Cannabinoid and Heroin Activation of Mesolimbic Dopamine Transmission by a Common μ_1 Opioid Receptor Mechanism

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The effects of the active ingredient of *Cannabis*, Δ^9-tetrahydrocannabinol (Δ^9-THC), and of the highly addictive drug heroin on in vivo dopamine transmission in the nucleus accumbens were compared in Sprague-Dawley rats by brain microdialysis. Δ^9-THC and heroin increased extracellular dopamine concentrations selectively in the shell of the nucleus accumbens; these effects were mimicked by the synthetic cannabinoid agonist WIN55212-2. SR141716A, an antagonist of central cannabinoid receptors, prevented the effects of Δ^9-THC but not those of heroin. Naloxone, a generic opioid antagonist, administered systemically, or naloxonazine, an antagonist of μ_1 opioid receptors, infused into the ventral tegmentum, prevented the action of cannabinoids and heroin on dopamine transmission. Thus, Δ^9-THC and heroin exert similar effects on mesolimbic dopamine transmission through a common μ_1 opioid receptor mechanism located in the ventral mesencephalic tegmentum.

Although *Cannabis* is the most commonly abused illicit substance, its reinforcing properties are difficult to demonstrate in animals, and efforts to obtain consistent self-administration of the active component of *Cannabis*, Δ^9-THC, in laboratory animals have been unsuccessful (*1*). Δ^9-THC acts in the brain on a G protein–coupled receptor (CB_1) whose putative endogenous ligand has been identified as anandamide, an arachidonic acid derivative (*2*, *3*). The reinforcing properties of Δ^9-THC may be mediated by its action on the mesolimbic dopamine (DA) system, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (*4*); this neuronal system is also one of the candidate substrates for the reinforcing and addictive actions of other drugs and substances of abuse (*5*). However, the evidence for an action of Δ^9-THC on central DA transmission has been contradictory (*6*).

The availability of specific cannabinoid receptor agonists (WIN55212-2, CP55940) (*7*) and antagonists (SR141716A) (*8*) allowed us to reexamine this issue. We used brain microdialysis with vertical concentric probes (*9*) to monitor changes in extracellular DA concentration in the two main subdivisions of the NAc, the shell and the core (*10*), elicited by the administration of the natural cannabinoid Δ^9-THC, the synthetic cannabinoid agonist WIN55212-2, and cannabinol, an inactive cannabinoid (*11*). To reduce the possibility of the occurrence of nonspecific changes resulting from the stress of injection, we administered Δ^9-THC and WIN55212-2 intravenously (iv) through chronically implanted catheters (*9*). The effects of cannabinoids were compared with those of the addictive drug heroin administered iv at doses that are able to maintain iv self-administration in rats (*12*).

Δ^9-THC significantly increased dialysate DA in the NAc shell at doses of 0.15 mg per kilogram of body weight (mg/kg) iv ($F_{8,27}$ = 2.502, $P < 0.05$) and 0.30 mg/kg iv ($F_{8,4}$ = 3.522, $P < 0.002$) (Fig. 1). These effects were dose-dependent ($F_{1,87}$ = 6.092, $P < 0.02$; post hoc, $P < 0.05$) and time-dependent ($F_{8,80}$ = 5.606, $P < 0.001$). No significant changes in dialysate DA were obtained in the NAc core after Δ^9-THC doses of 0.15 and 0.30 mg/kg iv ($F_{8,45}$ = 0.650, P = 0.73). Post hoc comparison of the effect of Δ^9-THC on dialysate DA in the NAc shell and core showed a preferential effect in the shell at both doses of Δ^9-THC (Fig. 1). The increase in dialysate DA in the NAc shell elicited by a Δ^9-THC dose of 0.30 mg/kg was prevented by pretreatment with the cannabinoid antagonist SR141716A (*13*) [1 mg/kg intraperitoneally (ip), 40 min in advance]. SR141716A by itself did not modify dialysate DA in the NAc shell (Fig. 1). Cannabinol, a nonpsychoactive cannabinoid, dissolved in the same vehicle as Δ^9-THC, failed to modify dialysate DA in the NAc shell at doses of 0.30 mg/kg iv ($F_{8,18}$ = 0.722; $P > 0.05$) or 1.0 mg/kg iv ($F_{8,27}$ = 0.511, $P > 0.05$).

