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Strain-specific facilitation of dopamine efflux by Δ^9-tetrahydrocannabinol in the nucleus accumbens of rat: an in vivo microdialysis study

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This study tests the hypothesis that Δ^9-tetrahydrocannabinol (Δ^9-THC) has a strain-specific facilitatory effect on dopamine (DA) efflux in rat nucleus accumbens, a crucial forebrain convergence of reward-relevant DA neural fibers that has been implicated as a focal brain locus mediating the euphorigenic properties of drugs of abuse. The dependent variable is presynaptic DA efflux measured by in vivo microdialysis in the nucleus accumbens. The independent variables are: (1) intraperitoneal injections of Δ^9-THC at 0.0 (vehicle), 0.5 and 1.0 mg/kg; (2) Sprague–Dawley vs Lewis strain rat. Results show that Δ^9-THC produces a dose-dependent, strain-specific enhancement of basal DA efflux in Lewis strain rats. These results suggest that genetic variation influences drug abuse vulnerability.

For many drugs of abuse (e.g. ethanol, morphine), genetic differences influence both drug preference and propensity for drug self-administration [1, 6, 11, 14, 16]. For example, mouse strains that show high ethanol preference and high ethanol self-administration appear to generalize this increased vulnerability to other drugs of abuse such as nicotine and opiates [5, 6, 10]. In addition, several inbred rat strains exhibit high ethanol preference and self-administration [1]. This suggests that some genetically inbred rat strains may have a generalized vulnerability to the rewarding effects of recreational and abused drugs.

Lewis strain rats have a high vulnerability for both ethanol and cocaine oral self-administration [5, 8]. Similarly, using the intracranial self-stimulation (ICSS) brain reward paradigm as a bio-behavioral probe, we demonstrated that Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive component of marijuana, acutely facilitates brain stimulation reward in the Lewis strain rat but not in Sprague–Dawley, Long Evans, or Fischer-344 strain rats [4].

While our previous work appears to implicate genetic variation in the neurobiological substrates of Δ^9-THC abuse using the ICSS brain reward paradigm, the present in vivo microdialysis study attempts to see if the strain-specific brain reward enhancing effects of Δ^9-THC mirrors the presynaptic dopamine (DA) efflux in the nucleus accumbens, a crucial anatomic convergence of brain-reward-relevant DA circuitry.

Male Sprague–Dawley (Taconic) and Lewis rats (Charles River) weighing between 300 and 350 g were used for the experiments. All rats were implanted under sodium pentobarbital anesthesia (50 mg/kg) with microdialysis probes in the left nucleus accumbens. The probes were implanted at stereotaxic coordinates [13] relative to bregma; A +2.0, L 1.2, and V −7.0 (from dura). Loop-shaped microdialysis probes [15] were implanted longitudinally parallel to the mid-sagittal line and positioned in the midline of the medial-lateral extent of the nucleus accumbens. Each dialysis probe had 5 mm of exposed dialysis membrane (Cellulose membrane, MWCO 5000, Spectrum Medical Industries) that covered the vertical extent of the nucleus accumbens. Before implantation, each dialysis probe was tested in vitro, and percent recoveries for DA and its metabolites established. Relative in vitro recovery rates for the probes used in these experiments were approximately 6% for DA, 8% for 3,4-dihydroxyphenylacetic acid (DOPAC) and 9% for homovanillic acid (HVA). Immediately following implantation, probe perfusion was initiated and continued for the duration of the experiment. All rats were given 20–24 h

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for recovery from surgery before sample collection was started in the freely moving preparation. The perfusate was a modified [9] Ringers solution (189 mM NaCl, 3.9 mM KCl, 3.37 mM $CaCl_2$ (pH = 6)), perfused through the dialysis probes at a flow rate of 2 μl/min.

Dialysis samples (40 μl) were collected every 20 min and immediately assayed for DA, DOPAC and 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) 100 × 3.2 mm column and a mobile phase (pH = 3.0) containing 0.1 M chloroacetic acid, 0.1 mM EDTA, 0.2 mM sodium octyl sulfate, and 6.5% (v/v) methanol. The mobile phase flow rate was 1.0 ml/min. Electrochemical measurements were made with a glassy carbon working electrode (Bioanalytical Systems model TL-5A) coupled to a LC-4B detector (Bioanalytical Systems) set at +0.7 V. The minimum detection limit was approximately 6–16 fmol for all chemicals measured.

