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Cannabis Sativa: Effects on Brain Function and Ultrastructure in Rhesus Monkeys

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Two studies, designed to control for as many variables as possible, were conducted in 21 rhesus monkeys, some with brain electrodes and some unoperated, to determine the effects of marijuana on brain function and ultrastructure. Some monkeys were exposed to smoke of active marijuana (using different dose schedules), some were administered Δ-9-THC (0.69 mg/kg iv), and others were exposed to smoke of inactive *marijuana (from which all cannabinols had been removed). To deliver smoke to the monkeys, two different apparatuses were used. Dose schedules comparable to those of human marijuana smokers were established, as determined by plasma levels of Δ-9-THC. After 2- to 3-months' exposure, the monkeys that were heavy- and moderate-smokers of active marijuana, and those administered Δ-9-THC iv, developed chronic recording changes at deep brain sites, most marked in the septal region, hippocampus, and amygdala. These changes persisted throughout the exposure period (6 or 8 months) and during the postexposure observation period (1 to 8 months). Brains of these electrode-implanted monkeys, as well as those without electrodes that were exposed to Δ-9-THC, showed ultrastructural alterations characterized by changes at the synapse, destruction of rough endoplasmic reticulum, and development of nuclear inclusion bodies. In contrast, brains of monkeys exposed to smoke of inactive marijuana and of unexposed controls showed no ultrastructural changes. The findings indicate that exposure to Δ-9-THC, the psychoactive ingredient of marijuana, at doses commensurate with those used by human marijuana smokers can produce permanent alterations in brain function and structure of monkeys.*

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INTRODUCTION

In the numerous published reports of the effects of marijuana-smoking on behavior, results have been conflicting (Brecher, 1975a, 1975b; Fink *et al.,* 1976; Grinspoon, 1971; Hollister, 1971; Kolansky and Moore, 1972; Rubin and Comitas, 1975). Similar controversy prevails over the effects of cannabis derivatives on brain function and structure (Barnes and Fried, 1974; Campbell *et al.,* 1971; Co *et al.,* 1977; Heath, 1976a, 1976b; Heath *et al.,* 1979). Heavily weighted with expressions of opinion, many reports lack substantial data, and studies in human subjects lack rigid controls. On the other hand, data extrapolated from more exact animal studies have often been received skeptically. Using many of the same techniques we employed previously in patients and animals to establish correlations between brain activity and behavior, and monitoring dosage of psychoactive cannabis material by measuring plasma levels, we have been able to relate Δ-9-tetrahydrocannabinol (Δ-9-THC) effects in rhesus monkeys to those in human smokers. In the monkeys, we observed behavior and brain function simultaneously with exposure to cannabis. We also imposed rigid controls, which were not possible in studies with human subjects. Further, the monkey studies have provided additional invaluable histopathologic data.

Our interest in marijuana was prompted by observations of its effects in patients being studied and treated with deep electrodes. Three patients in this special series were marijuana-users before admission to our service. Under our direction, each smoked a marijuana joint on three different occasions while brain recordings were being obtained. Their pleasurable response to the smoking was associated with distinct changes in deep recordings from sites where activity has been correlated with emotion, changes that were most pronounced in the septal region (Heath, 1954, 1972; Heath and Harper, 1976). Similar alterations in deep brain recordings had been observed in monkeys exposed to marijuana smoke (Heath, 1973).

On the basis of these preliminary findings, we presumed the rhesus monkey would be a useful model for gaining information relevant to man of the effects of chronic cannabis usage. We therefore designed two successive studies in monkeys to determine effects of chronic exposure to cannabis sativa derivatives on function and ultrastructure of their brains, attempting to control as many factors as possible and to simulate the smoking patterns of human subjects.

EXPERIMENTAL MONKEYS

Feral-raised monkeys, 3 to 6 years old and weighing 4 to 6 kg, were used in the studies. Some were prepared with brain electrodes and some were unoperated.

Monkeys with Brain Electrodes

For implantation of electrodes, the monkeys were anesthetized with Nembutal, with roentgenographic visualization of the ventricular system after pneumoencephalography. Two types of silver-ball electrodes were stereotaxically implanted into deep sites and over the cortex of the brain: (1) a single silver-ball electrode 0.025-inch diam and (2) a bipolar electrode composed of two silver balls 0.08-inch apart, each 0.015-inch diam. Each monkey had bipolar electrodes implanted into the septal region, hippocampus, and amygdala (sites where recording changes had been shown to be correlated with emotional expression in previous studies (Heath, 1976c), into sensory relay nuclei, and into nuclei where cells were known reservoirs for specific chemical transmitters. Single silver-ball electrodes were placed on the arachnoid under the dura at the selected cortical sites. In order to obtain recordings from all these sites, two different electrode arrays were used (Table I). After electrode implantation, the monkeys were allowed to rest for at least 2 weeks to ensure full recovery and normal electroencephalograms (EEGs) from all brain sites.