The effect of Δ^9-THC was mimicked by the centrally active cannabinoid agonist WIN55212-2. Thus, WIN55212-2 significantly increased dialysate DA in the NAc shell at doses of 0.15 mg/kg iv ($F_{8,27}$ = 6.976, $P < 0.001$) and 0.30 mg/kg iv ($F_{8,27}$ = 9.299, $P < 0.001$). This effect of WIN55212-2 was dose-dependent ($F_{1,70}$ = 6.093, $P < 0.02$; post hoc, $P < 0.05$) and time-dependent ($F_{8,63}$ = 10.24, $P < 0.001$). SR141716A (1.0 mg/kg, 40 min in

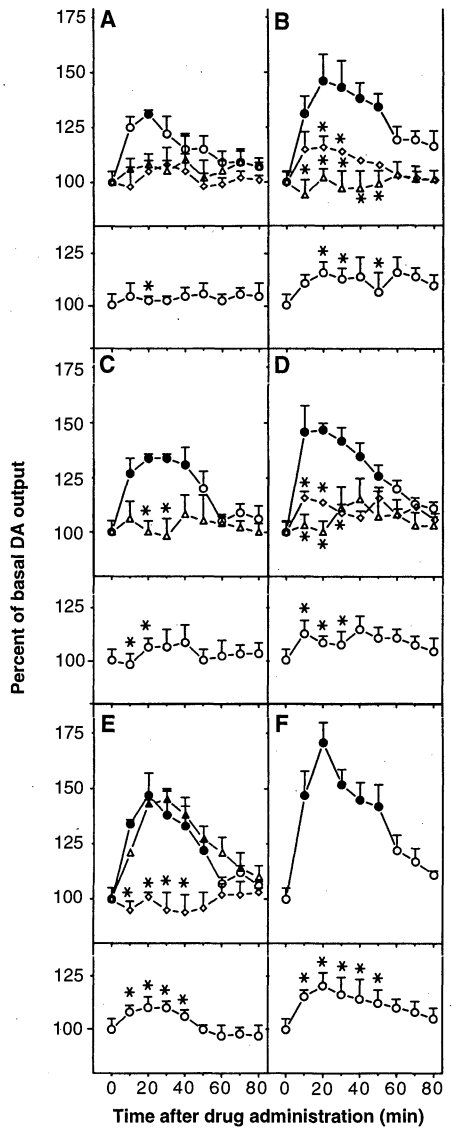

Fig. 1. Effect of intravenous Δ^9-THC, WIN55212-2, and heroin on dialysate DA in the shell (upper panels) and core (lower panels) of the NAc. (**A** and **B**) Δ^9-THC doses of 0.15 and 0.30 mg/kg iv; (**C** and **D**) WIN55212-2 doses of 0.15 and 0.30 mg/kg iv; and (**E** and **F**) heroin doses of 0.018 and 0.030 mg/kg iv. Rats were pretreated with saline (circles) or SR141716-A (triangles) (1 mg/kg sc) or with naloxone (diamonds) (0.1 mg/kg ip). Results are means ± SEM of the amount of DA in 10-min dialysate samples, expressed as percent of basal values. Solid symbols: $P < 0.05$ compared with basal values. Asterisks: $P < 0.05$ compared with the corresponding value obtained in the shell of saline-pretreated controls.