Δ^9-THC (NIDA, USPHS) was prepared for in vivo administration by an adaptation of the method of Fenimore and Loy [2]. Briefly, the Δ^9-THC was dissolved in a 20% solution of polyvinylpyrrolidone (PVP; Aldrich Chemical Company) prepared in ethanol. This mixed solution was then evaporated under nitrogen and the dried, concentrated Δ^9-THC-PVP solution was suspended in saline at a concentration of either 0.5 or 1.0 mg/ml. A 15% PVP solution was used for vehicle injections. All drugs and vehicle solutions were administered by intraperitoneal injection (i.p.).

Three stable 20 min baseline dialysis samples were collected prior to drug or vehicle injections, and dialysis sampling continued for 2 h postinjection. Mean levels of DA, DOPAC, and HVA for the three 20 min dialysis samples immediately preceding drug or vehicle administration were designated as baseline, and the remaining data were expressed as percent change from baseline after drug or vehicle injection. Statistical comparisons were made by analysis of variance with time as the within subjects factor and both drug and strain as between subjects factor. Post-hoc comparisons were made with the Tukey–Kramer test. Student *t*-tests (unpaired) were used to compare basal DA and metabolite levels between Lewis and Sprague–Dawley rats and also to make within strain comparisons.

After the completion of experiments, animals were sacrificed by anesthetic overdose. Their brains were removed, and standard histology was carried out to verify that the 5 mm of exposed dialysis membrane was localized within the borders of the nucleus accumbens.

Table I shows that pretreatment baseline levels of DA and metabolites do not differ between Lewis and Sprague–Dawley rats. However, response to Δ^9-THC was markedly different between the two strains, as illustrated in Figs. 1 and 2. Analysis of DA efflux data across strain, drug, and time shows a significant effect of strain ($F_{1,20} = 42.82$, $P < 0.0005$), a significant drug effect ($F_{2,20} = 38.18$, $P < 0.0005$), and a significant interaction ($F_{2,20} = 10.60$, $P < 0.001$) of drug × strain (Fig. 1A,B). Subsequent analysis of strain at 0.0 mg/kg Δ^9-THC (vehicle) is not significant ($P > 0.8$), but analyses of strain at 0.5 and 1.0 mg/kg Δ^9-THC are significant ($P < 0.0005$). Fig. 2 shows that the average maximal response in DA

TABLE I

PRE-DRUG BASAL CONCENTRATIONS OF DA, DOPAC, AND HVA IN NUCLEUS ACCUMBENS (UNCORRECTED FOR PERCENT RECOVERY)

Between strain basal levels are not statistically different. Units: fmol/20 min for DA; pmol/20 min for DOPAC and HVA (±S.E.M.)

	Lewis	*n*	Sprague–Dawley	*n*
DA	82.1±5.2	15	74.3±3.8	11
DOPAC	20.7±1.5	15	22.8±3.4	11
HVA	9.3±0.4	15	9.1±0.6	11

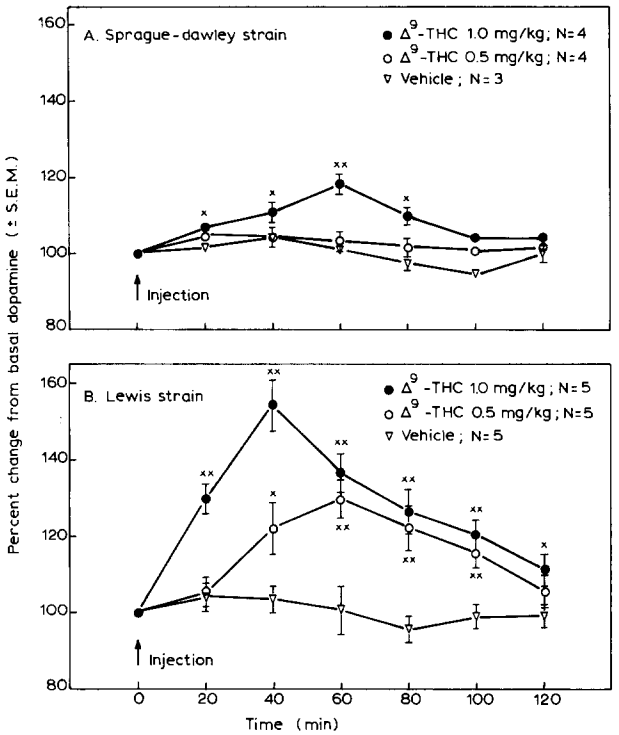

Fig. 1. Time course of basal dopamine (DA) efflux in the nucleus accumbens of Sprague–Dawley and Lewis strain rats following acute Δ^9-THC (0.5 and 1.0 mg/kg, i.p.) and vehicle injections. Values are expressed as mean (±S.E.M.) percentage of pre-injection DA levels. *Signifies comparison of individual samples with pre-injection DA level (*$P < 0.05$, **$P < 0.01$).