In addition to the deep and surface brain recordings, scalp recordings and an electrocardiogram were obtained routinely.

Unoperated Monkeys

The unoperated monkeys served as controls for the effects of implanted electrodes. If changes in brain function indeed occurred during the studies, we wanted to be sure they could not be attributed to the intracerebral electrodes. Further, the brains of the unoperated monkeys served as controls for the effects of long-term electrode implantation in the histopathologic studies carried out as the final procedure in this investigation.

Table I. Brain Electrode Arrays

Array #1	Array #2
Left temporal cortex	Left temporal cortex
Right temporal cortex	Right temporal cortex
Right occipital cortex	Left frontal cortex
Left hippocampus	Right hippocampus
Right lateral amygdala	Left medial amygdala
Right lateral geniculate	Right medial geniculate
Right anterior septal region	Right anterior septal region
Left posterior septal region	Left posterior septal region
Left mesencephalic reticulum	Left caudate nucleus
Right cuneiform nucleus	Left posterior ventral lateral thalamus
Left raphe nucleus	Left locus ceruleus
Left anterior hypothalamus	Right substantia nigra
Right cerebellar fastigial nucleus	Right cerebellar fastigial nucleus

TEST MATERIALS

Test materials were given to the monkeys in the form of smoke or by intravenous administration.

Smoke of Marijuana

Active marijuana used in the studies contained 2.45% to 3.0% Δ-9-THC.[2] To control for nonspecific effects of smoking, *inactive* marijuana, from which all cannabinols had been removed, was also used for some of the monkeys.

Intravenous Administration of Δ-9-THC

Some monkeys were prepared with indwelling intravenous catheters for administration of Δ-9-THC (95% pure) obtained from the National Institute of Drug Abuse. The Δ-9-THC was suspended in fatty serum and given through the catheter at a dose of 0.69 mg/kg. This dose was chosen because it induced acute behavioral and EEG changes comparable to the acute changes obtained in the heavy- and moderate-smoking monkeys (Study I) during a single smoke exposure. The intravenous administration controlled the variables of smoking effects, such as anoxia, and any inconsistencies in depth and duration of inhalation.

ESTABLISHING DOSE LEVELS FOR MARIJUANA-SMOKING

When these studies were begun, dependable measures for blood levels of Δ-9-THC were not available. We therefore based dosages of starting material on published reports of quantities consumed by human smokers (Tennant and Groesbeck, 1972), calculating on a weight basis (man *vs.* monkey) and using Freireich *et al.*'s (1966) factor for relative surface area. Later, we were able to measure blood Δ-9-THC levels[3] in the monkeys for comparison with levels obtained in human subjects who smoked known quantities of marijuana. In subsequent studies, therefore, dosage was established on the basis of blood levels of Δ-9-THC.

[2]Assays by Carlton E. Turner, Ph.D., Associate Director of the Research Institute of Pharmaceutical Science, School of Pharmacy, University of Mississippi, Oxford, Mississippi. Inactive marijuana was also obtained from Prof. Turner.

[3]Radioimmunoassays were performed by the Cannabinoid Quantification Serum Laboratory, White Memorial Center, Los Angeles, Calif., and gas chromatography-mass spectrometry analyses were performed by Battell Laboratories, Columbus, Ohio.

SMOKING PROCEDURES

Original Apparatus

The apparatus designed and fabricated in our laboratory that we first used to deliver smoke to the monkeys is shown in Fig. 1. The marijuana was weighed and packed into a pipe, which was attached to a modifed respirometer. The marijuana was then lighted and the smoke drawn into the respirometer by a variable-speed electric motor until combustion of the marijuana was completed. Smoke was delivered from the respirometer through a tube to the nasopharynx of the monkey at a rate commensurate with the rate observed in human smokers.

Use of the respirometer to trap smoke from accurately measured quantities of marijuana involved modification of the technique described by Renault and associates (1971). It was the first step towards establishing accurate dosage. Problems arose, however, concerning loss or alteration of the Δ-9-THC as it passed through the apparatus. Further, the monkey's efficiency in inhaling smoke and absorbing the psychoactive ingredients

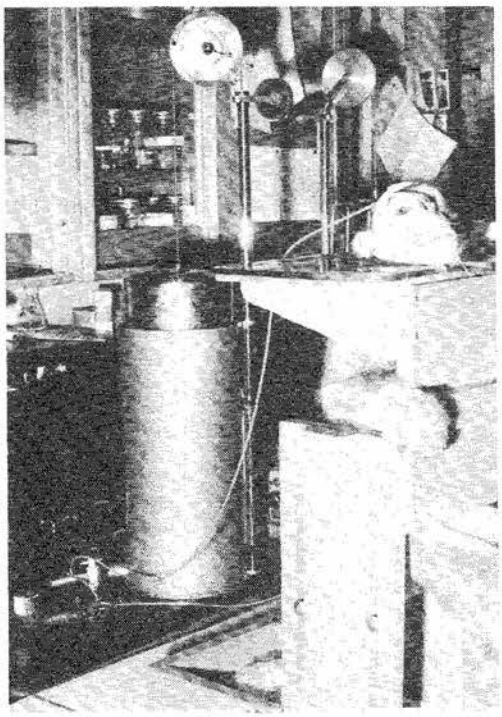

Fig. 1. Original smoking apparatus (see text).

