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Ventral tegmental microinjection of Δ^9-tetrahydrocannabinol enhances ventral tegmental somatodendritic dopamine levels but not forebrain dopamine levels: evidence for local neural action by marijuana's psychoactive ingredient

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Basal extracellular levels of dopamine (DA) and its metabolites in both ventral tegmental area (VTA) and nucleus accumbens (Acb) were simultaneously monitored by in vivo brain microdialysis in laboratory rats. Microinjection of 12 μg or 24 μg Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive ingredient in marijuana and hashish, into the VTA produced a dose-dependent increase in somatodendritic DA levels in VTA but failed to produce any simultaneous change in extracellular DA in Acb. Direct Δ^9-THC perfusion (5×10^{-5} and 2×10^{-4} M) into Acb via the microdialysis probes caused a significant increase in extracellular DA levels in Acb. These findings suggest that (1) Δ^9-THC has a facilitating effect on somatodendritic DA efflux, and (2) the elevation of Acb DA levels seen in our previous studies with systemic administration of Δ^9-THC may result from local actions of Δ^9-THC at or near the DA terminal projections in Acb.

INTRODUCTION

Several studies have shown that Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive principle of marijuana, augments functional extracellular dopamine (DA) levels in the brain. Thus, Δ^9-THC inhibits dopamine (DA) uptake[1,14,16] and increases both synthesis[2] and release[24] of DA in synaptosomes. Using in vivo techniques, we have previously reported that Δ^9-THC augments brain-stimulation reward[12], which is known to critically involve a mesotelencephalic DA substrate[9,31]. We have also shown, again using in vivo techniques, that Δ^9-THC enhances potassium-evoked DA efflux in caudate-putamen[21] and basal extracellular DA levels in both nucleus accumbens (Acb)[7] and medial prefrontal cortex[8]. The exact neural locus of activation of the mesotelencephalic DA system by Δ^9-THC remains unclear, however, since Δ^9-THC was administered systemically in previous in vivo studies[7,8,12,21]. Two working hypotheses become apparent: (1) Δ^9-THC might act at presynaptic DA terminals, inhibiting DA re-uptake or inducing DA release; or (2) Δ^9-THC might activate DA neurons in the ventral mesencephalon, the axons of which project to the forebrain, thereby increasing forebrain DA synthesis and terminal release. To shed light on these two possibilities, the present study was designed to examine the effects of direct administration of Δ^9-THC into the ventral tegmental area (VTA), the DA nuclear cell group for the mesolimbic DA projections to Acb, on extracellular DA levels in both the somatodendritic area in VTA and the terminal area in Acb, monitored simultaneously by using two in vivo brain microdialysis probes in the same rats. Additionally, direct administration of Δ^9-THC was also made into the Acb, and local extracellular Acb DA levels were monitored by in vivo brain microdialysis.

MATERIALS AND METHODS

Male Lewis rats (Charles River) weighing 250–300 g were used. For the first experiment (local VTA Δ^9-THC administration), rats were implanted under sodium pentobarbital anesthesia (50 mg/kg)

