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Chronic exposure to Δ^9-tetrahydrocannabinol fails to irreversibly alter brain cannabinoid receptors

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The effects of chronic Δ^9-tetrahydrocannabinol (Δ^9-THC) and marijuana administration on the properties of brain cannabinoid receptor populations of the rat and monkey, respectively, were examined in this study. It was determined that the properties of the cannabinoid receptors in the striatum, cerebral cortex, cerebellum, hippocampus, and brainstem/spinal cord of the rat do not appear to be irreversibly altered by chronic exposure to Δ^9-THC. Similarly, the cannabinoid receptors in the caudate, prefrontal cortex, and cerebellum of the monkey do not appear to be irreversibly altered by chronic exposure to marijuana smoke.

Marijuana (*Cannabis sativa*) is a widely used substance known for both its psychoactive abuse potential[9,12] and therapeutic benefits[27]. Δ^9-Tetrahydrocannabinol (Δ^9-THC) has been determined to be the major CNS-active constituent of marijuana[29]. In human subjects, its effects include analgesia, antiemesis, and drowsiness, as well as alterations in mood, perceptions, cognition, memory, and psychomotor activity[12,28]. In animals, the actions of Δ^9-THC include altered behavior in monkeys, static ataxia in dogs, and hypothermia, analgesia, immobility, and changes in spontaneous locomotor activity in rodents[26,29].

Previous descriptions of the structure–activity relationships for cannabinoid activity in animals and cultured neuronal cells supported the hypothesis that natural and synthetic cannabinoid compounds act through a unique cannabinoid receptor[15–17,22,26]. At the cellular level, CNS-active cannabinoid compounds inhibit adenylate cyclase via a receptor associated with the G_i protein[13,14]. The subsequent development of a ligand binding assay has made it possible to quantify and characterize the cannabinoid receptor[8], including its modulation of cyclic AMP accumulation in the adult rat[4]. Recent autoradiographic studies using [^{3}H]CP-55,940 have shown anatomical localization of cannabinoid receptors in the brain of rat, rhesus monkey, human, and other species[11]. The abundance of receptors in the basal ganglia, hippocampus, cortex, and cerebellum is consistent with observed behavioral effects of cannabinoids[11,17]. There are various reports in the literature concerning the interactions of Δ^9-THC with several neurotransmitter receptor systems in the brain, many of which have reported neurotransmitter perturbations in vitro, after short-term, and in a few studies, after long-term cannabinoid administration (see refs. 1, 25, 28, and refs. therein). Investigators have reported that after high dose cannabinoid administration there is a decrease in the mean volume of rat hippocampal neurons and their nuclei, and after low dose administration there is a shortening of hippocampal dendritic spines[21,30]. However, it has been recently demonstrated that there are few, if any, residual or irreversible effects of Δ^9-THC on brain chemistries using chronic treatment protocols[1,2]. The studies reported herein attempted to determine whether irreversible changes in brain cannabinoid receptor characteristics could be demonstrated following chronic cannabinoid administration.

Desacetyllevonantradol (DALN) and CP-55,940 were provided by Pfizer Central Research. [^{3}H]CP-55,940 (88 Ci/mmol) was custom tritiated by Dupont-New England Nuclear. Δ^9-THC was a gift from NIDA. All other compounds were obtained from Sigma Chemical Company.