posed a variable when compared to a human subject's smoking pattern. Consequently, dosages used in our initial study were crude estimates based on the following considerations. Application of Freireich *et al.*'s (1966) factor to the monkey required a 300% increase in dosage (per kg) over the dosage calculated on a per kilogram basis used by a human subject. Further, in initiating Study I, we estimated that 50% of the psychoactive ingredient (Δ-9-THC) would be lost in the respirometer and tubing of our smoking apparatus, and we therefore again doubled the dose. The amount of starting material per exposure used in our original smoking apparatus was therefore about six times that contained in the average joint of a human smoker (per kg of body weight). In our initial studies, no allowance was made for differences in smoking patterns of monkey and man, that is, willingness to inhale deeply and hold the smoke before exhaling.

Dosage Problems and Resolution

Early in Study I, the pattern of the monkey's inhaling the smoke became an obvious problem. Despite the uniformity of starting material, we suspected significant variability in the quantity of active material being absorbed because of the inconsistent responses of the monkeys. When assays for Δ-9-THC levels in blood and in smoke became available to us, we were able to relate dosage more meaningfully for monkey and man by (i) accurately measuring quantities of Δ-9-THC in the smoke delivered to the monkey by the smoking apparatus *vs.* that inhaled by man from the same amount of marijuana, and (ii) measuring the quantities of Δ-9-THC absorbed into the blood from the smoke exposure.

To appraise the efficiency of our smoking procedure for monkeys, we conducted two experiments with the original smoking apparatus after Study I, when plasma assays of Δ-9-THC became available.

First, to evaluate the effectiveness of the smoking apparatus, the smoke of a standard human dose of marijuana (1 g) was delivered through the apparatus into an ethanol bath to trap the Δ-9-THC for assay. For comparison, a man, using the same standard dose (1 g), drew the smoke from a pipe into his mouth and, without inhaling, blew the smoke through a short tube into the ethanol solution for assay of Δ-9-THC content.[4] The results shown in Table II indicate that man was able to capture slightly more than 25% of the Δ-9-THC in the starting material, whereas the smoking apparatus delivered only 3.4% of the Δ-9-THC in the starting material. With both smoking procedures (man and apparatus), there was residual Δ-9-THC in the pipe as noncombustible marijuana and ashes. These

[4]Sent to Carleton E. Turner, Ph.D. for assay of Δ-9-THC in both samples.

Table II. Smoking Efficiency: Δ-9-THC Captured by Man *Vs.* Δ-9-THC Delivered by Smoking Apparatus[a]

Variable	Smoke mg Δ-9-THC	Ashes mg Δ-9-THC
Human (n = 4)	6.45 (25.10%)	3.78 (14.70%)
Smoking apparatus (n = 12)	0.86 (03.42%)	Trace (0.10%)

[a]Starting material each smoke = 1 g marijuana (2.45% Δ-9-THC = 24.5 mg Δ-9-THC)

findings demonstrate that the smoking apparatus delivered only one-eighth the quantity of Δ-9-THC that the man obtained from the same amount of starting material, a significant quantity of the active ingredient having been dissipated and/or remaining attached to the apparatus. Although the dose originally used in the monkey, based on rough estimates, was six times that of man on a per weight basis, we found, after the radioimmune assay technique became available to us, that our rough estimate was too low. The original smoking apparatus was so inefficient that a dose eight times that for man on a per weight basis was required for delivery of a weight equivalent dose to the monkey.

The purpose of the second experiment was to compare the smoking efficiency of the monkey with that of the human subject. Results of some of these experiments are shown in Table III. In these examples, plasma levels

Table III. Δ-9-THC Plasma Levels: Man *Vs.* Monkey

Variable		Number of joints	Δ-9-THC content (mg)	Time after smoking (min)	Plasma level[b] (ng/ml)	Method
Human subject	1	1	5.0	5	30-70	RIA
	2	1	18.5	15	140-150	RIA
	3	1	10.0	5	100	GC-MS
	4	1	10.8	15	15-66	GC-MS
	5	1	10.0	10	19-26	GC-MS
Monkey						
Original apparatus	1	1	5.4	5	8	RIA
	2	1	21.0	5	35	RIA
	3	3[a]	23.5	5	40	RIA
	4	1	21.0	5	3.2	GC-MS
New apparatus	5	1	6.13	5	60	GC-MS
	6	1	6.13	5	95	GC-MS
	7	2[a]	12.26	5	100	GC-MS
	8	2[a]	12.26	5	140	GC-MS

[a]One-hour interval between smoke exposures.
[b]When two figures are shown for plasma levels, they represent the range from minimal to maximal values for consumption of one joint with repeated smoking on different days.

of Δ-9-THC were determined by radioimmune assay (RIA) or gas chromatography-mass spectrometry (GC-MS), or both, in monkeys and man at various times after exposure to smoke of varying amounts of marijuana. The smoke was delivered to the monkeys by the original smoking apparatus (four monkeys) or the new apparatus (four monkeys), and the human subjects smoked joints conventionally.