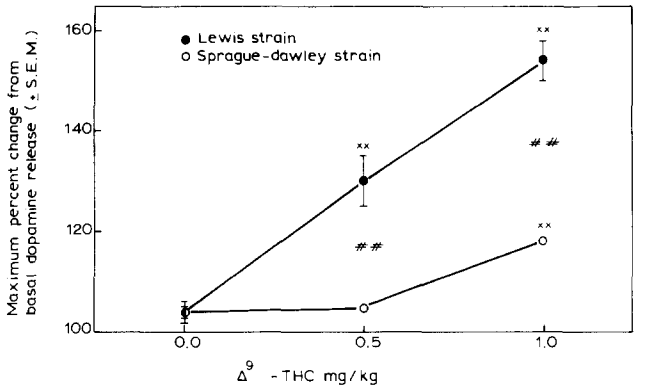

Fig. 2. Maximum effect of Δ^9-THC on basal dopamine (DA) efflux in Lewis ($n=5$ for vehicle group, $n=5$ for both 0.5 and 1.0 mg/kg groups) and Sprague–Dawley strain rats ($n=3$ for vehicle group, $n=4$ for both 0.5 and 1.0 mg/kg groups). Values are the maximum mean (±S.E.M.) increase in DA efflux within 2 h after Δ^9-THC treatment in both strains and are expressed as percentage of pre-injection levels. *Signifies comparison of maximum DA increase with pre-injection level of each strain (**$P<0.01$). *Signifies comparison of maximum DA increase between Lewis and Sprague–Dawley strain rats)**$P<0.01$).

efflux within 2 h after injection is greatest in Lewis rat and is significant at both low and high doses of Δ^9-THC (Student t-test, $P<0.01$). Δ^9-THC did not produce significant alterations in DOPAC or HVA levels in either strain rat. Likewise, vehicle injections of 15% PVP solution did not alter DA, DOPAC, or HVA levels in either strain rat.

Neither strain showed stereotypy or psychomotor activation following injections of Δ^9-THC. While animals seemed slightly quiescent following Δ^9-THC administration, they retained full motoric capability.

We previously showed [4] that Lewis strain rats are uniquely sensitive to the enhancing effect of Δ^9-THC on brain-stimulation reward (assessed by titrating-threshold electrical ICSS of the reward-associated DA projections to the nucleus accumbens) as compared to Sprague–Dawley rats. That previous finding implicated significant genetic variation in vulnerability to the brain-reward facilitating effects (and thus, presumably, to the euphorigenic effects) of Δ^9-THC and of the Δ^9-THC-containing recreational drug marijuana. Such genetic variation in vulnerability to the rewarding effects in laboratory animals of another commonly abused recreational drug — ethanol —has been previously suggested, on the basis of strain differences in ethanol preference and in propensity to self-administer ethanol [e.g. 1, 6, 11, 14]. Thus, in mice, the C57BL/6J strain shows high ethanol preference and high ethanol self-administration while the BALB/cJ strain shows low ethanol preference [6]. Provocatively, such genetically determined increased vulnerability to ethanol reward and ethanol self-administration in C57BL/6J mice appears to generalize to other drugs of abuse such as nicotine and opiates [10], suggesting that some genetically inbred strains may have a generalized vulnerability to the rewarding effects of recreational and abused drugs. In rats, a number of inbred strains have also been shown to exhibit high ethanol preference and high ethanol self-administration (e.g. the M520, ALKO, AA, and UChA strains) while other strains (e.g. the WKY, ALKO ANA, ACI, and UChB strains) show low ethanol preference [1, 6, 11, 14]. Provocatively, Lewis strain rats, shown by us previously to be highly vulnerable to the reward-enhancing effects of Δ^9-THC, also have been found to exhibit increased vulnerability for high ethanol self-administration [6, 16], to be more sensitive to the stimulant effects of cocaine [7], and to show high levels of responding for oral self-administration of cocaine [8], as compared to other strains, suggesting that this strain of rat may possess a generalized vulnerability to the rewarding effects of recreational and abused drugs similar to that observed in the C57BL/6J mouse strain.

