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Neurotoxicology of Cannabis and THC: A Review of Chronic Exposure Studies in Animals[1]

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SCALLET, A. C. *Neurotoxicology of cannabis and THC: A review of chronic exposure studies in animals.* PHARMACOL BIOCHEM BEHAV **40**(3) 671–676, 1991.—Several laboratories have reported that chronic exposure to delta-9-tetrahydrocannabinol (THC) or marijuana extracts persistently altered the structure and function of the rat hippocampus, a paleocortical brain region involved with learning and memory processes in both rats and humans. Certain choices must be made in designing experiments to evaluate cannabis neurotoxicity, such as dose, route of administration, duration of exposure, age at onset of exposure, species of subjects, whether or how long to allow withdrawal, and which endpoints or biomarkers of neurotoxicity to measure. A review of the literature suggests that both age during exposure and duration of exposure may be critical determinants of neurotoxicity. Cannabinoid administration for at least three months (8–10% of a rat's lifespan) was required to produce neurotoxic effects in peripubertal rodents, which would be comparable to about three years exposure in rhesus monkeys and seven to ten years in humans. Studies of monkeys after up to 12 months of daily exposure have not consistently reported neurotoxicity, and the results of longer exposures have not yet been studied.

Neurotoxicology Morphometry Cannabis Marijuana THC Hippocampus Rats Monkeys Golgi Electron microscopy

MARIJUANA is among the most frequently used illicit drugs in the United States and worldwide (39), and has often been proposed for legalization [e.g., (30)]. Although the presumed neurotoxic effects of marijuana enter into the legalization argument, surprisingly few experimental studies of marijuana neurotoxicity have been published. Human marijuana research has been hampered by limited sensitivity of measurements and the retrospective nature of most of the studies [e.g., (54,55)]. Careful neurobehavioral studies of rodents chronically treated orally or by injection with cannabis extracts or THC (12, 44–48) implied potential toxicity to the hippocampus, suggestions that have been reinforced by neurohistological studies in THC-treated rats (26,37). However, chemical constituents, pyrolysis products, exposure kinetics, and perhaps physiological responses are different between humans smoking marijuana and rodents injected or ingesting THC or cannabis extracts. The rhesus monkey (*M. mulatta*) has provided suggestive neurophysiological evidence, as well as neurohistological findings from two treated monkeys, of a limbic system toxicity that included the hippocampus (14–16). Quantitatively, this was described as a widening of the synaptic clefts. Establishing comparability between human exposure conditions and an appropriate animal model may be somewhat easier in monkeys than in rodents. Here, we will review the available literature on the toxicity of chronic exposure to marijuana and THC in experimental animal models, with an emphasis on studies of neurohistological morphometry. Based on critical factors such as developmental stage at onset of exposure and duration of exposure, the design of studies to address the neurotoxicity of cannabis in a risk assessment context will be discussed.

METHODOLOGICAL ISSUES

Definition of Neurotoxicity

A working definition adopted by the Interagency Committee on Neurotoxicology (ICON), comprised of representatives of the Environmental Protection Agency, the Food and Drug Administration, and others, states "Neurotoxicity is any adverse effect on the structure or function of the central and/or peripheral nervous system by a biological, chemical, or physical agent and may result from direct or indirect actions or reflect permanent or reversible changes in the nervous system." This definition includes temporary and reversible effects on the nervous system as "neurotoxic." A number of marijuana studies have shown acute behavioral effects, although the issue of whether these effects are "adverse" has not often been addressed [e.g., (40,41)]. However, the permanence or at least long-term persistence of effects is still a useful distinction to make while considering chronic exposure scenarios. The present review will restrict its consideration to prolonged or irreversible effects.