Two points become apparent from the data of the second experiment: (1) Δ-9-THC plasma levels varied considerably from given amounts of marijuana in both the monkeys and human subjects, a reflection of depth and frequency of inhalation during the smoking period. (ii) Despite differences in total body weight, monkey and man require similar amounts of starting marijuana to produce similar plasma levels of Δ-9-THC.

These two experiments show that the quantity of starting material used for our dose in Study I (0.82 g) was within the range required to induce a plasma level of Δ-9-THC in the monkeys that approximated plasma levels induced in human subjects who smoked an average-size joint (0.8 to 1.2 g of marijuana containing 3.0% Δ-9-THC). Man's weight is 15 to 20 times that of the rhesus monkey. For the monkey to achieve a plasma level of Δ-9-THC in the range of a man's plasma level, approximately the same total amount of starting material was required (15 to 20 times the dose per unit of weight). The inefficiency of the original smoking apparatus alone required an eightfold increase to deliver an equivalent amount of Δ-9-THC to the monkey. The additional increase up to 16 to 20 times was necessary because of the monkey's inability to inhale smoke as man inhales.

New Apparatus for the Delivery of Smoke

Because of the obvious inefficiency of our original smoking apparatus, we designed a new one to allow closer simulation of man's smoking pattern. This apparatus, shown in Fig. 2, which we continue to use for our studies, consists of a modified infant Isolette respirator (Model 2900070), along with a specially adapted anesthesia mask and a series of valves. The apparatus accurately and consistently reproduces the smoking pattern of a human subject. It meets the following criteria: (i) Artificially compels the monkeys to inhale and to hold the marijuana smoke in its lungs; (ii) Flushes the smoke out of a face mask to allow the monkey free breathing between puffs; (iii) Accurately repeats (programmable system) the time sequence of drawing in the smoke and holding it in the lungs.

Steps in the smoking procedure are as follows: (i) The monkey is restrained in a chairlike device, which is then placed in the chamber of the Isolette respirator with only the monkey's head exposed. A modified, disposable foam strip, attached around the monkey's neck, acts as

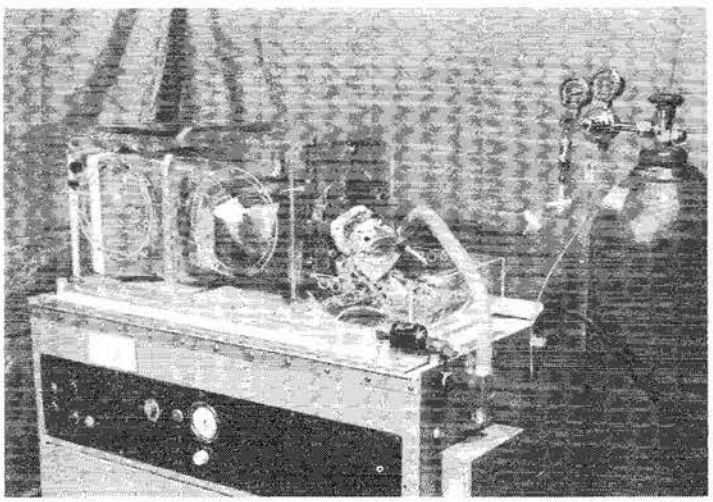

Fig. 2. New smoking apparatus (see text).

a seal between the head and the restraining plate attached to the respirator. (ii) With the monkey in the chamber, an oxygen supply tube and a breathing hose are attached to the mask. (iii) A lighted "joint" is then inserted into a fitting attached to the anterior end of the mask. (iv) By depression of a start-cycle switch, the machine (respirator and attached valves) is activated in the following sequence: (a) With the oxygen supply solenoid in the closed position, the breathing valve closes. (b) The vacuum motor is activated, thereby creating a vacuum of 6-sec duration in the chamber, thus expanding the monkey's lungs and causing it to inhale the smoke as it is sucked from the joint into the face mask. (c) When maximal vacuum is achieved, exhalation thereby being restricted, the oxygen supply solenoid and the breathing valve open, flushing the mask of smoke and preparing the monkey to breathe air uncontaminated by marijuana after the vacuum in the chamber is released. (d) The vacuum is then released, the monkey resuming normal breathing through the breathing valve. The possibility of anoxia from rebreathing air in the tubing is eliminated by supplying oxygen to the monkey during this period. (e) After about 24 sec, the oxygen supply closes and the cycle resumes. The procedure is repeated until the joint is completely burned.