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with two microdialysis probes, one in the VTA and one in the Acb, using standard surgical and stereotaxic techniques. Concentric microdialysis probes were used, having exposed cellulose dialysis membranes (Spectrum, MCO 6000) of 1 and 2 mm for VTA and Acb, respectively. A 25 μl microsyringe with 225 μm outside diameter needle was pre-loaded with Δ^9-THC solution or vehicle and implanted into the VTA immediately adjacent and lateral to the VTA microdialysis probe, with the microsyringe needle cemented parallel to the microdialysis probe such that the needle tip was positioned halfway down the exposed piece of microdialysis membrane in the VTA (see Fig. 1). The stereotaxic implant coordinates (according to the atlas of Paxinos and Watson[22]) relative to bregma were: A -5.3, L 1.0, V -7.7 (from dura) for VTA, and A 2.0, L 1.2, V -8.0 (from dura) for Acb. A 3 h recovery time was allowed following implantation surgery before sample collection started. Animals were maintained under surgical anesthesia for the duration of microdialysis sample collections. For the second experiment (local Acb Δ^9-THC administration), rats were implanted with microdialysis probes (2 mm exposed dialysis membrane) in Acb 20–24 h prior to Δ^9-THC administration at the same stereotaxic coordinates as in the first experiment. Animals were allowed to recover from surgery and the experiment was carried out on conscious, freely moving rats. The brain perfusate, a modified Ringer's solution (165 mM NaCl, 3.7 mM KCl, 2.5 mM $CaCl_2$, pH adjusted to 7.4) was pumped through the probes at a flow rate of 2 μl/min. Dialysis samples (40 μl) were collected every 20 min and immediately assayed for DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by high-performance liquid chromatography with electrochemical detection. The compounds were separated by reverse-phase chromatography with a Brownlee Velosep RP-18 (3 μm particles) 100×3.2 mm column and a mobile phase containing 0.1 M chloroacetic acid, 0.1 mM EDTA, 0.2 mM sodium octyl sulfate, and 6–6.5% methanol, with pH adjusted to 3.0 with 10 N NaOH. The mobile phase flow rate was 1.0 ml/min. Electrochemical measurements were made with a dual glassy carbon electrode (Bioanalytical Systems) coupled to two LC-4C (Bioanalytical Systems) detectors, one for detection of DA and the other for DA metabolites, set at $+0.7$V.

Δ^9-THC (National Institute on Drug Abuse) was evaporated with nitrogen and then dissolved in 10% v/v emulphor, 10% v/v propylene glycol and 80% v/v perfusate. This mixed suspension was then further diluted with the modified Ringer's solution to a final suspension containing 4 μg/μl or 8 μg/μl Δ^9-THC for the first experiment. For the second experiment, Δ^9-THC was prepared in the same way and diluted to 5×10^{-5} and 2×10^{-4} M as the final administered concentrations. Vehicle solutions were prepared by the same procedure, but without the addition of Δ^9-THC.

For the first experiment, following the 3 h recovery period after surgery, sample collection was started and continued until 3 consecutive stable 20 min samples were collected simultaneously from both VTA and Acb. Then, microinjection of Δ^9-THC or vehicle into the VTA was made. 3.0 μl of the Δ^9-THC suspension (4 μg/μl or 8 μg/μl for a total injection of 12 or 24 μg Δ^9-THC) were slowly injected by hand over a 10 min period. Manual microsyringe injection was used instead of an automated microinjection pump because Δ^9-THC binds readily to the plastic tubing of automated microinjection apparatuses. Supplemental sodium pentobarbital (14 mg/kg) was given every hour to maintain a steady state of anesthesia throughout the experiment, and body temperature was maintained at 37°C. Possible influences of sodium pentobarbital itself on VTA and Acb DA levels were excluded by control experiments in which repeated intraperitoneal administrations of 14 mg/kg sodium pentobarbital were given to rats every hour for 4 consecutive hours without any significant changes in extracellular levels of DA and its metabolites in either VTA or Acb (data not shown).