Route of Exposure and Cannabis Metabolism

Exposure to humans is most typically via inhalation of smoke from combustion of the flowering tops of the female plant, *Can-*

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nabis sativa L. Extracts of cannabis include more than 60 terpene compounds termed cannabinoids, as well as hundreds of other constituents (49). The most abundant of the cannabinoids are cannabinol (CBN), cannabidiol (CBD), and delta-9-tetrahydrocannabinol (THC), the major psychoactive component (28). Considering losses due to pyrolysis and side-stream smoke, the bioavailability of THC from smoked plant material is only about 20%, but may range from 13–40% depending on the experience of the user (10). Bioavailabilities of CBD (31%) and CBN (41%) are similar. Lower ratios of parent cannabinoids to their metabolites are observed following oral administration (9,10) compared to smoke inhalation or intravenous injection because of initial metabolism of the parent compounds by the liver prior to their reaching the systemic circulation ("first-pass" effect). Cannabinoids are extensively metabolized by the liver, and some metabolites (e.g., 11-OH-THC) are psychoactive, whereas others are not (e.g., 11-nor-9-COOH-THC). Therefore, depending on route of administration, the relative amounts, as well as the specific identities of the cannabinoids, will be different, resulting in a "complex mixture exposure" problem for the toxicologist.

Duration and Timing of Exposure and Assessment

Duration of exposure may be for decades of daily or near-daily use in humans, and age of onset is often during the preadolescent or adolescent period. Some adult humans are heavy, chronic users, whereas others may quit for long periods of time or be occasional users. Since tolerance and withdrawal effects of cannabis and THC have been reported (7, 27, 50, 56), these factors also must be considered in experimental designs that model human exposures in animals. A special case may also be presented by "in utero" and/or early postnatal exposure of offspring, since THC and metabolites cross the placenta and are present in maternal milk (5, 6, 10).

Dose, Blood Levels and Target Tissues

Extensive pharmacokinetic studies have been done that relate administered dose to blood and tissue levels (6, 9, 10, 33, 43). In humans, a single marijuana cigarette (2.5% THC) results in peak blood levels of about 150 ng/ml THC immediately after smoking, which initially decline rapidly within minutes, followed by a slower elimination phase (9). In rats, oral dosing with 20 mg/kg synthetic THC produces a much slower increase in plasma levels, peaking around the same 150 ng/ml at one hour after dosing, but plasma levels remain elevated for nearly 6 hours (37). Thus oral dosing of rats designed to match peak blood THC levels in humans after smoke exposure not only produces a greater total THC exposure to the rat in terms of area under the concentration versus time curve, it also increases the exposure to THC metabolites produced by "first-pass" liver metabolism. Cannabinoids in blood appear to be freely available to brain tissue (6), especially the phospholipid membrane bilayer compartment, since they are highly lipophilic compounds (18). These results indicate that blood levels of THC can be used as an index of exposure to cannabis compounds, but underscore the fact that oral administration of THC or cannabis represents a very different model from smoke exposure.

Receptor Mediation

The issue of potential receptor mediation of any suspected neurotoxicity of either marijuana or THC is a particularly interesting question at present. Marijuana's combustion and/or metabolic products may interact with a stereoselective receptor binding site coupled to G-proteins that can inhibit adenylate cyclase (11), as well as a separate nonstereoselective but high-affinity binding site (31). Moreover, a number of studies in rats [e.g., (22, 24, 32)] has shown that exposure to marijuana may increase plasma corticosterone, which in its own right may be neurotoxic via specific glucocorticoid receptors [e.g., (26)]. Moreover, the high lipid solubility of cannabinoids referred to above indicates the need to consider properties of the phospholipid bilayer in the potential neurotoxicity of marijuana exposure (18). The neuroanatomical localizations of glucocorticoid receptors [hippocampal dentate granule cells and CA1 pyramidal cells, deep layers of cortex, cerebellar granule cells, basomedial amygdala: (13, 35, 53)] and the stereoselective CP-55,940 binding site (17) are rather similar to one another, and either or both could contribute to a potential receptor-mediated hippocampal neurotoxicity. It is also interesting that the hippocampus has been implicated in the behavioral expression of drug-produced withdrawal symptoms (21). Therefore, a number of possibilities exist whereby marijuana could exert direct or indirect, but nevertheless receptor-mediated, neurotoxicity.