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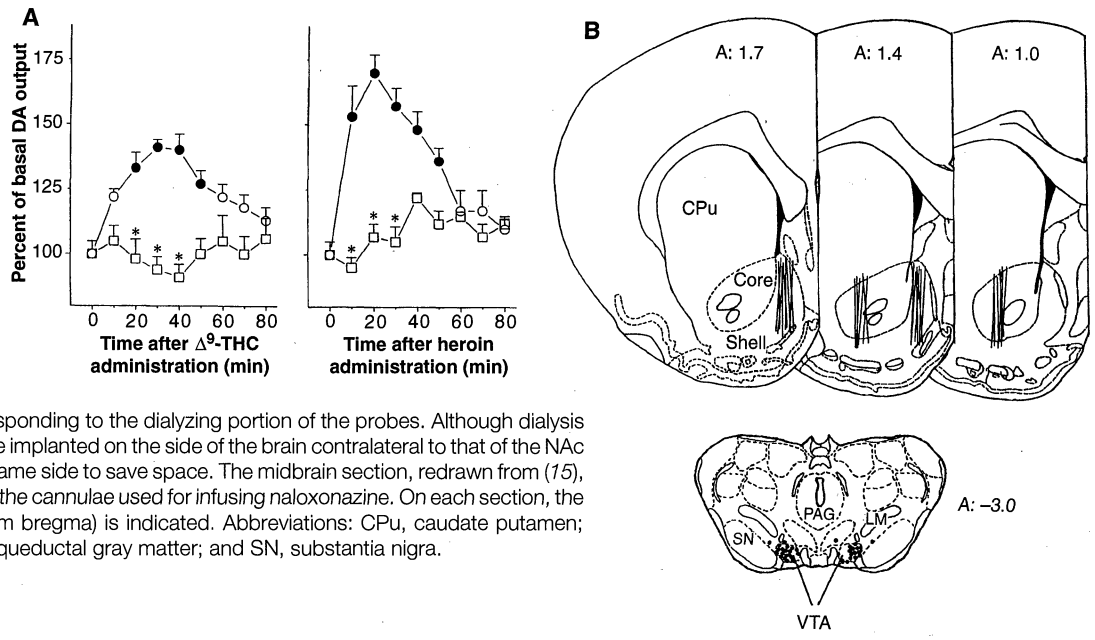

Fig. 2. (**A**) Effect of naloxonazine (squares) or vehicle (circles), bilaterally infused in the VTA, on dialysate DA in the NAc stimulated by Δ^9-THC and by heroin. Results are means ± SEM of the amount of DA found in 10-min dialysate samples expressed as percent of basal values uncorrected for probe recovery. Solid symbols: $P < 0.05$ compared with basal values. Asterisks: $P < 0.05$ compared with the corresponding value of saline-pretreated controls. (**B**) Forebrain sections, redrawn from (*9*), represent the track corresponding to the dialyzing portion of the probes. Although dialysis probes aimed to the NAc shell were implanted on the side of the brain contralateral to that of the NAc core, they are shown here on the same side to save space. The midbrain section, redrawn from (*15*), represents the location of the tip of the cannulae used for infusing naloxonazine. On each section, the anterior coordinate (measured from bregma) is indicated. Abbreviations: CPu, caudate putamen; LM, medial lemniscus; PAG, periaqueductal gray matter; and SN, substantia nigra.

advance) prevented this effect of WIN55212-2 (main effect of group, $F_{1,61} = 48.208$, $P < 0.001$; time × group interaction, $F_{1,45} = 2.953$, $P < 0.01$) (Fig. 1). As in the case of Δ^9-THC, no significant change in dialysate DA was obtained in the core after a WIN55212-2 dose of 0.30 mg/kg iv ($F_{8,45} = 0.73$, $P > 0.05$). Post hoc comparison of the effect of WIN55212-2 on dialysate DA in the NAc shell and in the core showed a preferential effect in the shell at both doses of WIN55212-2 (Fig. 1).

Heroin increased dialysate DA in the NAc shell at doses of 0.018 mg/kg iv ($F_{8,27} = 7.141$, $P < 0.001$) and 0.030 mg/kg iv ($F_{8,27} = 8.9999$, $P < 0.001$) in a dose-related ($F_{1,70} = 12.991$, $P < 0.001$; post hoc, $P < 0.05$) and time-related fashion ($F_{8,63} = 16.008$, $P < 0.001$) (Fig. 1). As in the case of Δ^9-THC and WIN55212-2, heroin was ineffective in modifying DA in the NAc core at doses of 0.018 mg/kg iv ($F_{8,18} = 2.405$, $P < 0.06$) and 0.03 mg/kg iv ($F_{8,27} = 0.983$, $P = 0.47$). Post hoc comparison of the effect of heroin on dialysate DA in the NAc shell and core showed a preferential effect in the shell at both doses of the drug (Fig. 1).

SR141716A antagonized the effects of Δ^9-THC and of WIN5521-2 but failed to modify the effect of heroin ($F_{1,79} = 0.719$, $P = 0.45$). On the other hand, a low dose of the opiate antagonist naloxone [0.1 mg/kg subcutaneously (sc), 15 min in advance] prevented the effect of heroin (main effect of group, $F_{1,61} = 62.94$, $P < 0.001$; group × time interaction, $F_{8,45} = 4.386$, $P < 0.0001$) (Fig. 1).