Oxygen was added to eliminate any possibility of carbon monoxide accumulation in the tubing. With this smoking program (as well as with the original apparatus), blood gases remained within normal range.

By measuring plasma levels of Δ-9-THC, we determined that a 0.25-g joint (6.1 to 7.5 mg Δ-9-THC) smoked by the monkey in the new apparatus

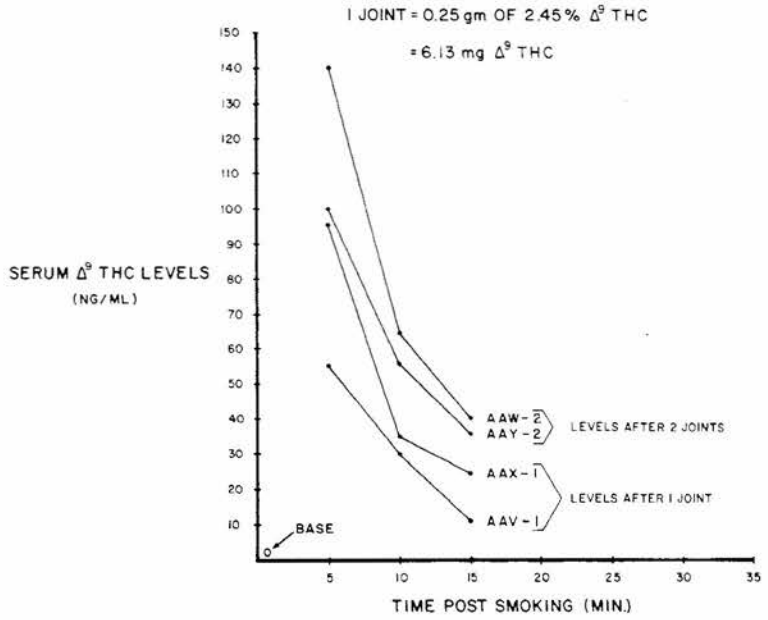

Fig. 3. Plasma levels of Δ-9-THC in four monkeys at various times after initial exposure to smoke of marijuana. For comparison with plasma levels in human smokers of marijuana, see Table III.

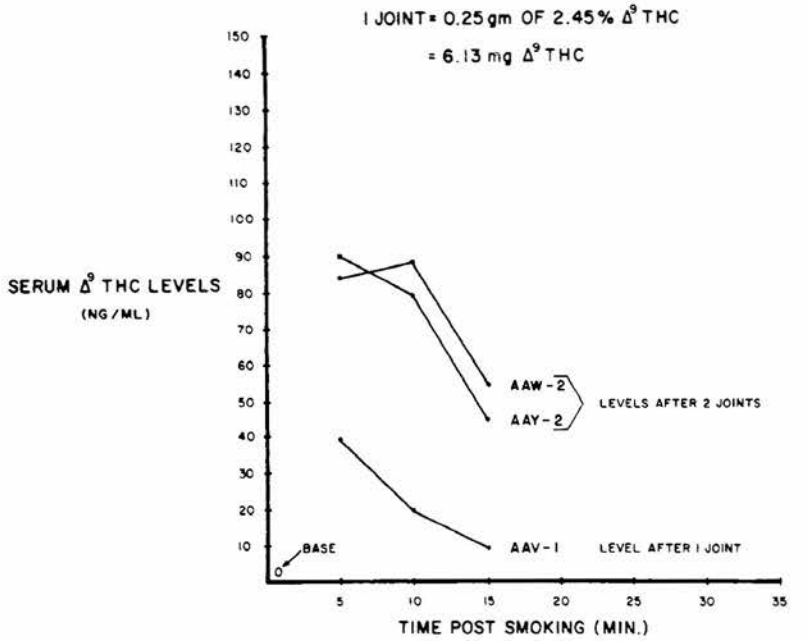

Fig. 4. Plasma levels of Δ-9-THC at various times 2 months after regular smoking.

induced a plasma level within the range of the plasma level of a man who smoked a 1.0-g joint of the same Δ-9-THC content (Fig. 3). With this smoking technique, using the 0.25-joint, the monkey was exposed to smoke containing 1 to 2 mg/kg of Δ-9-THC per joint. As these data indicate, the loss of active material with the new smoking apparatus is considerably less than with the original apparatus. Even after chronic smoking, the plasma level consistently increased with exposure to smoke of a 0.25-g joint, although to a slightly lesser degree than with the original exposure (Fig. 4). Because the 0.25-g joint produced plasma levels similar to those of a man smoking a standard joint, we standardized on the 0.25-g joint for monkey Study II, using the new apparatus.

