A single *in-vivo* exposure to Δ9THC blocks endocannabinoid-mediated synaptic plasticity

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Endogenous cannabinoids (eCB) mediate synaptic plasticity in brain regions involved in learning and reward. Here we show that in mice, a single *in-vivo* exposure to Δ9-tetrahydrocannabinol (THC) abolishes the retrograde signaling that underlies eCB-mediated synaptic plasticity in both nucleus accumbens (NAc) and hippocampus *in vitro*. This effect is reversible within 3 days and is associated with a transient modification in the functional properties of cannabinoid receptors.

There is strong evidence that a single exposure to addictive drugs can alter synaptic plasticity in the brain reward pathway[1–3]. Whether a single exposure to cannabis derivatives also modifies endocannabinoid-mediated synaptic plasticity has not been explored. Here we evaluated the consequences of a single *in-vivo* exposure to THC, the principal psychoactive ingredient of cannabis, in the NAc and the hippocampus, two brain areas where we have previously characterized eCB-mediated, long-lasting forms of synaptic plasticity[4–6].

Repetitive activation of prelimbic cortex afferents to the NAc induces an eCB-mediated long-term depression of excitatory transmission (eCB-LTD) that might be part of a negative feedback loop reducing the strength of glutamatergic synapses during sustained cortical activity[5]. In the hippocampus, eCBs mediate long-term depression of inhibitory synaptic transmission (I-LTD), a phenomenon that could underlie the effects of cannabinoids on learning and memory[4]. To test whether eCB-mediated retrograde signaling could be part of the early synaptic changes to THC exposure, we compared eCB-LTD and I-LTD in NAc and hippocampal slices prepared from vehicle- and THC-treated mice (**Fig. 1**). Tolerance to cannabinoids develops rapidly and behavioral tolerance is observed after one day of THC treatment[7,8]. Thus, mice were injected once with a non-aversive dose of THC (3 mg/kg)[7] or vehicle 15–20 h before the experiment. We found that eCB-LTD (**Fig. 1a**) and I-LTD (**Fig.1b**) were both abolished in THC-injected animals. This action could be due to a persistent change in eCB release or in the CB1 receptor (CB1R) itself. Because the eCB release that triggers eCB-LTD and I-LTD occurs as a result of the activation of postsynaptic metabotropic glutamate receptors (mGluR)[4,5], it is conceivable that THC-induced effects could be due to a modification of the mGluR-dependent release of eCBs. This possibility is unlikely because another CB1R-mediated phenomenon in which the release of eCBs does not require mGluR activation, the depolarization-induced suppression of inhibition (DSI)[9,10], was markedly reduced in the THC-injected animals (**Fig. 1c**). The THC-induced effects were reversible as eCB-LTD, I-LTD and DSI were entirely normal in slices prepared 3 d after injection (**Fig. 1d**). The effects of a single exposure to THC were completely prevented when the CB1 antagonist SR141716A (1 mg/kg) was injected 30 min before THC, demonstrating the role of CB1R in the THC-induced blockade of synaptic plasticity (**Fig. 1d**). THC injection did not cause a shift from an eCB-mediated to an eCB-independent form of synaptic plasticity, as 1 μM SR141716A bath application prevented eCB-LTD and I-LTD in slices prepared after 3 d or 1 week recovery (3 d after single THC, eCB-LTD was 77.1 ± 5.5% of baseline, $n = 3$; compared to 100.7 ± 3.0%, $n = 3$, in SR141716A, $P < 0.05$, and I-LTD was 76.7 ± 1.9%, $n = 4$, compared to 101.4 ± 1.4 %, $n = 5$ in SR141716A, $P < 0.05$; data not shown). Thus, a single *in-vivo* exposure to a low dose of THC transiently blocks eCB-mediated retrograde signaling in structures