The treatment protocol for the rats consisted of the

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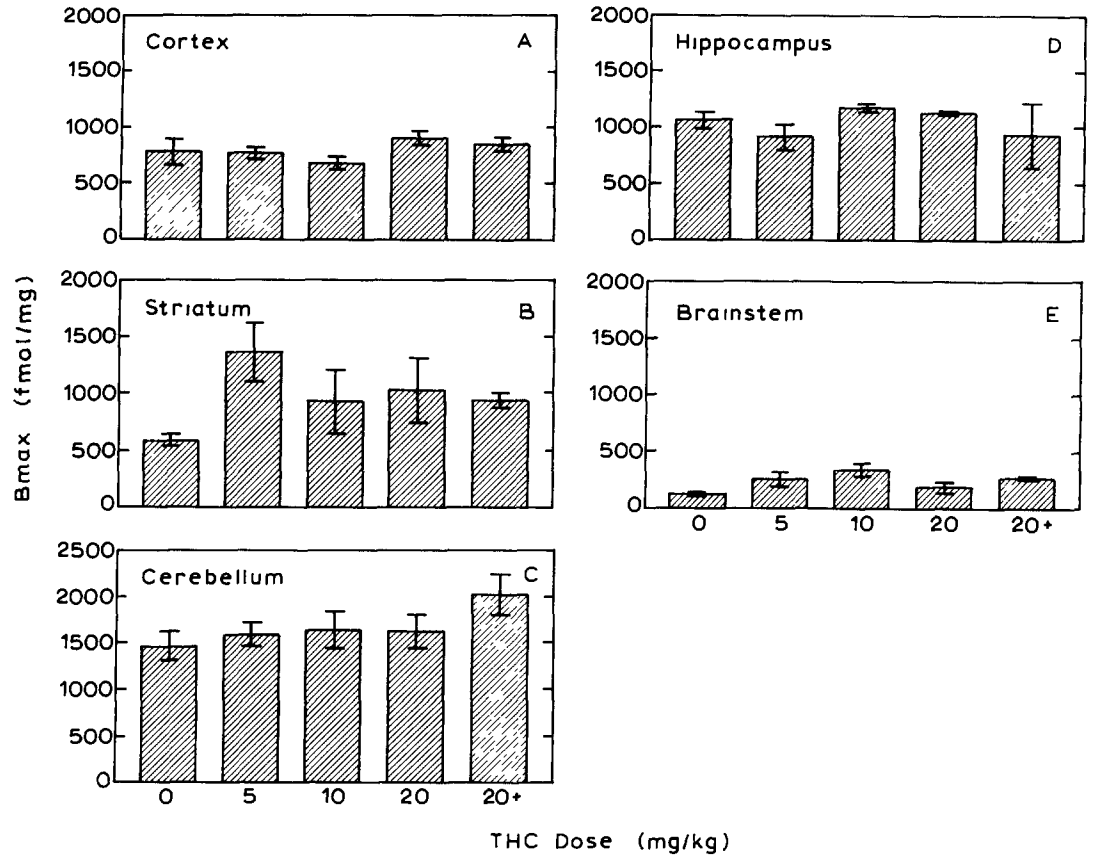

Fig. 1. Cannabinoid receptor density in brain regions from rats chronically treated with Δ^9-THC. Values are mean ± S.E.M. for (A) cerebral cortex, (B) striatum, (C) cerebellum, (D) hippocampus, and (E) brainstem/spinal cord. For all groups $n = 5$, except for the striatum, where $n = 3$–5 No experimental values were significantly different between groups at $P < 0.05$

following[2]: male Sprague–Dawley rats, 8–10 weeks old, were divided into 5 treatment groups. Four groups received either 0, 5, 10, or 20 mg of Δ^9-THC per kg of body weight in a vehicle of Triton X-100:EtOH:saline (1:10:89), daily, Monday–Friday. The fifth group received 20 mg/kg of Δ^9-THC daily, Monday–Thursday, and received 60 mg on Friday (the weekend dose). All treatment groups were dosed for 90 days. Three or four animals were caged together in temperature (22–23 °C) and photoperiod (12/12 h light/dark cycle) controlled rooms. Rat chow and tap water were provided ad libitum; all animals were observed to gain weight at similar rates during the dosing period. After 90 days of dosing, Δ^9-THC administration was stopped and the rats were maintained 60 days prior to sacrifice. Brains were dissected on ice for cerebral cortex, striatum, hippocampus, cerebellum, and brainstem (plus a variable portion of the spinal cord), and samples were placed into coded cryogenic vials and stored at −70 °C.

The monkey treatment protocol consisted of the following[31]: male periadolescent rhesus monkeys (initial body weights 3.7 ± 0.5 kg), were housed in an appropriate facility and living conditions. Rates of weight gain (approximately 0.1 kg/month) were the same for all groups throughout the entire experiment. One group of monkeys was exposed to sham smoke (ethanol-extracted marijuana cigarettes), 7 days/week for a year, and the other group of monkeys was exposed to the smoke of one marijuana cigarette (2.6% THC) 7 days/week for one year. Smoke exposure was accomplished using an inhalation mask that covered only nose and mouth. Plasma cannabinoids (Δ^9-THC and 11-nor-9-carboxy-Δ^9-THC) were constant in the marijuana group throughout the dosing period and were both undetectable in the sham group. Seven months after the last smoke exposure, the monkeys were sacrificed with an overdose of pentobarbital and brains dissected for caudate, prefrontal cortical, and cerebellar samples. These were placed into coded vials and frozen at −70 °C.