For the second experiment, after baselines for DA, DOPAC and

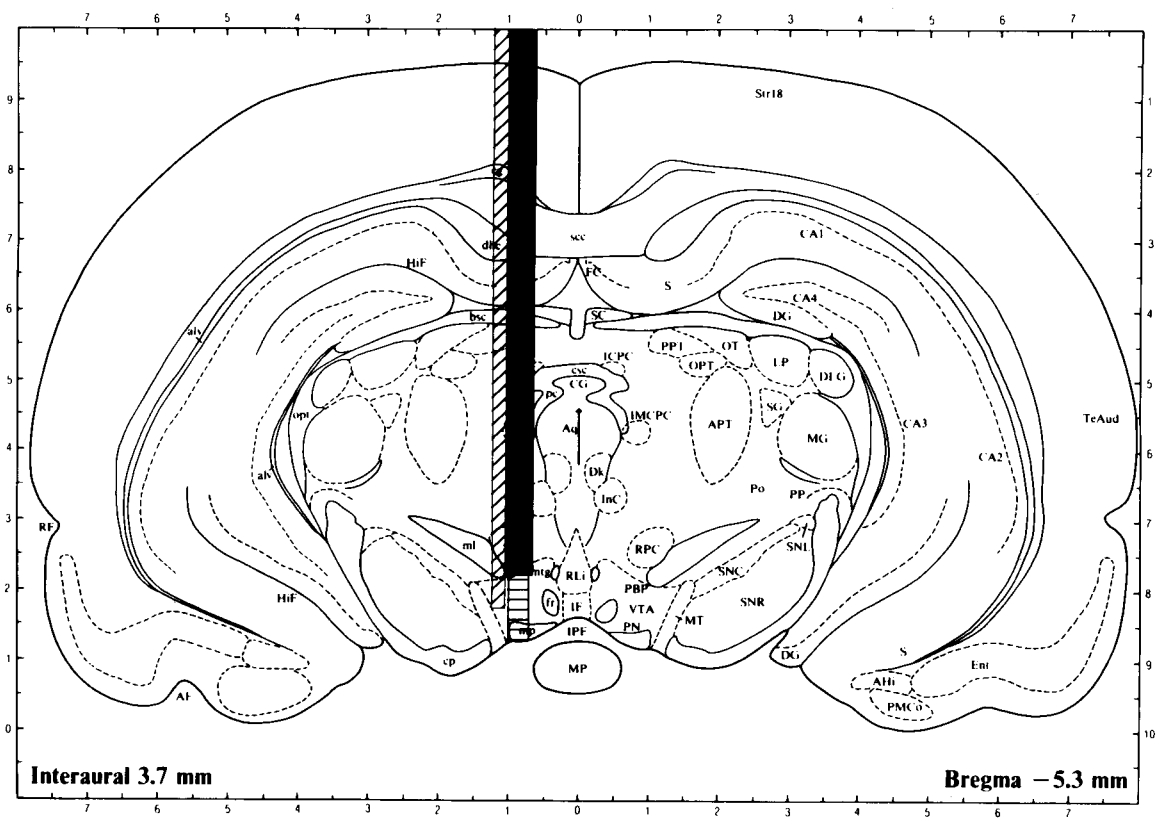

Fig. 1. Reconstruction of representative implant of microdialysis probe and microinjector in ventral tegmental area (VTA), mapped according to the brain atlas of Paxinos and Watson[22]. The impermeable stainless-steel portion of the microdialysis probe is shown in solid black. The 1 mm exposed microdialysis tubing, shown in horizontal hatching, extended vertically over the entire extent of the VTA. The microinjector needle, shown in diagonal hatching, was cemented to the stainless-steel tubing of the microdialysis probe, with needle tip positioned immediately lateral to and halfway down the vertical extent of the exposed microdialysis tubing.

HVA were established, perfusate containing Δ^9-THC (5×10^{-5} or 2×10^{-4} M) was perfused through the dialysis probes for 20 min and then switched back to normal perfusate. Six more samples were then collected.

Mean levels of DA, DOPAC, and HVA of the three pre-injection samples were used as baselines and analysis was performed on percent changes after drug or vehicle injections. Data were analyzed by two-way analysis of variance (ANOVA) for repeated measures, followed by post-hoc Dunnett and Tukey–Kramer tests of individual comparisons[17]. Standard histology was used to verify dialysis probe locations after the completion of experiments.