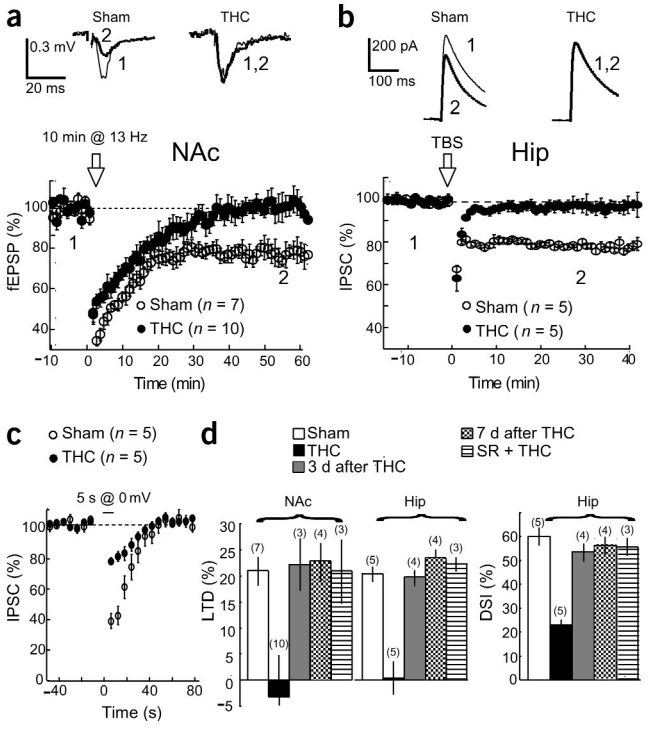

Figure 1 Single *in-vivo* administration of THC abolishes eCB-mediated synaptic plasticity in NAc and hippocampus. (**a**,**b**) Summary graphs (lower panels) of the time course of field excitatory postsynaptic potentials (fEPSPs) in the NAc (**a**) and IPSCs (inhibitory postsynaptic currents) from CA1 pyramidal cells (**b**), showing the effects of repetitive stimulation in THC-injected and sham animals. Upper panels show sample traces of representative experiments (numbers indicate the corresponding time point in the bottom graphs). (**c**) DSI was markedly reduced in THC-injected animals. (**d**) Bar histograms of the magnitude of eCB-LTD, I-LTD and DSI in sham and THC-treated animals, 1 d, 3 d and 1 week after injection. SR141716A (SR) injection (1 mg/kg) 30 min before THC abolished eCB-mediated plasticity in NAc and the hippocampus. Brain slices were prepared as previously described[4,5] (see **Supplementary Methods** online). All experimental procedures were in accordance with the Society for Neuroscience and European Union guidelines and were approved by the institutional animal care and use committees.

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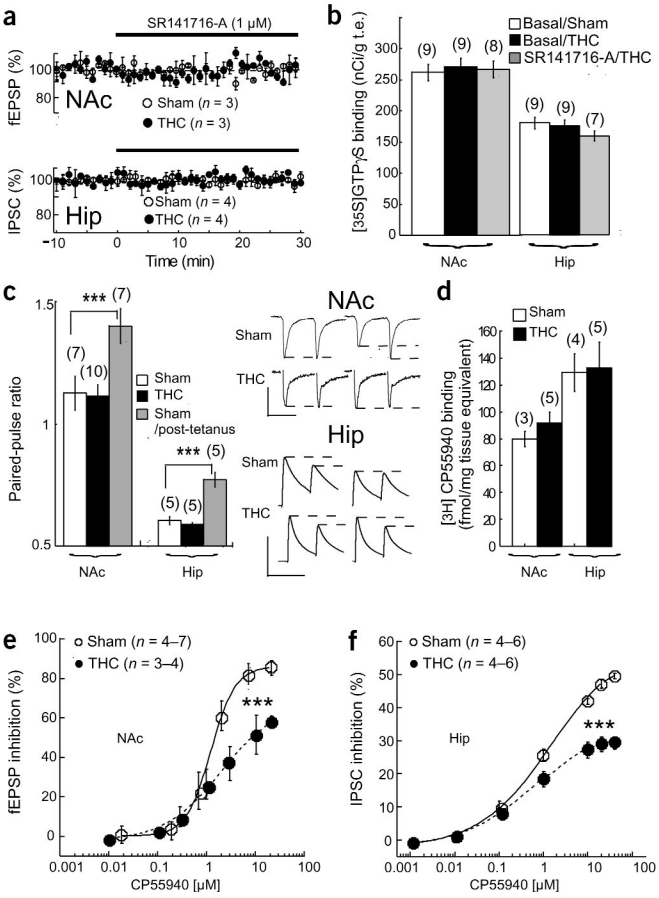

Figure 2 THC single injection causes functional tolerance. (**a**) CB1R blockade with SR141716-A did not affect basal synaptic transmission in NAc or hippocampus from vehicle- or THC-injected mice. (**b**) After THC single injection, basal [^{35}S]GTPγS binding was neither enhanced (vehicle-treated, white bar vs. THC-treated, black bar) nor reduced by SR141716-A (basal, black bar vs. SR141716-A, gray bar). (**c**) Paired-pulse ratio was identical in vehicle and THC-treated animals suggesting that basal probability of transmitter release was unchanged after THC injection. Right side: sample traces of representative experiments in NAc (scale bar: 0.2 mV, 40 ms) and hippocampus (scale bar: 1,000 pA, 200 ms), before and after LTD-induction from sham and THC-injected animals. (**d**) Specific binding of the cannabinoid agonist [^{3}H]CP55940 was similar in vehicle- and THC-treated animals. (**e**,**f**) Dose-response curves for CP55940 inhibition of fEPSP in the NAc (**e**) or IPSC in the hippocampus (**f**) from vehicle- or THC-treated mice. [^{35}S]GTPγS and [^{3}H]CP55940 autoradiography were as indicated elsewhere[11] (**Supplementary Methods**).

fundamental to reward-related behaviors (the NAc), and learning and memory (the hippocampus).