RODENT STUDIES

Fehr, Kalant and Le Blanc (12) reported that a residual learning deficit existed when peripubertal (40-day-old) rats were treated for 6 months (about 15–20% of their lifespan) with cannabis extract containing 20 mg/kg THC (and other cannabinoid compounds). The design of this study addressed several issues relevant to neurotoxicological evaluation of marijuana and THC. First, 1–3 months (but not 6 months) of treatment was insufficient to produce effects on Hebb-Williams maze problems, indicating that substantial duration of treatment might be a prerequisite for neurotoxicity. Exposure for six months altered performance in a maze, as well as the rats' ability to walk on a "moving belt" to avoid footshock. Secondly, testing was scheduled so that "animals were allowed to recover from drug effects for (at least) 1 month." It was thus recognized that persistent or "residual" alterations were of major interest, although the issue of whether or not withdrawal was required for expression of persistent cannabis effects was not addressed. Subsequent studies established that even 3 months exposure to cannabis extract was sufficient to produce residual effects on performance in a different type of maze [an 8-arm radial maze, (44)]. Other studies identified performance deficits on a DRL (differential reinforcement of low lever-pressing response rates) operant schedule (45). Also observed was an improvement in shuttlebox avoidance (48). As noted by these investigators, the pattern of inability to withhold responses on a DRL schedule together with increased responses leading to improved active avoidance has been the hallmark of hippocampal lesions in many physiological psychology investigations. Using these behavioral methods, Stiglick and Kalant (46) also reported synthetic THC was neurotoxic but less potent than cannabis extract when administered PO at 20 mg/kg to 40-day-old rats for a three-month period. Furthermore, cannabis treatment initiated at 70 days of age was less effective than treatment initiated at 40 days of age (47), as indicated by cannabis effects restricted to shuttlebox avoidance performance in the absence of altered radial maze, DRL, or open-field behavior in the former case.

In abstract form, Kallman et al. (23) reported that daily inhalation of marijuana smoke for 1–3 months (1 or 2 2.85% THC cigarettes) produced a decrement in rotorod performance 60 days later, a measure not unlike the moving belt test of Fehr et al. (12). A more complete report of this research including neurohistological and neurochemical endpoints is in preparation.

TABLE 1

STUDIES EXAMINING THE NEUROTOXICITY OF THC OR MARIJUANA IN RODENT MODELS

Reference	Dose (mg/kg)	Route	Age During Exposure (days)	Age at Evaluation (days)	Endpoints	Toxic
Rat THC						
Abel (1)	50, 150	PO	GD1–21	36–80	Behavior	–
Mokler (29)	10	SC	PND 4, 6, 8	130	Neuroendocr.	+
Hutchings (8,20)	15, 50	PO	GD8–22	2–32	Behavior	–
Walters (52)	10	PO	GD0-PND 20	10–60	Neurochem.	+
Stiglick (46)	20	PO	PND40–130	165–275	Behavior	+
Scallet (37)	10,20	PO	PND47–137	197	Neurohistol.	+
	20/60		PND150–240	450	Neurohistol.	+
Holson (19)	5, 10, 20 20/60	PO	PND56–146	206	Behavior	+
Landfield (26)	4, 10	SC	PND100–348	362	Neurohistol.	+
Rat Cannabis Extract						
Fehr (12)	20	PO	PND40–226	276	Behavior	+
Stiglick (44, 45, 48)	20	PO	PND40–130	165–275	Behavior	+
Stiglick (47)	20	PO	PND70–160	190–280	Behavior	+

Several additional studies have examined the possibility of "sensitive periods" during development during which THC exposure might result in heightened neurotoxicity. However, exposure during gestation following maternal administration of THC has not produced developmental neurotoxicity in the offspring with regard to a number of behavioral endpoints, e.g. rotorod, 2-way avoidance, water maze, locomotor activity, and nipple attachment (1, 8, 20).

Nevertheless, these studies do report a reproductive toxicity expressed as a decrease in size of litters of THC-treated dams, despite the absence of any similar effect in pair-fed controls. Abel et al. (2,3) have reported similar findings (no behavioral toxicity, but increased neonatal mortality) from dosing with cannabis extract during gestation.

With regard to other reproductive endpoints, the development of normal adult estrous cycles was compromised in neonatal rats exposed to THC (3.8, 19, or 38 mg/kg), which also elevated mediobasal hypothalamic opioid peptides while lowering hypothalamic LHRH and plasma LH (25).