The same dose of naloxone given 15 min in advance also reduced the effect of Δ^9-THC (0.30 mg/kg iv) on dialysate DA in the NAc shell (main effect of group, $F_{1,61} = 62.940$, $P < 0.001$; post hoc, $P < 0.05$) as well as that of WIN55212-2 (main effect of group, $F_{1,61} = 50.107$, $P < 0.001$; post hoc, $P < 0.05$). Naloxone (0.1 mg/kg sc) by itself failed to modify dialysate DA in the NAc shell.

Naloxonazine, a pseudo-irreversible μ_1 antagonist (*14*), bilaterally infused at the dose of 3 μg per side in the VTA (*15*) 20 to 24 hours before the microdialysis experiment, prevented the effect of Δ^9-THC (0.015 mg/kg iv) and of heroin (0.030 mg/kg iv) on dialysate DA in the NAc shell (vehicle in VTA plus Δ^9-THC iv versus naloxonazine in VTA plus Δ^9-THC iv: main effect of group, $F_{1,61} = 67.72$, $P < 0.001$; post hoc, $P < 0.05$; time × group interaction, $F_{8,45} = 3.293$, $P < 0.005$; vehicle in VTA plus heroin iv versus naloxonazine in VTA plus heroin iv: main effect of group, $F_{1,61} = 68.138$, $P < 0.001$; time × group interaction, $F_{8,45} = 7.540$, $P < 0.001$) (Fig. 2). The effect of naloxonazine was specific because intra-VTA naloxonazine failed to modify the increase of dialysate DA in the NAc by the DA receptor antagonist haloperidol (0.025 mg/kg iv) ($F_{1,58} = 0.231$, $P = 0.635$).

Our data show that Δ^9-THC and its synthetic analog WIN55212-2 increase extracellular DA selectively in the NAc shell but not in the NAc core. This effect was attributable to an action on specific cannabinoid receptors because it was prevented by the CB_1 antagonist SR141716A. This marked topographical selectivity of Δ^9-THC on DA transmission within the NAc may account for the inconsistencies in the effect of Δ^9-THC on DA transmission reported in the literature (*6*).

Δ^9-THC and heroin can now be added to the list of drugs of abuse (morphine, cocaine, amphetamine, and nicotine) that increase DA transmission preferentially in the NAc shell relative to the core (*16*). Given the extensive connections of the NAc shell with limbic brain areas involved in emotion (*10*), the activation of DA transmission in the NAc shell may be involved in the affective and motivational properties of Δ^9-THC.

Although SR141716-A prevented the action of the cannabinoids but not that of heroin, the opioid antagonists naloxone and naloxonazine prevented the effects of both (*17*). Therefore, stimulation of specific cannabinoid receptors may activate DA transmission in the NAc by activating an endogenous opioid system impinging on μ_1 opioid receptors of the VTA (*18*). These homologies between Δ^9-THC and heroin may be relevant to the issue, raised by epidemiological studies, of the relation between the degree and frequency of *Cannabis* use and the probability of subsequent heroin self-administration (*19*). Although our results do not provide direct evidence for a causal relation between *Cannabis* and heroin use, they are nonetheless consistent with this possibility.

REFERENCES AND NOTES

1. R. S. Mansbach, K. L. Nicholson, B. R. Martin, R. L. Balster, *Behav. Pharmacol.* **5**, 219 (1994).
2. L. A. Matsuda, S. J. Lolait, M. J. Brownstein, A. C. Young, T. I. Bonner, *Nature* **346**, 561 (1990).
3. W. A. Devane *et al.*, *Science* **258**, 1946 (1992).
4. U. Ungerstedt, *Acta Physiol. Scand.* (suppl.) **367**, 1 (1971); A. Bjorklund and O. Lindvall, in *Handbook of Chemical Neuroanatomy*, A. Bjorklund and T. Hok-

