003$, unpaired Student's t-test). **D.** Mean relationship between strength of current passed through the whole-cell electrode (square-wave, 1.5 sec duration), and the number of action potentials observed following chronic Δ^9-THC or vehicle. Although there is a trend toward increased excitability following chronic Δ^9-THC, the treatment x injected current interaction was not statistically significant ($F_{10,290} = 0.66$, $p > 0.99$, repeated measures ANOVA). **E.** Representative current clamp traces from individual NAcs medium spiny neurons from rats chronically treated with vehicle (gray) or Δ^9-THC (green). The family of square-wave currents injected through the recording pipette are shown at bottom. Number of neurons/Rat per group: **A.** Veh: 21/7, THC: 5/4. **B.** Veh: 12/7, THC: 5/4. **C.** Veh: 30/9, THC: 13/6. **D.** Veh: 25/9, THC: 11/6. Vm and Rm were unaltered in MSNs from chronic Δ^9-THC animals (Fig. S2A, S2B). However, the threshold current to generate action potentials was significantly lower in cells from chronic Δ^9-THC animals (Fig. S2C). Analysis of the relationship between the amount of injected current and number of action potentials generated showed a trend toward increased action potential output in the chronic Δ^9-THC animals (Fig. S2D, S2E), but this was not significant. Thus, chronic Δ^9-THC increased only action potential threshold in NAcs MSNs.

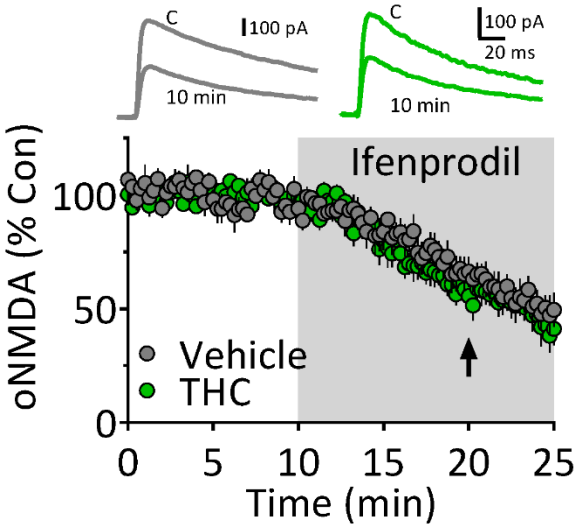

Fig. S3. Chronic Δ^9-THC does not alter NMDAR subunits at vHipp à NAcs synapses.
NMDA currents were elicited by laser activation of ChR2 in vHipp afferents to NAcs in neurons obtained from chronic vehicle or chronic Δ^9-THC-treated rats. The NR2B antagonist ifenprodil (5 µM) was applied after 10 min of steady baseline. Representative waveforms from single neurons in each group (gray = chronic vehicle, green = chronic Δ^9-THC) were averaged from 5 sweeps during the control baseline period (C), and 10 min after beginning ifenprodil application (top panel). The mean (± s.e.m.) time course for all neurons from both groups showed no significant difference in the inhibition produced by ifenprodil ($F_{3,16}$ = 0.83, p = 0.50, one-way ANOVA; n = 6 neurons in each group).

Supplementary Methods

Brain slices

Six to 8 weeks after virus injection, and 24 hours after the final drug injection, rats were anesthetized with isoflurane, then transcardially perfused with ice-cold (2-4°C), gassed (95% O_2/ 5% CO_2) artificial cerebrospinal fluid (aCSF) (1) (in mM: N-Methyl-D-Glucamine (NMDG), 93; KCl, 2.5; NaH_2PO_4, 1.2; $NaHCO_3$, 30; HEPES, 20; glucose, 25; sodium ascorbate, 5; sodium pyruvate, 3; $MgCl_2$, 10; $CaCl_2$ 0.5, pH 7.4, 300-310 mOsm). The rats were then decapitated, the brain removed and coronal slices (250 μm) of NAc prepared in ice-cold NMDG-aCSF, using a vibratome (Leica VT1200S, Heidelberg, Germany) (1, 2). Slices were then hemisected, transferred to 34°C NMDG-aCSF for 10-15 min, then to a chamber containing modified-aCSF (in mM: NaCl, 109; KCl, 4.5; $MgCl_2$, 1; $CaCl_2$ 2.5; NaH_2PO_4, 1.2; $NaHCO_3$, 35; glucose, 11; HEPES, 20; sodium ascorbate, 0.4; pH 7.4, 300-310 mOsm). A NAc slice was submerged in a recording chamber in a fixed-stage of a microscope and perfused with gassed aCSF (2-3 ml/min; in mM) NaCl, 126; KCl, 3.0; $MgCl_2$, 1.5; $CaCl_2$, 2.4; NaH_2PO_4, 1.2; glucose, 11.0; $NaHCO_3$, 26, at 30-32°C.