RESULTS

For the first experiment (anesthetized rats; local Δ^9-THC administration into VTA), basal DA and metabolite levels (uncorrected for probe recovery rate) preceding drug or vehicle injection were 46.5 ± 3.9 fmol/40 μl/20 min DA ($n = 13$), 1.26 ± 0.13 pmol/40 μl/20 min DOPAC ($n = 13$), and 2.31 ± 0.20 pmol/40 μl/20 min HVA ($n = 13$) for VTA, and 58.7 ± 6.9 fmol/40 μl/20 min DA ($n = 13$), 14.94 ± 1.24 pmol/40 μl/20 min DOPAC ($n = 13$), and 2.72 ± 0.31 pmol/40 μl/20 min HVA ($n = 13$) for Acb. For the second experiment (conscious, freely moving rats; local Δ^9-THC administration into Acb), basal DA and metabolite levels (uncorrected for probe recovery rate) preceding drug perfusion were 24.8 ± 3.8 fmol/40 μl/20 min DA ($n = 9$), 6.74 ± 0.51 pmol/40 μl/20 min DOPAC ($n = 9$), and 2.91 ± 0.48 pmol/40 μl/20 min HVA ($n = 9$) for Acb. Local VTA microinjections of vehicle did not alter extracellular DA, DOPAC, or HVA levels in either VTA or Acb (Figs. 2 and 3). As shown in Fig. 2, however, local VTA microinjection of 12 μg and 24 μg Δ^9-THC caused significant dose-dependent increases in extracellular somatodendritic DA overflow in VTA (Δ^9-THC effect, $F_{2,10} = 7.38$, $P < 0.05$; time effect, $F_{5,50} = 74.6$ $P < 0.0001$; drug × time interaction effect, $F_{10,50} = 38.8$ $P < 0.0001$), with 24 μg Δ^9-THC causing an increase to approximately 1,300% of pre-in-

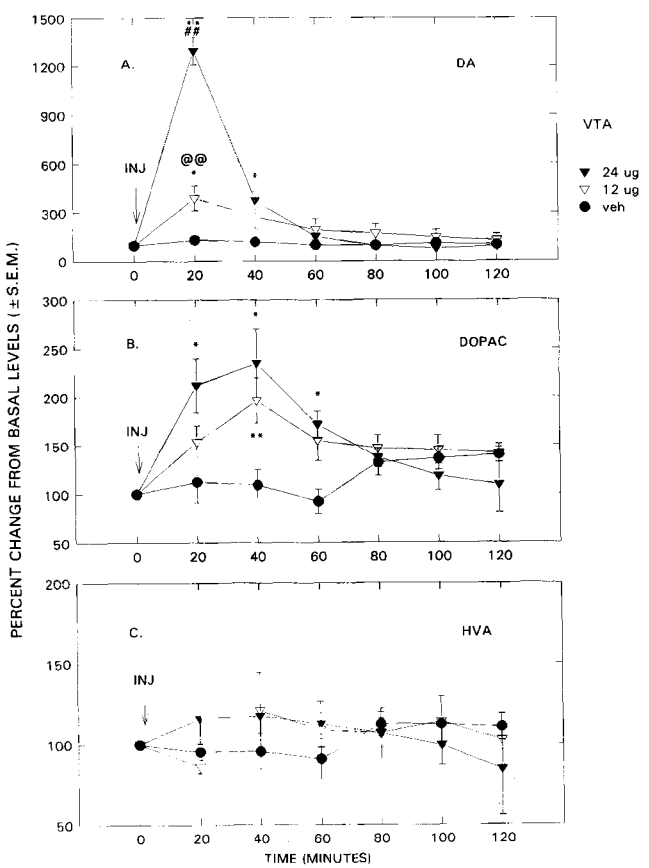

Fig. 2. Effects of local microinjection of Δ^9-THC (12 or 24 μg) or vehicle into ventral tegmental area (VTA) on somatodendritic levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in VTA. Values are expressed as mean percentages (±S.E.M., $n = 4$–5 for each group) of pre-injection basal levels. Asterisks signify statistical significance of individual Dunnett comparisons with pre-injection basal level (* $P < 0.05$, ** $P < 0.01$). ## Statistical significance of individual Tukey–Kramer comparisons between individual Δ^9-THC data points and vehicle data points ($P < 0.01$). @@ Statistical significance of individual Tukey–Kramer comparisons between individual high dose and low dose Δ^9-THC data points ($P < 0.01$).