Scallet et al. (37) utilized neurohistological and morphometric approaches to evaluate 9 rats seven months after their last oral dose of THC or vehicle. Although these rats were labeled only with identification codes not indicating treatment groups, electron micrographs of the CA3 subregion of their hippocampi could easily be sorted into two characteristic groups. In one group, the axodendritic contact regions appeared short and broken, there was considerable extracellular space, and subcellular organelles such as vesicles and mitochondria, though well fixed, were not (as is usually the case) always separated from the extracellular space by intact membranes. This group contained all 5 of the THC-treated rats. The second group exhibited little extracellular space, had intact membranes, distinct mossy-fiber synapses containing numerous vesicles, and neuropil typical of the untreated ultrastructural appearance of this region. This group contained all 4 of the vehicle-treated controls of the study.

These observations were supported by morphometric estimation of the size of hippocampal CA3 neuronal perikarya as well as the synaptic density (number of synapses per unit volume) in the hippocampi of the THC-treated rats. Statistical analysis revealed significantly smaller neurons and significantly decreased synaptic density in the THC-treated rats. These rats had been considerably older (about 5 months) than the 40-day-old rats usually used by Stiglick and Kalant (see Table 1), but were treated for the same duration (90 days) with the same dose of THC (20 mg/kg PO) Monday through Thursday. A notable difference, however, is that these rats had received a 60-mg/kg dose of THC each Friday in lieu of weekend dosing. To more closely match the conditions reported by Stiglick and Kalant, additional rats were dosed 5 days per week with vehicle, 10, or 20 mg/kg/day THC (see Table 1 for details). It was found that no ultrastructural abnormalities or change in synaptic density took place with THC treatment under these conditions, although neurons stained via the Golgi-Cox approach revealed dendrites that were consistently and measurably shortened in a dose-related fashion (37).

Landfield et al. (26) reported the effects of a longer duration of dosing (8 months) with lower doses of THC (4 or 8–10 mg/kg, 5 days/week) administered subcutaneously to animals that were 3–4 months old at the onset of treatment. No ultrastructural abnormalities or altered synaptic density as extreme as reported in Scallet et al.'s highest dose group (up to 60-mg/kg doses) were observed in this study, but 8–10 mg/kg THC re-

TABLE 2

STUDIES EXAMINING THE NEUROTOXICITY OF THC OR MARIJUANA IN NONHUMAN PRIMATE ANIMAL MODELS

Reference	Dose (mg/kg)	Route	Age During Exposure (months)	Age at Evaluation (months)	Endpoints	Toxic
Monkey THC						
Heath (15)	0.7	IV	48–54	57–64	Neurophys.	+
					Neurohistol.	+
Andrews (4)	0.1,1	IV	30–33	33	Neurohistol.	–
Monkey Cannabis Smoke Inhalation						
Heath (15)	1–3 cig/day		48–56	57–64	Neurophys.	+
					Neurohistol.	+
Scallet (38)	1 cig/day (7 days/week)		36–48	55	Neurohistol.	–
Paule (34)	1 cig/day (7 days/week)		36–48	55	Behavior	–

duced neuronal density as estimated by the number of nucleoli per 100 microns length of the CA1 stratum pyramidale. These changes may be more relevant to effects on the second set of rats studied by Scallet et al. (37) in which dendrite length was reduced even though synaptic density and ultrastructural appearance were not significantly altered. Moreover, both dose groups studied by Landfield et al. (26) had a significant increase in the proportion of opaque material, perhaps scavenged structural proteins, found within the cytoplasmic compartment of their astroglia.

The authors noted that the effects from THC on relatively young rats resembled the neurohistological effects of normal aging they had described in a series of previous investigations. Moreover, they noted that signs of pituitary adrenocorticotropic hormone (ACTH) and adrenal corticosterone "hyperresponsivity" to restraint stress were present in their THC-treated rats measured 2 and 4 months after initiation of treatment.

MONKEY STUDIES

Relatively few evaluations of chronic exposure to marijuana or THC have been conducted in nonhuman primate species. Sassenrath (36) indicates effects of doses as low as 2.5 mg/kg on endpoints such as visual attention and the aggressive behavior of low-ranking THC-treated rhesus monkey members of small social groups.