STUDY I

Thirteen monkeys were used in the first study conducted with the original smoking apparatus before methods were developed for determining plasma levels of Δ-9-THC. Each standardized dose of *active* marijuana consisted of smoke of 0.82 g of marijuana assayed at 3.0% Δ-9-THC equaling 24.6 mg Δ-9-THC. Each dose of *inactive* marijuana smoke (from which cannabinols, Δ-9-THC and Δ-8-THC, had been removed) was also from 0.82 g of marijuana.

Heavy Smokers

Four monkeys (two implanted, one with electrode Array #1 and the other with Array #2, and two without electrodes in which subsequent histopathologic examination was uncomplicated by electrode tracks) were exposed to three smokes of *active* marijuana per day (0.82 of marijuana per joint, with a rest period of at least 1 hr between smokes), 5 days a week, for 6 months.

Moderate Smokers

Two monkeys (one implanted with electrode Array #1 and the other with Array #2) were exposed to one smoke of *active* marijuana (0.82 g of marijuana per joint) on each of 2 days per week for 6 months.

Light Smokers

Three monkeys (one implanted with Array #1, one with Array #2, and a third unoperated) were exposed to smoke of a lower dose (0.24 g containing 7.2 mg Δ-9-THC) of *active* marijuana once per day, 5 days a week, for 6 months. The dose per day was therefore one-tenth that of the heavy-smoking monkeys.

Inactive Marijuana Smokers

Two monkeys (one with Array #1 and the other with Array #2) smoked *inactive* marijuana three times a day (with a rest period of at least 1 hr between smokes), 5 days a week for 6 months, the same schedule that was used for the heavy smokers of active marijuana.

Intravenous Administration

Two monkeys (one with Array #1 and the other with Array #2) were given Δ-9-THC suspended in fatty serum through the intravenous catheter at a dose of 0.69 mg/kg once per day, 5 days a week, for 6 months.

Duration of Study

After continuing the above-described schedule for 6 months, the monkeys were rested for 8 additional months before being sacrificed and their brains removed for histopathologic studies.

Electroencephalograms

With a 16-channel Grass Model 6 or 8 electroencephalograph, base-line recordings were obtained on all monkeys at least three times before exposure to cannabis sativa. Recordings were obtained weekly throughout the exposure period and during the 8-months' postexposure, resting period. Both deep and surface recordings were obtained in those monkeys prepared with brain electrodes. In the unoperated monkeys, scalp recordings only were obtained.

STUDY II

In Study II, designed to augment Study I and to answer at least some of the questions arising from it, the new smoking apparatus was used in six monkeys: three were implanted with deep and surface electrodes (two with Array #1 and one with Array #2) and three were unoperated. Only active marijuana was used, each joint containing 0.25-g marijuana assayed at 2.45% to 3.0% Δ-9-THC (6.1 to 7.5 mg Δ-9-THC).

Smoking Schedule

All six monkeys were exposed to marijuana smoke 5 days per week for 8 months. Three of the six monkeys (one implanted with Array #1 and two

without brain electrodes) were exposed to smoke of two joints per day (with a rest period of at least 1 hr between), 5 days per week, and three (one implanted with Array #1, one with Array #2, and one without electrodes) were exposed to smoke of one joint per day, 5 days per week.

Electroencephalograms

The same schedule of recordings was followed as for Study I (three base-line recordings before exposure and weekly recordings during the 8 months of exposure).

Postexposure Observation Period

Postexposure periods varied. One of the six monkeys (with electrodes) was sacrificed 1 day after the last smoke exposure, three (two with electrodes and one unoperated) were sacrificed on the 5th day after the last smoke exposure, one (unoperated) was sacrificed 2 months after, and one (with electrodes) 7½ months after. Two of the monkeys (the ones sacrificed at 2 months and 7½ months) were administered Δ-9-THC intravenously 15 min before being sacrificed.

HISTOPATHOLOGIC STUDIES OF MONKEY BRAINS

For histopathologic studies, the monkeys were anesthetized with sodium pentobarbital (30 mg/kg) and perfused at ambient temperature under a pressure of 115 mm Hg. A short flush of heparinized normal saline preceded the fixation solutions. The initial 30 min of the perfusion was done with a 1% paraformaldehyde/1.5% gluteraldehyde mixture made up in 0.1 M cacodylate buffer. This was followed for 60 min by a 4% paraformaldehyde/4% gluteraldehyde mixture and the same buffer. The brain was then removed from the skull and soaked in the more concentrated fix for an additional 3½ hr. It was then washed five times in 0.1 M cacodylate buffer with 10% sucrose for a total of 60 min. During this time, pledgets were removed from the designated loci.

The cut tissue was postfixed for 90 min in 1% osmium tetroxide in 0.1 M cacodylate buffer. *En bloc* staining was accomplished during the first step of dehydration with 1% uranyl acetate made up in 50% ethanol. Graded ethanol completed the dehydration before the tissue was embedded in Epon 812.