felt, Eds. (Elsevier, Amsterdam, 1984), pp. 55–122.
5. R. A. Wise and M. A. Bozarth, *Psychol. Rev.* **94**, 469 (1987); G. F. Koob and F. E. Bloom, *Science* **242**, 715 (1988); G. Di Chiara, *Drug Alcohol Depend.* **38**, 95 (1995).
6. In Sprague-Dawley rats, no change in dialysate DA in the NAc was observed after a Δ^9-THC dose of 0.5 mg/kg ip; a 20% increase occurred after a dose of 1.0 mg/kg ip [J. P. Chen *et al., Neurosci. Lett.* **129**, 136 (1991)]. No change in dialysate DA in the NAc and in the caudate-putamen of Long-Evans rats was observed after Δ^9-THC doses of 1.0 and 10.0 mg/kg administered by gavage [E. Castañeda *et al., Pharmacol. Biochem. Behav.* **40**, 587 (1991)]. In the Lewis strain of rats, however, Δ^9-THC has been reported to increase dialysate DA in the NAc at doses of 0.5 and 1.0 mg/kg ip (*20*).
7. D. R. Compton, L. H. Gold, S. J. Ward, R. L. Balster, B. R. Martin, *J. Pharmacol. Exp. Ther.* **263**, 1118 (1992); L. H. Gold, R. L. Balster, R. L. Barrett, D. T. Britt, B. R. Martin, *ibid.* **262**, 479 (1992).
8. M. Rinaldi-Carmona *et al., FEBS Lett.* **350**, 240 (1994); J. L. Wiley, J. A. Lowe, R. L. Balster, B. R. Martin, *J. Pharmacol. Exp. Ther.* **275**, 1 (1995).
9. Concentric dialysis probes were prepared with AN 69 fibers (Hospal Dasco, Italy) as described (*21*). All animal experimentation was conducted in accordance with European Economic Community guidelines for care and use of experimental animals. Male Sprague-Dawley rats (280 to 300 g; Charles River, Calco, Como, Italy) were anesthetized with ketamine (100 mg/kg ip) and placed in a stereotaxic apparatus. The skull was exposed and a small hole drilled to expose the dura on each side. Each rat was implanted with one dialysis probe on each side, aimed at the NAc shell on one side and at the core on the other side, according to the rat brain atlas of G. Paxinos and C. Watson [*The Rat Brain in Stereotaxic Coordinates* (Academic Press, Sydney, 1987)] [uncorrected coordinates: shell, A = +2.0, L = 1.4, V = 8.0; core, A = +1.4, L = 2.0, V = 7.8 (A, anterior; L, lateral; and V, ventral)]. Under halothane anesthesia, a polyethylene catheter was inserted into the right femoral vein and then tunneled subcutaneously to exit at the nape of the neck. A femoral vein was catheterized and experiments were performed 24 hours after implant of probes. Ringer's solution (147 mM NaCl, 2.2 mM $CaCl_2$, and 4 mM KCl) was pumped through the dialysis probes at a constant rate of 1 μl/min. Dialysate samples (10 μl) were taken every 10 min and injected without purification into a reversed-phase high-performance liquid chromatography apparatus (LC-18 DB, 15 cm, particle size 5 μm; Supelco) and a coulometric detector (ESA; Coulochem II, Bedford, MA) to quantify dopamine. The oxidation and reduction electrodes of the detector were set at +130 mV and −175 mV, respectively. The mobile phase contained 50 mM NaH_2PO_4, 0.1 mM Na_2EDTA, 0.5 mM *n*-octyl sodium sulfate, and 18% (v/v) methanol (pH adjusted to 5.5 with Na_2HPO_4). The mobile phase was pumped with an LKB 2150 pump at 1.0 ml/min. Assay sensitivity for dopamine was 2 fmol per sample.
10. L. Heimer *et al., Neuroscience* **41**, 89 (1991).
11. Δ^9-THC and cannabinol (Makor Chemicals, Jerusalem, Israel) and WIN55212-2 (RBI Chemicals, Amersham, Milano, Italy) were suspended in 0.3% Tween 80 in saline and administered iv (1 ml/kg).
12. Heroin (Salars, Milano, Italy) was dissolved in saline with the aid of a drop of glacial acetic acid and administered iv (1 ml/kg).
13. SR141716A was suspended in 0.3% Tween 80 in saline and administered ip (3 ml/kg). Naloxone (Sigma, Milano, Italy) was dissolved in saline and administered sc (1 ml/kg).
14. E. F. Hahn, M. Carroll-Buatti, G. W. Pasternak, *J. Neurosci.* **2**, 572 (1982); N. Johnson and G. W. Pasternak, *Mol. Pharmacol.* **26**, 477 (1984).
15. Naloxonazine (RBI Chemicals) was infused bilaterally into the VTA through stainless steel cannulas (uncorrected coordinates; A = −3.0 from bregma, L ± 1.0, V = −8.5 from dura) [L. J. Pellegrino *et al., A Stereotaxic Atlas of the Rat Brain* (Plenum, New York, 1979)].
16. F. E. Pontieri, G. Tanda, F. Orzi, G. Di Chiara, *Nature* **382**, 255 (1996); see also (*21*).
17. The observation that in the Sprague-Dawley rats naloxone reduces the effect of Δ^9-THC on dialysate DA in the NAc is consistent with observations in Lewis rats (*20*).
18. S. W. Johnson and R. A. North [*J. Neurosci.* **12**, 483 (1992)] provided electrophysiological evidence for the existence of non-DA neurons in the VTA that tonically inhibit the DA neurons and are depressed by μ-opiates such as morphine. This neural system might also be the substrate of the DA-stimulating action of Δ^9-THC. Δ^9-THC may promote the release of endogenous opioids in the VTA, but its primary site of action could be outside the VTA, on neurons projecting to the VTA.
19. R. R. Clayton and H. L. Voss, *NIDA Res. Monograph 39* (U.S. Government Printing Office, Washington, DC, 1981); J. A. O'Donnell and R. R. Clayton, *Behav. Biomed. Issues* **4**, 229 (1982); D. Kandel, *Arch. Gen. Psychiatry* **41**, 200 (1984).
20. J. P. Chen *et al., Psychopharmacology* **102**, 156 (1990).
21. F. E. Pontieri, G. Tanda, G. Di Chiara, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 12304 (1995).
22. We thank R. Frau, P. Loddo, and A. Marchioni for expert assistance, and P. Soubrié for providing SR141716A. Supported by funds from CNR, "Disease Factors" project, from Ministero Ricerca Scientifica and from European Community project BMH 4-CT 96-0203.