A P_2 preparation was made from each sample; all were assayed individually. Depending on the region of brain, rat samples weighed from 25 to 900 mg and monkey samples weighed from 1 to 3 g. The binding of [^{3}H]CP-55,940 was performed as previously described[8], with the exception that the final concentration of fatty acid-deficient bovine serum albumin was 0.1 mg/ml, and that all assays were performed directly in siliconized microfuge tubes. The final 1-ml volume of each assay tube

TABLE I

Affinity of the cannabinoid receptor for CP-55,940 and Δ^9-THC in cerebral cortex, hippocampus, and cerebellum of rats treated with various doses of Δ^9-THC

Brain region	*Treatment*	*K_i (nM) mean ± S.E.M.*	
	Δ^9-THC	*CP-55,940*	*Δ^9-THC*
Cerebral cortex	control	0.37 ± 0.24 (n = 4)	13 ± 9.7 (n = 3)
	5	0 30 ± 0 11 (n = 3)	17 ± 0.9 (n = 4)
	10	0.22 ± 0.06 (n = 4)	21 ± 5.9 (n = 4)
	20	0.22 ± 0.05 (n = 4)	6.3 ± 5.0 (n = 5)
	20 (+60)	0.22 ± 0.05 (n = 4)	11 ± 6.0 (n = 3)
Hippocampus	control	0 16 ± 0 03 (n = 3)	5.3 ± 4.1 (n = 2)
	5	0.36 ± 0 17 (n = 3)	8.4 ± 4 2 (n = 4)
	10	0 32 ± 0.16 (n = 3)	16.5 ± 10 (n = 3)
	20	0.32 ± 0.02 (n = 2)	2.0 ± 0.09 (n = 2)
	20 (+60)	0.20 ± 0.17 (n = 3)	4.2 ± 0 20 (n = 3)
Cerebellum	control	1 1 ± 0.68 (n = 4)	11 0 ± 3.2 (n = 4)
	5	1.3 ± 0.84 (n = 4)	9.4 ± 2 4 (n = 4)
	10	0.85 ± 0.67 (n = 3)	4.1 ± 1.6 (n = 4)
	20	0 53 ± 0.30 (n = 3)	12 0 ± 2.2 (n = 5)
	20 (+60)	2 7 ± 2 1 (n = 4)	30.0 ± 20 (n = 5)

contained 25–35 μg membrane protein and 40–70 pM [^{3}H]CP-55,940. Non-specific binding was that obtained from tubes containing 10 nM DALN. Homologous and heterologous displacement studies were performed by incubating with various concentrations of CP-55,940 and Δ^9-THC. A standard cortical rat brain P_2 preparation sample was included with each assay as a control. Vehicle and non-specific data points were quadruplicates; all other data points were duplicates. Protein determinations were made by the method of Bradford[3].

Values of IC_{50} and the slope factor were calculated using the Hill equation[20], and values of K_i were calculated using the assumptions of Cheng and Prusoff[5]. The data are expressed as mean ± S.E.M. from 5 animals, except where noted. The data were analyzed using a one-way analysis of variance (ANOVA).

The cannabinoid receptor binding capacity from the regions of the rat brains are shown in Fig. 1. The B_{max} values obtained from the rat controls (the first bar of each graph) were in fmol/mg: cortex, 774; striatum, 595; hippocampus, 1068; cerebellum, 1465; and brainstem, 125. These values are consistent with previous findings reported for rats[4]. Using the ANOVA, it was observed that the treated values were not significantly different from the control values at the 0.05 confidence level. Although the striatal value may appear to be higher than control, the variance was large and precluded a significant difference. The affinities of the cannabinoid receptor for the agonist ligands CP-55,940 and Δ^9-THC are shown in Table I. The rat control K_i value for CP-55,940 (0.58 ± 0.27 nM) and for Δ^9-THC (10.3 ± 3.3 nM) did not vary between brain regions. The K_i values of the treated animal groups were not found to be significantly different from controls at the 0.05 confidence level.