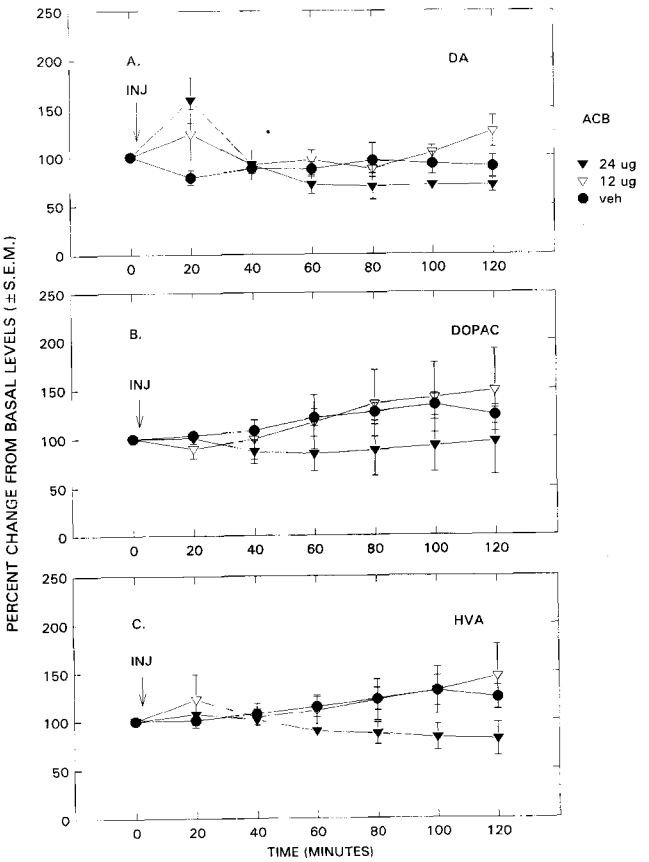

Fig. 3. Effects of local microinjection of Δ^9-THC (12 or 24 μg) or vehicle into ventral tegmental area (VTA) on extracellular levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in nucleus accumbens (Acb). Values are expressed as mean percentages (±S.E.M., $n = 4$–5 for each group) of pre-injection basal levels. There were no statistically significant changes in extracellular levels of DA, DOPAC or HVA in Acb after administration of Δ^9-THC into VTA.