The studies of Harper, Heath and colleagues (14–16) included both functional neurophysiological analyses via surface and depth electrode EEG recordings as well as postmortem structural evaluation by electron microscopy. They reported changes in patterns of electrical activity recorded by depth electrodes: increased slow waves and high-amplitude spiking that was most pronounced in the hippocampus, septal region, and amygdala. These were persistent effects that lasted up to 8 months after a 6-month exposure period to marijuana smoke.

Several of these monkeys were evaluated by morphometric electron microscopic methods following fixation by perfusion with mixed aldehydes (14,15). Ultrastructural changes such as synaptic widening, accumulation of dark material in the synaptic clefts, reduction of percent area of cytoplasm occupied by rough endoplasmic reticulum (RER), and development of nuclear inclusion bodies were reported. These were most prominent in the septal region and in hippocampus. Unfortunately, there were several methodological problems with this study. EEG results were reported as illustrations of sample polygraph chart recordings rather than analysed quantitatively. With regard to the neurohistology results, the sample size was very small, comprising only one monkey treated with marijuana smoke and one animal treated with THC IV compared to two controls and one monkey that had smoked only marijuana from which the ethanol-soluble cannabinoids had been extracted. The monkey treated with THC IV and the monkey exposed to extracted smoke had also been implanted with depth EEG electrodes prior to perfusion. The synaptic widths, as well as the fraction of cytoplasmic RER, were significantly different between the pair of actively treated monkeys and the controls both in hippocampus and septal area.

Using identical or comparable neurotoxic endpoints as applied in the rodent studies, further studies of rhesus monkeys have been conducted. The design of these studies and the effects of marijuana smoke on clinical chemistry endpoints, plasma THC and metabolite concentrations, and carboxyhemoglobin levels are reported by Slikker et al. (43). Paule et al. (34) consider the neurobehavioral effects of chronic exposure to marijuana smoke, and Ali et al. (this volume) describe neurochemical studies. Andrews et al. (4) and Scallet et al. (38) have made preliminary reports of the neurohistological results. Thus far, these studies have not revealed any neurotoxic effects from exposure to marijuana comparable to the effects reported in the rodent studies.

SUMMARY

Chronic oral exposure of peripubertal rats to cannabis extract or to THC resulted in a behavioral syndrome very similar to the effects of adult hippocampal lesions. Histological evidence demonstrated that hippocampal pyramidal neurons were measurably altered by chronic exposure to THC according to dosing regimens similar to those that produced behavioral effects. Therefore, in peripubertal rats, oral exposure to cannabis or THC appears to be neurotoxic, producing persistent functional and structural changes. There may be a sensitive period for these neurotoxic effects, since 70-day-old subjects were less affected than 40-day-old subjects and gestational administration produced reproductive but not neurobehavioral effects. Periods of cannabis or THC exposure shorter than 3 months have not yet been demonstrated to cause neurotoxic effects in rats. It is uncertain at present whether these effects require withdrawal or if their mechanism involves THC, other cannabinoids, glucocorticoids,

or their receptors either alone or in combination. Studies of up to one-year exposures to marijuana smoke have not yet identified neurotoxic effects in peripubertal monkeys comparable to the rodent findings. However, it should be noted that even a one-year exposure to the rhesus monkey (with a lifespan of about forty years) is relatively short as a percent of lifetime compared with the three months exposure that thus far has been the minimum required for neurotoxicity in the rat.