Electrophysiology

MSNs were visualized in the medial nucleus accumbens shell (NAcs) with infrared-differential interference contrast (IR-DIC) microscopy and a 40x objective (Olympus, Tokyo, Japan). Cells were targeted in NAcs at sites medial and ventral to the anterior commissure and ventromedial to the lateral ventricle (Fig. S1). Borosilicate glass electrodes (3-4 MΩ) were used for whole-cell voltage- and current-clamp recordings. Intra-pipette solutions were (in mM): 117.5 $CsMeSO_3$, 10 HEPES, 10 TEA-Cl, 8 CsCl, 0.25 EGTA, 5 QX-314 (Br), 10 Na^+-Phosphocreatine,

4 MgATP, 0.3 NaGTP; pH7.3, 290-300 mOsm, for voltage-clamp, and 140 K-gluconate, 10 HEPES, 0.2 EGTA, 2 $MgCl_2$, 0.1 $CaCl_2$, 4 Na_2-ATP, 0.3 Na-GTP; pH7.3 and 280-290 mOsm, for current-clamp. A Multiclamp 700B amplifier (Molecular Devices, San Jose, CA) was used, and signals acquired with software (WinLTP Ltd; www.winltp.com) and a National Instruments (Austin, TX) PCI-6251 A/D board. Series resistance (R_s; 10-20 MΩ), monitored with hyperpolarizing steps (-5 mV, 200 ms), was not compensated. If R_s showed >10% change the recording was abandoned. Unless indicated, cells were voltage clamped at -70 mV.

Virus injections

Rats were anesthetized with isoflurane and injected (0.7 µl over 5 min) with AAV5-CaMKII-ChR2-eYFP (4.6x1012 virus molecules/ml; University of North Carolina vector core) into vmPFC (AP: +3.3; ML: ±0.8; DV: -5.0), vHipp (AP: -5.6; ML: ±5.0; DV: -6.8) or BLA (AP: -2.8; ML: ±5.0; DV: -8.5) using a 10-µl Hamilton syringe, an UltraMicroPump, and SYS-Mico4 controller (WPI, Sarasota, FL). A *cre*-dependent construct, AAV5-EF1α-DIO-ChR2-eYFP (5.5x1012 virus Molecules/ml, University of North Carolina Vector Core), was injected into medial VTA (AP: -5.4; ML: ±2.0; DV -8.2; 10° angle) of TH*cre* rats. The same batches of AAV5-CaMKII-ChR2-eYFP were used in mPFC, vHipp, and BLA, and a single vial of AAV5-EF1α-DIO-ChR2-eYFP was used for all VTA injections to maintain consistency of viral titer. Incisions were closed with absorbable sutures, and body temperature was maintained with a heating pad until anesthesia recovery. In addition, we intentionally used the same volume injections (0.7 µl) of the ChR2 constructs in each of the brain regions.

Drug Application

Drugs were prepared in H_2O or DMSO, diluted in aCSF to final concentration, and delivered via gravity-driven perfusion.

Data Analysis

Representative traces are averages of 10 consecutive sweeps measured as a percentage of baseline, and data are presented as mean ± s.e.m. GraphPad Prism 6.0 (San Diego, CA) and Clampfit (v9.0, Molecular Devices) were used for figures and statistics. Unless indicated, statistical significance was determined using one-way, or two-way repeated measures ANOVA and Holm-Sidak post hoc tests, and a minimum significance level of $p < 0.05$. The numbers of neurons recorded from the number of rats (n/R) is reported in the figure captions.

Supplemental References

1. Ting JT, Lee BR, Chong P, Soler-Llavina G, Cobbs C, Koch C, et al. (2018): Preparation of Acute Brain Slices Using an Optimized N-Methyl-D-glucamine Protective Recovery Method. *J Vis Exp*.
2. Zhang S, Qi J, Li X, Wang HL, Britt JP, Hoffman AF, et al. (2015): Dopaminergic and glutamatergic microdomains in a subset of rodent mesoaccumbens axons. *Nat Neurosci*. 18:386-392.