However, the nature of this vulnerability of Lewis rats has remained obscure. In recent years, the hypothesis that drugs of abuse derive a significant portion of their abuse liability from direct neuropharmacological augmentation of brain-reward circuits, and that this augmentation involves a portion of the mesotelencephalic DA projections to the nucleus accumbens, has become the most compelling hypothesis available on the neurobiology of drug abuse [18–22]. That these ascending DA neurons to the nucleus accumbens constitute a crucial 'second-stage' anatomic convergence within the forebrain reward circuitry upon which drugs of abuse act to enhance brain reward is equally compelling [18–22]. In support of this hypothesis, many lines of evidence can be adduced [18, 20, 22]. First, virtually all drugs of abuse that have been adequately studied (including opiates, barbiturates, benzodiazepines, amphetamines, cocaine, phencyclidine-like dissociative anesthetics, ethanol, nicotine, and marijuana) augment brain-stimulation reward (ICSS) or lower ICSS reward threshold in brain loci closely associated with these ascending DA projections. Second, virtually all drugs of abuse that have been adequately studied have been found to augment either neuronal firing or DA release (or both) in these ascending DA projections. Third, animals will work to receive microinjections of drugs of abuse into mesocorticolimbic DA loci associated with these ascending DA projections, but for the most part not into other brain loci. Fourth, lesions or pharmacological blockade of this DA circuitry markedly inhibit the rewarding properties of systemically administered drugs of abuse.

Thus, the present findings that Δ^9-THC, at doses relevant to human recreational marijuana use, specifically

enhances DA efflux in nucleus accumbens of Lewis rats as compared to Sprague–Dawley rats suggests a preliminary explanation for the previously demonstrated brain-reward vulnerability of Lewis rats to Δ^9-THC, and perhaps to other abusable drugs as well. While the present results are suggestive of a genetic difference in DA neuronal sensitivity and/or DA regulatory responsiveness to Δ^9-THC (because basal pre-drug DA and metabolite levels are similar in both strains, see Table I), it is nonetheless possible that pharmacokinetic mechanisms may be involved in the observed effects. Some of the presently observed strain-specific effects may, in fact, be partly or totally due to strain differences in the rates of drug absorption, distribution, biotransformation, and/or excretion.

It must also be noted that the calcium concentration in the perfusate used in the present study was 3.37 mM, which is within the range of plasma calcium concentrations. It is also the perfusate calcium concentration heretofore used standardly by a majority of workers in brain microdialysis (e.g. ref. 9) and was deliberately chosen to make the data generated by the present experiments comparable to the extant brain microdialysis literature. However, Moghaddam and Bunney [12] have recently demonstrated that rat brain extracellular calcium concentrations are closer to 1.2 mM, and that basal DA release, and DA release under pharmacological manipulation, are dependent upon perfusate calcium concentration. Therefore, the present data must be interpreted with caution, since the 'basal' DA release measured in these experiments may have been to some extent a calcium-enhanced presynaptic DA efflux. Likewise, it must similarly be noted that the presently used perfusate was somewhat hypertonic and acidic, but whether these differences have significant influence on basal DA release is not definitively established.

However, it must be stressed that the present experiments were designed to compare the responsiveness of these two rat strains to exogenously applied pharmacological manipulations, rather than to compare basal DA functions in nucleus accumbens between the two strains. Thus, even if the present data reflect a partial calcium-enhanced presynaptic DA efflux, the point still remains that the Lewis rats were very significantly more affected in this regard by Δ^9-THC than were the Sprague–Dawley rats.

Finally, a comment on the lack of responsiveness of the DA metabolites to Δ^9-THC is in order. While extracellular DOPAC and HVA rise in correlated fashion with DA itself in some instances of pharmacological challenge (e.g. opiates), these DA metabolites *decrease* while DA itself rises in other instances of pharmacological challenge (e.g. amphetamines) [17]. Thus, Δ^9-THC's lack of effect on DOPAC and HVA is neither unprecedented nor particularly surprising. If, at the cellular level, Δ^9-THC's effect is partially opiate-like and partially amphetamine-like, a net result of no change in DOPAC and HVA could conceivably manifest itself. Such notions, though, are obviously highly speculative.

In summary, Lewis rats manifest a very significantly increased extracellular DA overflow in nucleus accumbens after Δ^9-THC challenge as compared to Sprague–Dawley rats. This augmented vulnerability of nucleus accumbens DA responsiveness to Δ^9-THC in Lewis rats appears to parallel the unique vulnerability of Lewis rats to Δ^9-THC-induced augmentation of brain-stimulation reward in the DA neural system projecting to nucleus accumbens, and may reflect a neural substrate for the Lewis rat's heightened vulnerability to, and preference for, not only marijuana but other drugs of abuse as well.

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