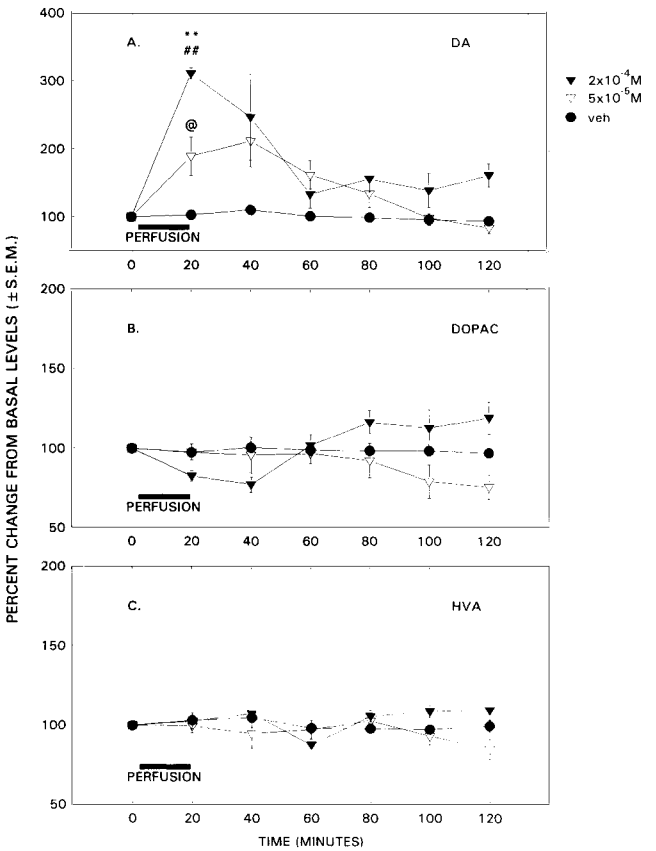

Fig. 4. Effects of local perfusion of Δ^9-THC (5×10^{-5} or 2×10^{-4} M) or vehicle into nucleus accumbens (Acb) on extracellular levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in Acb. Values are expressed as mean percentages (± SEM, $n = 3$ for each group) of pre-perfusion basal levels. ** Statistical significance of individual Dunnett comparisons with pre-injection basal level ($P < 0.01$). ## Statistical significance of individual Tukey–Kramer comparisons between individual Δ^9-THC data points and vehicle data points ($P < 0.01$). @ Statistical significance of individual Tukey–Kramer comparisons between individual high dose and low dose Δ^9-THC data points ($P < 0.05$). There were no statistically significant changes in extracellular levels of DOPAC or HVA in Acb after administration of Δ^9-THC into Acb.

jection basal DA levels. Δ^9-THC also produced a dose-dependent increase in the extracellular levels of DOPAC in VTA, but no significant change in extracellular HVA levels in VTA (Fig. 2). In contrast, microinjection of Δ^9-THC into the VTA did not significantly alter extracellular levels of DA, DOPAC or HVA in Acb (Fig. 3). Local Acb perfusion of vehicle did not alter extracellular DA, DOPAC, or HVA levels in Acb (Fig. 4), however, local perfusion of Δ^9-THC into Acb caused a dose-dependent increase in extracellular DA levels in Acb (Δ^9-THC effect, $F_{2,6} = 6.35$, $P < 0.05$; time effect, $F_{5,10} = 8.80$, $P < 0.0001$; drug × time interaction effect, $F_{10,30} = 2.97$, $P < 0.001$) without significantly altering DOPAC or HVA levels in Acb (Fig. 4).

DISCUSSION

The present findings of Δ^9-THC-induced augmentation of extracellular DA overflow in VTA appear to be in fundamental agreement with previous reports from other groups that Δ^9-THC augments extracellular DA neurotransmitter levels in both in vitro and in vivo preparations[1,24,27], and in close agreement with our own previous in vivo findings that Δ^9-THC augments both potassium-evoked and basal extracellular DA overflow in forebrain DA loci[7,8,21], although the present study is the first to explore Δ^9-THC's effects in the ventral mesencephalic DA nuclear groups.

Very few studies have reported on either basal or drug-influenced extracellular DA levels in the DA nuclear groups of the ventral mesencephalon, and it is difficult to compare the presently observed basal extracellular levels of DA and its metabolites in VTA to values reported by others, due to differences in microdialysis probe recovery rates and experimental conditions. For example, the basal values we observed in the present study are almost 3-times the basal extracellular DA levels in VTA reported by Bradberry and Roth[5]. One cause for this difference might well be the higher calcium concentration (2.5 mM) in the present perfusate, making the present 'basal' DA levels to some degree calcium-evoked levels. It should be also noted that the microdialysis probe placement used in the present study was 1.0 mm distant within the VTA from the site sampled by Bradberry and Roth[5].

Δ^9-THC is the principal psychoactive constituent of marijuana, the most widely used illicit habit-forming drug in the world[19]. That habit-forming drugs act as either direct or indirect DA agonists on a portion of the mesotelencephalic DA systems involved in the neural mediation of reward and reinforcement, and derive their euphorigenic properties and abuse potential therefrom, is at present the most compelling hypothesis of the neurobiology of drug abuse[9,18,28,30,31]. Many lines of evidence support this hypothesis. First, virtually all habit-forming drugs augment electrical brain-stimulation reward in brain loci associated with the mesotelencephalic DA system[9]. Second, most indirect DA agonists are euphorigenic (and have consequent habit-forming potential) while most DA antagonists are dysphorigenic[29,31] (*direct* DA agonists, while self-administered by rodents, dogs, and monkeys, appear to have low habit-forming potential in humans, perhaps because in humans they additionally activate other brain systems mediating nausea, emesis, and other subjectively unpleasant effects). Third, virtually all habit-forming drugs augment extracellular DA synaptic levels in the mesotelencephalic DA system[9]. Fourth, animals

will voluntarily self-administer habit-forming drugs into mesotelencephalic DA brain loci, but for the most part not into other brain loci[4,13,15,23,31]. Fifth, lesions or pharmacological blockade of the mesotelencephalic DA system dramatically inhibit the reinforcement derived from voluntary systemic (e.g. intravenous) self-administration of habit-forming drugs[4,20,25,26,32].