One-micron sections were cut on a Porter-Blum MT2 microtome for light microscopy. Differential staining was obtained with basic fuchsin and methylene blue (Huber *et al.*, 1968) or with methylene blue *p*H 4 followed by methylene blue *p*H 11, or by Del Rio Hortega's silver method (Goldblatt

and Trump, 1965). On some sections, the epoxy was removed before staining with hematoxylin and eosin (Lane and Europa, 1965) or with methylene blue-basic fuchsin and malachite green (Berkowitz *et al.*, 1968). Blocks were trimmed to discrete sites as determined from the thick sections. Light gold to silver sections were cut and stained with uranyl acetate and lead citrate before being viewed on a Philips EM-300 electron microscope.

The septal region and hippocampus of five representative brains were studied to determine effects of cannabis sativa on ultrastructure of the synapse and on the organelles of the neurons. Three were brains from monkeys in Study I: one from an unoperated heavy-smoking monkey of *active* marijuana, a second from a monkey with electrodes that had received intravenous Δ-9-THC, and a third from an electrode-implanted heavy-smoking monkey of *inactive* marijuana. The other two brains were from unoperated control monkeys that had not been used previously for any study.

Small pieces of the subcallosal gyrus of the septal region at approximate stereotaxic coordinates A 24, D + 7, and L 2 and of the hippocampus at approximate stereotaxic coordinates A 9, D −8, and L 12 were taken from the coronal plane (Snider and Lee, 1961). These tissues were selected for detailed, time-consuming ultrastructural study because the septal region and hippocampus were two of the three brain sites that showed the most consistent, lasting changes in recordings (see Results). In the two monkeys with chronically implanted electrodes, tissues were obtained on the side opposite the site of implantation to minimize the possible complicating effects of the implanted electrode. Samples for quantitative study were randomly coded so that the investigators were unaware of the monkey source of the tissue under study.

The Synapses

Photographic plates taken for measurement were standardized for magnification (39,000 X) with a carbon-grating replica (Fullam No. 1002), and enlarged with a 3.75 print factor. Ensuing prints were measured with Mitutoyo calipers under a 3 X dissecting microscope; 600 prints were found acceptable for measurement. The distance between outer leaflets of the confronting pre- and postsynaptic membranes was taken at the synaptic cleft width. The mean of three measurements, at the center and near each edge, was used to determine the final width value for each cleft.

We chose to compare synapses of the same morphologic type from septal and hippocampal tissues of the five representative brains. Axodendritic synapses, described by Colonnier (1968) and by others

(Møllgaard *et al.*, 1971) as corresponding closely to Gray's (1959) asymmetric Type I synapses, were measured. These synapses are characterized by a thickened postsynaptic cytoplasmic opacity bordering the region of synaptic contact and by a population of spherical vesicles. Only those fine structures with clear pre- and postsynaptic membranes were included, so as to ensure exact cross-sectional measurement of the cleft. Layer 5 of the septal region and CA III of the hippocampus, the large pyramidal neuron layers, were the specific sites chosen for study. The cytoarchitectural boundaries of these sites, particularly when seen first in semithin reference sections, are distinct, and these layers were therefore localized within each thin section (of each of the brains) with minimal difficulty.

The Organelles

Photographic plates taken for measurement were standardized for magnification (6840 X) with a carbon-grating replica (Fullam No. 1002). In order to construct a montage, we photographed all cells in parts and then enlarged with a 2.75 print factor to give a final magnification of 18,800 X.

The technique of stereologic morphometry (Loud, 1968; Weibel *et al.*, 1966; Rosiwal, 1898; Myers and Heath, 1979) was used to measure volume density. Volume density is defined as the volume of the components related to the containing volume (also called the relative or fractional volume; for example, mitochondrial volume contained in the unit volume of cytoplasm).

An analysis of variance with a significance ($F_{1,3}$) of $p < 0.01$ was used for statistical comparison in calculating the mean values for each monkey brain.

Ultrastructural Examination of the Nuclei

In addition to the five brains included in the other ultrastructural studies, the brain of a sixth monkey was added for this study. A Study II monkey, it was exposed to smoke once per day, 5 days per week, for 8 months. After being rested for 2 months after exposure, the monkey was given 0.7 mg/kg iv dose of Δ-9-THC 15 min before being sacrificed.

For this study, nerve cell nuclei from seven brain sites were viewed: septal region, posterior ventral lateral thalamus, hippocampus, amygdala, fastigial nucleus, caudate nucleus, and motor cortex. At each of these sites of each brain, 140 to 480 nuclei of nerve cells were examined and the number of inclusion bodies in these nuclei counted.