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Activation of Corticotropin-Releasing Factor in the Limbic System During Cannabinoid Withdrawal

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Corticotropin-releasing factor (CRF) has been implicated in the mediation of the stress-like and negative affective consequences of withdrawal from drugs of abuse, such as alcohol, cocaine, and opiates. This study sought to determine whether brain CRF systems also have a role in cannabinoid dependence. Rats were treated daily for 2 weeks with the potent synthetic cannabinoid HU-210. Withdrawal, induced by the cannabinoid antagonist SR 141716A, was accompanied by a marked elevation in extracellular CRF concentration and a distinct pattern of Fos activation in the central nucleus of the amygdala. Maximal increases in CRF corresponded to the time when behavioral signs resulting from cannabinoid withdrawal were at a maximum. These data suggest that long-term cannabinoid administration alters CRF function in the limbic system of the brain, in a manner similar to that observed with other drugs of abuse, and also induces neuroadaptive processes that may result in future vulnerability to drug dependence.

Cannabis continues to be a major drug of abuse, and as many as 9% of *Cannabis* users may meet criteria for substance dependence (*1*). Short-term exposure to *Cannabis* derivatives (hashish, marijuana) produces subjective emotional responses ranging from mild relaxation to panic reactions (*1, 2*); long-term use of *Cannabis* may result in mental lethargy and anhedonia (*3*). A clear-cut abstinence syndrome is rarely reported, presumably because of the long half-life of cannabinoids, which precludes the emergence of abrupt abstinence symptoms (*1*), although nervousness, tension, restlessness, sleep disturbances, and anxiety have been described in humans, monkeys, and rats after termination of long-term cannabinoid administration (*4*). A distinct abstinence syndrome can, however, be elicited in animals treated with cannabinoids over a long period (*5*) by administering a competitive cannabinoid antagonist (*6*). This antagonist-precipitated withdrawal may unmask the development of underlying neuroadaptive processes that contribute to the development of cannabinoid dependence. The neurobiological substrates of cannabinoid-induced emotional responses remain to be elucidated, although they are likely to be mediated by activation of CB_1 cannabinoid receptors, which are present in the limbic system and brain nuclei that have been implicated in stress responses (*7*). Psychotropic cannabinoids are potent activators of the hypothalamic-pituitary-adrenal (HPA) axis (*8*), and this property may contribute to the unpleasant side effects described by users of *Cannabis*.

A common element of withdrawal from drugs of abuse is a negative affective state that is characterized in humans by dysphoria and anxiety and in animals by a reward deficit and enhanced behavioral reactivity to stressors (*9*). We report here that cannabinoid withdrawal, induced by administration of a cannabinoid CB_1 antagonist, re-

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