Within this context of the DA neural substrates of reinforcement and reward activated by habit-forming drugs, it is clear that habit-forming drugs of different classes activate the mesotelencephalic DA reward circuitry by varying and different mechanisms. Thus, amphetamines appear to release DA from presynaptic synthesis pools, cocaine to block presynaptic re-uptake of DA, and opiates to augment synaptic release of DA by increasing neuronal firing of presynaptic DA neurons[9]. The questions obviously arise – at which neural locus and by which neural mechanism within the mesotelencephalic DA system does Δ^9-THC act?

While we[7,8,21] and others[27] have previously reported that Δ^9-THC augments extracellular DA levels in mesotelencephalic DA projection loci, with particular sensitivity in the mesolimbic DA fibers projecting to the Acb[7,8], the specific neuroanatomical locus of action within the mesolimbic DA system has remained obscure. Few definitive conclusions can be made when Δ^9-THC is given systemically. Therefore, in the present study, direct application of Δ^9-THC into the somatodendritic region of the DA neurons in VTA was made. Noteworthily, such application augmented somatodendritic DA levels in VTA but failed to augment axon terminal DA levels of the same neural population in Acb. With respect to the lack of effect in Acb, two possibilities exist: (1) that Δ^9-THC augments neuronal firing of DA neurons but that the Δ^9-THC doses used in the present study were not sufficient to produce such augmentation and resulting augmented terminal release of DA in Acb; or (2) that Δ^9-THC acts locally on DA nerve membranes to either release DA or prevent DA re-uptake within just the local area of Δ^9-THC diffusion. We favor the latter interpretation for many reasons. First, the present Δ^9-THC doses were sufficiently large to produce enormous augmentation of somatodendritic extracellular DA overflow (to approximately 400% of pre-drug baseline by 12 μg Δ^9-THC and to approximately 1,300% of pre-drug baseline by 24 μg Δ^9-THC - see Fig. 2). If such augmentation of somatodendritic extracellular DA overflow were in any way correlated with augmented neuronal firing, increased extracellular DA overflow from Acb terminals should have been evident. Second, as noted above, in vitro studies have shown that Δ^9-THC inhibits the re-uptake of DA[1,14,16], suggesting a localized synaptic action rather than a generalized action on neuronal firing patterns. Third, previous in vivo brain electrochemistry studies show that Δ^9-THC produces augmented voltammetric signals corresponding to extracellular DA that are identical to the augmented voltammetric signals produced by nomifensine, a well-characterized DA re-uptake blocker[21]. Fourth, previous in vivo microdialysis studies from this laboratory demonstrate an additive or even synergistic effect on extracellular DA overflow in Acb between Δ^9-THC and haloperidol-induced augmentation of presynaptic axonal firing[10], which is congruent with Δ^9-THC acting (perhaps indirectly) as a DA re-uptake blocker. Fifth, direct local Δ^9-THC administration into the Acb caused a significant increase in extracellular DA levels in Acb in the present study. Sixth, as noted above, *systemic* Δ^9-THC administration produces significant augmentation of extracellular DA overflow in Acb[7]. Of course, the present data would also be compatible with a *combined* action by Δ^9-THC on *both* somatodendritic DA release or re-uptake blockade *and* augmentation of neuronal firing, with the latter effect counteracted by activation of somatodendritic DA autoreceptors which inhibit DA neuronal firing[6].

In summary, the present findings appear to be compatible with the hypothesis that Δ^9-THC acts locally within DA-releasing regions of the mesotelencephalic DA system to augment synaptic DA levels. To the extent that such actions are localized within subportions of the mesotelencephalic DA system mediating reward and reinforcement, such actions would appear to contribute to marijuana's euphorigenic actions and its abuse liability.

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