What cellular/molecular mechanism could account for the THC-induced effects on the eCB retrograde signaling? Persistent CB1R activation by residual THC could depress synaptic transmission, thereby occluding eCB-mediated changes in synaptic efficacy. This possibility was excluded based on electrophysiological and biochemical evidence. First, bath application of the selective CB1R antagonist SR141716-A (1 μM) did not increase baseline synaptic transmission in the NAc or the hippocampus of THC-injected mice (**Fig. 2a**). Second, [^{35}S]GTPγS autoradiography was performed in both structures to quantify the coupling efficiency between CB1R and Gi/o transduction proteins[11]. After THC single-injection, basal [^{35}S]GTPγS binding was unaltered and SR141716-A (1 μM) did not reduce basal [^{35}S]GTPγS binding (**Fig. 2b**).

Exogenous THC could trigger chemical LTD and occlude eCB-mediated synaptic plasticity. This possibility is also unlikely because paired-pulse ratio, a form of short-term plasticity that changes during eCB-LTD and I-LTD, was unaffected in THC-injected animals (**Fig. 2c**). It has been reported that multiple injections with high THC doses can trigger CB1R downregulation and uncoupling from Gi/o proteins[12,13]. However, we found no change in CB1 binding sites in the NAc and the hippocampus measured by *in-vitro* autoradiography[11], suggesting that a single THC treatment was not sufficient to cause significant internalization of CB1R (**Fig. 2d**). Moreover, CB1-agonist-stimulated [^{35}S]GTPγS autoradiography did not reveal significant uncoupling from Gi/o proteins after single THC treatment (**Supplementary Fig. 1** online)[14]. Finally, we explored whether a functional modification of the CB1R (tolerance) could explain the THC-mediated effects on synaptic plasticity[15]. We found that the depression induced by the CB1R-selective agonist CP55940 was clearly reduced in THC-treated mice, as compared to vehicle-treated mice, in both the NAc and the hippocampus (**Fig. 2e,f**). Taken together, these findings indicate that functional tolerance of the CB1R can account for the suppression of eCB-mediated synaptic plasticity after acute THC exposure.

In conclusion, our study shows that a single exposure to THC has profound repercussions—albeit transient—on synaptic plasticity in key brain areas that are involved in reward or learning. Thus, our findings reveal a mechanism by which cannabis derivatives may alter cognitive functions and motivational behaviors.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Ungless, M.A., Whistler, J.L., Malenka, R.C. & Bonci, A. *Nature* **411**, 583–587 (2001).
2. Saal, D., Dong, Y., Bonci, A. & Malenka, R.C. *Neuron* **37**, 577–582 (2003).
3. Melis, M., Camarini, R., Ungless, M.A. & Bonci, A. *J. Neurosci.* **22**, 2074–2082 (2002).
4. Chevaleyre, V. & Castillo, P.E. *Neuron* **38**, 461–472 (2003).
5. Robbe, D., Kopf, M., Remaury, A., Bockaert, J. & Manzoni, O.J. *Proc. Natl. Acad. Sci. USA* **99**, 8384–8388 (2002).
6. Gerdeman, G.L. & Lovinger, D.M. *Br. J. Pharmacol.* **140**, 781–789 (2003).
7. Maldonado, R. *Pharmacol. Ther.* **95**, 153–164 (2002).
8. Hutcheson, D.M. *et al. Br. J. Pharmacol.* **125**, 1567–1577 (1998).
9. Wilson, R.I. & Nicoll, R.A. *Nature* **410**, 588–592 (2001).
10. Ohno-Shosaku, T., Maejima, T. & Kano, M. *Neuron* **29**, 729–738 (2001).
11. Mato, S., Del Olmo, E. & Pazos, A. *Eur. J. Neurosci.* **17**, 1747–1754 (2003).
12. Romero, J. *et al. Brain Res. Mol. Brain Res.* **46**, 100–108 (1997).
13. Breivogel, C.S. *et al. J. Neurochem.* **73**, 2447–2459 (1999).
14. Sim, L.J., Hampson, R.E., Deadwyler, S.A. & Childers, S.R. *J. Neurosci.* **16**, 8057–8066 (1996).
15. Hoffman, A.F., Oz, M., Caulder, T. & Lupica, C.R. *J. Neurosci.* **23**, 4815–4820 (2003).