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REFERENCES

1. Abel, E. L. Effects of delta-9-THC on pregnancy and offspring in rats. Neurobehav. Toxicol. Teratol. 6:29–32; 1984.
2. Abel, E. L.; Bush, R.; Dintcheff, B. A.; Ernst, C. A. S. Critical periods for marihuana-induced intrauterine growth retardation in the rat. Neurobehav. Toxicol. Teratol. 3:351–354; 1981.
3. Abel, E. L.; Dintcheff, B. A.; Day, N. Effects of marihuana on pregnant rats and their offspring. Psychopharmacology (Berlin) 71: 71–74; 1980.
4. Andrews, A. M.; Scallet, A. C.; Uemura, E.; Ali, S. F.; Bailey, J. R.; Paule, M. G.; Brown, R. M.; Slikker, W., Jr. Ultrastructural evaluation of hippocampus in rhesus monkeys after intravenous delta-9-tetrahydrocannabinol (THC): 90 Day study. EMSA Proc. 47: 956–957; 1989.
5. Asch, R. H.; Smith, C. G. Effects of delta-9-THC, the principal psychoactive component of marijuana, during pregnancy in the rhesus monkey. J. Reprod. Med. 31:1071–1081; 1986.
6. Bailey, J. R.; Cunny, H. C.; Paule, M. G.; Slikker, W., Jr. Fetal disposition of delta-9-tetrahydrocannabinol (THC) during late pregnancy in the rhesus monkey. Toxicol. Appl. Pharmacol. 90:315–321; 1987.
7. Beardsley, P. M.; Balster, R. L.; Harris, L. S. Dependence on tetrahydrocannabinol in rhesus monkeys. J. Pharmacol. Exp. Ther. 239:311–319; 1986.
8. Brake, S. C.; Hutchings, D. E.; Morgan, B.; LaSalle, E.; Shi, T. Delta-9-tetrahydrocannabinol during pregnancy in the rat: II. Effects on ontogeny of locomotor activity and nipple attachment in the offspring. Neurotoxicol. Teratol. 9:45–49; 1987.
9. Chiang, C. N.; Barnett, G. Marijuana effect and delta-9-tetrahydrocannabinol plasma level. Clin. Pharmacol. Ther. 36:234–238; 1984.
10. Chiang, C. N.; Rapaka, R. S. Pharmacokinetics and disposition of cannabinoids. Natl. Inst. Drug Abuse Res. Monogr. Ser. 79:173–188; 1987.
11. Devane, W. A.; Dysarz, F. A. III; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34:605–613; 1988.
12. Fehr, K. A.; Kalant, H.; LeBlanc, A. E. Residual learning deficit after heavy exposure to cannabis or alcohol in rats. Science 192: 1249–1251; 1976.
13. Fuxe, K.; Cintra, A.; Harfstrand, A.; Agnati, L. F.; Kalia, M.; Zoli, M.; Wikstrom, A.-C.; Okret, S.; Aronsson, M.; Gustafsson, J.-A. Central glucocorticoid receptor immunoreactive neurons: New insights into the endocrine regulation of the brain. Ann. NY Acad. Sci. 512:362–393; 1987.
14. Harper, J. W.; Heath, R. G.; Myers, W. A. Effects of cannabis sativa on ultrastructure of the synapse in monkey brain. J. Neurosci. Res. 3:87–93; 1977.
15. Heath, R. G.; Fitzjarrell, A. T.; Fontana, C. J.; Garey, R. E. Cannabis sativa: Effects on brain function and ultrastructure in rhesus monkeys. Biol. Psychiatry 15:657–691; 1980.
16. Heath, R. G.; Fitzjarrell, A. T.; Garey, R. E.; Meyers, W. A. Chronic marihuana smoking: Its effect on function and structure of the primate brain. In: Nahas, G. G.; Paton, W. D. M., eds. Marihuana: Biological effects. Oxford: Pergamon Press; 1979:713–730.
17. Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; DeCosta, B. R.; Rice, K. C. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA 87:1932–1936; 1990.
18. Hillard, C. J.; Harris, R. A.; Bloom, A. S. Effects of the cannabinoids on physical properties of brain membranes and phospholipid vesicles: Fluorescence studies. J. Pharmacol. Exp. Ther. 232:579–588; 1985.
19. Holson, R. R.; Ali, S. F.; Scallet, A. C.; Slikker, W., Jr.; Paule, M. G. Benzodiazepine-like behavioral effects following withdrawal from chronic delta-9-tetrahydrocannabinol administration in rats. Neurotoxicology 10:605–620; 1989.