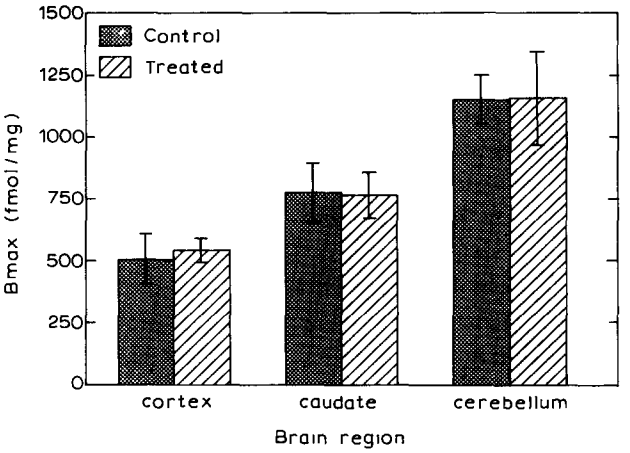

Fig. 2. Cannabinoid receptor density in brain regions from monkeys chronically treated with marijuana. Values are mean ± S.E.M. (n = 5), for the prefrontal cortex, caudate, and cerebellum from control (stippled) and treated (hatched) monkeys. Values from treated animals were not significantly different from controls at $P < 0.05$.

Data obtained from the study of monkeys that had been chronically exposed to marijuana smoke are shown in Fig. 2. The B_{max} values obtained from the monkey controls were (fmol/mg protein): cortex, 500 ± 100; caudate, 770 ± 119; and cerebellum, 1120 ± 97. The B_{max} values of the treated groups were not statistically different from controls. The K_i values for CP-55,940 (control

TABLE II

Affinity of the cannabinoid receptor for CP-55,940 in prefrontal cortex, caudate, and cerebellum of control and treated monkeys

	K_i (nM) mean ± S.E.M.		
	Prefrontal cortex	*Caudate*	*Cerebellum*
Control	0 63 ± 0.33 (n = 5)	0.27 ± 0 05 (n = 5)	0.27 ± 0.03 (n = 5)
Treated	0 29 ± 0.11 (n = 6)	0.39 ± 0.10 (n = 5)	0.37 ± 0.06 (n = 6)

= 0.42 ± 0.1 nM) in Table II from homologous CP-55,940 displacement studies indicate no differences between regions. No differences could be demonstrated between treated groups.

We conclude that in the regions of the rat and monkey brains that were examined, the cannabinoid receptor properties are not irreversibly altered by chronic exposure to Δ^9-THC or marijuana smoke according to the treatment protocols outlined in the methods section.

A previous study was designed to determine whether acute or chronic exposure to Δ^9-THC produced significant alterations in several neurotransmitter systems[1]. Rats gavaged with vehicle, 10 or 20 mg THC/kg for 90 days were sacrificed either 24 h or two months after the last drug dose. Examination of dopamine, serotonin, acetylcholine, GABA, benzodiazepine and opioid neurotransmitter systems via HPLC analysis or binding studies revealed that no significant changes occurred[1] Although a significant decrease in GABA receptor binding was initially demonstrated in the hippocampus of the high dose group animals given a two-month recovery period prior to sacrifice, subsequent experiments could not replicate this initial finding[1].

A larger study designed to determine if chronic Δ^9-THC and marijuana smoke administration produced neurochemical alterations in rat and monkey brain has recently been completed[2]. Binding to several neurotransmitter receptors (dopamine, serotonin, acetylcholine, GABA, opioid, and benzodiazepine) and HPLC analyses of tissue levels of monoamines and their metabolites were examined. An initial report[2] from this study revealed that no significant irreversible changes could be demonstrated in the rats chronically treated with Δ^9-THC. Monkeys exposed to a chronic treatment of marijuana smoke for one year and then sacrificed after a 7-month recovery period were found to have no changes in various neurotransmitter concentrations in caudate, frontal cortex, hypothalamus, or brainstem regions[2]. Thus, the important findings of these studies are that significant irreversible alterations in major neuromodulator pathways in the rat and monkey brain are not apparent after long-term exposure of the animal to the active compound in marijuana. The present data augment the larger study to indicate that no irreversible alterations in cannabinoid receptor binding properties are evident following chronic treatment with Δ^9-THC.

Tolerance is expected to develop after chronic exposure to agonist ligands as has been demonstrated for many neuroreceptors[24,32,33]. One cellular mechanism for tolerance is the down-regulation of receptors[7] as has been shown for β-adrenergic, muscarinic cholinergic and opioid receptors (see refs. 6, 7, 19, 23). Thus, one would predict that chronic exposure to Δ^9-THC would result in a decreased number of cannabinoid receptors in the brain. The recovery periods in the above protocols (60 days for rats; 7 months for monkeys) are probably sufficient amounts of time to allow for the full recovery of any receptors that would have been lost during the treatment period. Future studies will be designed to detect the reversible alterations in cannabinoid receptor number that may result from exposure to cannabinoid agonists.

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