20. Hutchings, D. E.; Morgan, M.; Brake, S. C.; Shi, T.; LaSalle, E. Delta-9-tetrahydrocannabinol during pregnancy in the rat: I. Differential effects on maternal nutrition, embryotoxicity, and growth in the offspring. Neurotoxicol. Teratol. 9:39–43; 1987.
21. Isaacson, R. L.; Lanthorn, T. H. Hippocampal involvement in the pharmacologic induction of withdrawal-like behaviors. Fed. Proc. 40:1508–1512; 1981.
22. Jacobs, J. A.; Dellarco, A. J.; Manfred, R. A.; Harclerode, J. The effect of delta-9-tetrahydrocannabinol, cannabidiol, and shock on plasma corticosterone concentrations in rats. J. Pharm. Pharmacol. 31:341–342; 1979.
23. Kallman, M. J.; Jones, A. B.; Ali, S. F.; Scallet, A. C.; Paule, M. G.; Slikker, W., Jr. Neurobehavioral effects of repetitive marihuana smoke exposure in rats. Soc. Neurosci. Abstr. 15:1351; 1989.
24. Kubena, R. K.; Perhach, J. L., Jr.; Barry, H., III. The effects of delta-9-tetrahydrocannabinol on plasma corticosterone in rats. Eur. J. Pharmacol. 14:88–92; 1971.
25. Kumar, A. M.; Solomon, J.; Patel, V.; Kream, R. M.; Drieze, J. M.; Millard, W. J. Early exposure to delta-9-tetrahydrocannabinol influences neuroendocrine and reproductive functions in female rats. Neuroendocrinology 44:260–264; 1986.
26. Landfield, P. W.; Cadwallader, L. B.; Vinsant, S. Quantitative changes in hippocampal structure following long-term exposure to delta-9-tetrahydrocannabinol: Possible mediation by the glucocorticoid system. Brain Res. 443:47–62; 1988.
27. McMillan, D. E.; Harris, L. S.; Frankenheim, J. M.; Kennedy, J. S. Delta-9-trans-tetrahydrocannabinol in pigeons: Tolerance to the behavioral effects. Science 169:501–503; 1970.
28. Mechoulam, R. Marihuana chemistry. Science 168:1159–1166; 1970.
29. Mokler, D. J.; Robinson, S. E.; Johnson, J. H.; Hong, J. S.; Rosecrans, J. A. Neonatal administration of delta-9-tetrahydrocannabinol (THC) alters the neurochemical response to stress in the adult Fischer-344 rat. Neurotoxicol. Teratol. 9:321–327; 1987.
30. Moran, T. Society should take the risk. JAMA 261:2443; 1989.
31. Nye, J. S.; Seltzman, H. H.; Pitt, C. G.; Snyder, S. H. High affinity cannabinoid binding sites in brain membranes labeled with (3-H)5′-trimethyl-ammonium delta-8-tetrahydrocannabinol. J. Pharmacol. Exp. Ther. 234:784–791; 1985.
32. Patel, V.; Borysenko, M.; Kumar, M. S. A.; Millard, W. J. Effects of acute and subchronic delta-9-tetrahydrocannabinol administration on the plasma catecholamine, beta-endorphin, and corticosterone levels and splenic natural killer cell activity in rats. Proc. Soc. Exp. Biol. Med. 180:400–404; 1985.
33. Paule, M. G.; Bailey, J. R.; Fogle, C. M.; Gillam, M. P.; Slikker, W. Jr.; Brown, R. M. Plasma distribution of delta-9-tetrahydrocannabinol (THC) in the rhesus monkey after marijuana smoke exposure. Proc. West. Pharmacol. Soc. 30:397–399; 1987.
34. Paule, M. G.; Allen, R. R.; Bailey, J. R.; Scallet, A. C.; Ali, S. F.; Brown, R. M.; McMillan, D. E.; Slikker, W. Jr. Chronic smoke exposure in the rhesus monkey II: Effects on conditioned position responding. J. Pharmacol. Exp. Ther.; in press.
35. Pavlik, A.; Buresova, M. The neonatal cerebellum: The highest level of glucocorticoid receptors in the brain. Dev. Brain Res. 12: 13–20; 1984.
36. Sassenrath, E. N. Assessment of acute vs. long-term effects of delta-9-tetrahydrocannabinol on social behavior and adaptability in group-living macaques. In: Miczek, K. A., ed. Ethopharmacology: Primate models in neuropsychiatric disorders. New York: Alan R. Liss; 1983:277–291.

37. Scallet, A. C.; Uemura, E.; Andrews, A. M.; Ali, S. F.; McMillan, D. E.; Paule, M. G.; Brown, R. M.; Slikker, W., Jr. Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). Brain Res. 436:193–198; 1987.
38. Scallet, A. C.; Uemura, E.; Andrews, A. M.; Craven, J. M.; Rountree, R. L.; Wilson, S. W.; Ali, S. F.; Bailey, J. R.; Paule, M. G.; Slikker, W., Jr. Morphometric neurohistological studies of rhesus monkeys after chronic marijuana smoke exposure. Soc. Neurosci. Abstr. 16:1116; 1990.
39. Schuster, C. R. The United States' drug abuse scene: An overview. Clin. Chem. 33/11(B):7B–12B; 1987.
40. Schulze, G. E.; McMillan, D. E.; Bailey, J. R.; Scallet, A. C.; Ali, S. F.; Slikker, W., Jr.; Paule, M. G. Acute effects of delta-9-tetrahydrocannabinol (THC) in rhesus monkeys as measured by performance in a battery of complex operant tests. J. Pharmacol. Exp. Ther. 245:178–186; 1988.
41. Schulze, G. E.; McMillan, D. E.; Bailey, J. R.; Scallet, A. C.; Ali, S. F.; Slikker, W., Jr.; Paule, M. G. Acute effects of marijuana smoke in rhesus monkeys as measured by performance in a battery of complex operant tests. Life Sci. 45:465–475; 1989.
42. Slikker, W., Jr.; Ali, S. F.; Scallet, A. C.; Frith, C. H.; Newport, G. D.; Bailey, J. R. Neurochemical and neurohistological alterations in the rat and monkey produced by orally administered methylenedioxymethamphetamine (MDMA). Toxicol. Appl. Pharmacol. 94: 448–457; 1988.
43. Slikker, W., Jr.; Paule, M. G.; Ali, S. F.; Scallet, A. C.; Bailey, J. R. Chronic marijuana smoke exposure in the rhesus monkey I: Plasma cannabinoid and blood carboxyhemoglobin concentrations. Fundam. Appl. Toxicol.; in press.
44. Stiglick, A.; Kalant, H. Learning impairment in the radial-arm maze following prolonged cannabis treatment in rats. Psychopharmacology (Berlin) 77:117–123; 1982.
45. Stiglick, A.; Kalant, H. Residual effects of prolonged cannabis administration on exploration and DRL performance in rats. Psychopharmacology (Berlin) 77:124–128; 1982.
46. Stiglick, A.; Kalant, H. Behavioral effects of prolonged administration of delta-9-tetrahydrocannabinol in the rat. Psychopharmacology (Berlin) 80:325–330; 1983.
47. Stiglick, A.; Kalant, H. Residual effects of chronic cannabis treatment on behavior in mature rats. Psychopharmacology (Berlin) 85: 436–439; 1985.
48. Stiglick, A.; Llewellyn, M. E.; Kalant, H. Residual effects of prolonged cannabis treatment on shuttle-box avoidance in the rat. Psychopharmacology (Berlin) 84:476–479; 1984.
49. Turner, C. E.; Elsohly, M. A.; Boeren, E. G. Constituents of cannabis sativa L., XVII: A review of the natural constituents. J. Nat. Prod. 43:169–234; 1980.
50. Verberne, A. J. M.; Fennessy, M. R.; Lewis, S. J.; Taylor, D. A. Involvement of brain histamine in delta-9-tetrahydrocannabinol tolerance and withdrawal. Pharmacol. Biochem. Behav. 23:153–159; 1985.
51. Walters, D. E.; Carr, L. A. Changes in brain catecholamine mechanisms following perinatal exposure to marijuana. Pharmacol. Biochem. Behav. 25:763–768; 1986.
52. Walters, D. E.; Carr, L. A. Perinatal exposure to cannabinoids alters neurochemical development in rat brain. Pharmacol. Biochem. Behav. 29:213–216; 1988.
53. Warembourg, M. Radioautographic study of the rat brain after injection of [1,2-3H]corticosterone. Brain Res. 89:61–70; 1975.
54. Wert, R. C.; Raulin, M. L. The chronic cerebral effects of cannabis use. I. Methodological issues and neurological findings. Int. J. Addict. 21:605–628; 1986.
55. Wert, R. C.; Raulin, M. L. The chronic cerebral effects of cannabis use. II. Psychological findings and conclusions. Int. J. Addict. 21:629–642; 1986.
56. Wikler, A. Aspects of tolerance to and dependence on cannabis. Ann. NY Acad. Sci. 282:126–147; 1976.