RESULTS

Brain Recordings, Study I

Immediate Effects

The smoke of active marijuana in heavy and moderate dose levels and intravenous Δ-9-THC induced distinct and immediate (acute) changes in behavior, the monkeys responding less to all forms of sensory stimuli and tending to stare blankly into space. In the four heavy- and moderate-smoking monkeys prepared with deep and surface electrodes, distinct changes occurred at many brain sites, but they were most consistent in the septal region, hippocampus, and amygdala (Fig. 5). Changes in scalp recordings in these monkeys and in the two intact (without implanted electrodes) heavy-smoking monkeys were not significant. When they did occur, they were nonspecific, in the form of generalized slowing of the type correlated with reduction in level of awareness (such as drowsiness).

Changes in recordings of the two monkeys that received intravenous Δ-9-THC were more pronounced than those obtained from monkeys exposed to smoke of active marijuana (Fig. 6). High-amplitude spiking or slow activity, or both, occurred at specific subcortical sites. When the monkeys displayed catatoniclike behavior, the recordings resembled those we had previously obtained from the septal region of severely disturbed psychotic patients (Heath, 1975). At other times, when the monkeys behaved as if they might be hallucinating, these recording changes were most pronounced in the sensory relay nuclei, but recordings (spike and slow-wave) from nuclei-containing cells for specific transmitter chemicals were also affected (Fig. 7). The migration of the abnormal electrical activity was among brain sites previously described as integral sites in the neural network for emotional expression (Heath, 1976c).

In the monkeys exposed to smoke, these behavioral and recording changes came on gradually, being quite pronounced at the end of the smoke exposure, whereas they occurred within 1 min after intravenous administration of Δ-9-THC. In both instances, there was a gradual return to base line in 30 to 40 min.

No behavioral or recording effects developed in the light-smoking monkeys or in the heavy smokers of *inactive* marijuana.

Persistent Effects

Persistent (chronic) changes in brain activity, that is, changes that remained beyond the acute effects, developed in the monkeys exposed to

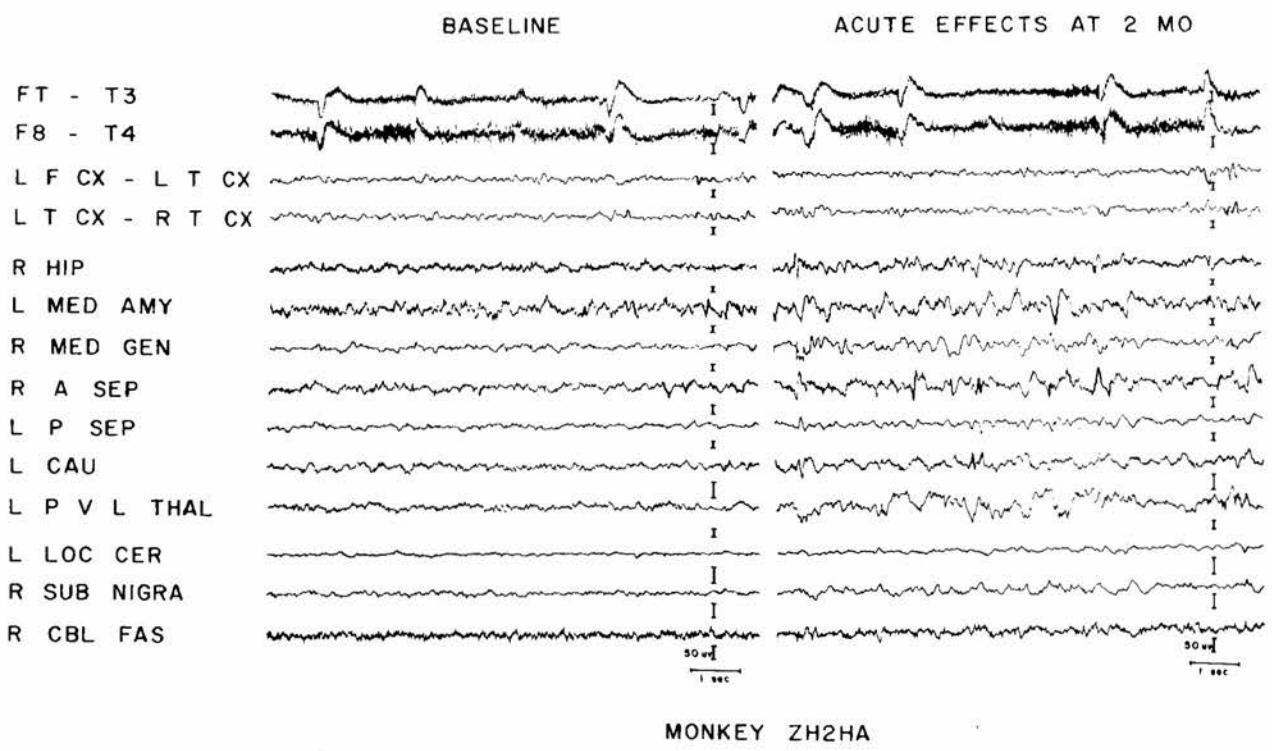

Fig. 5. Sample recordings obtained from an electrode Array #2 monkey (see Table 1) before (base line) and 10 min after exposure to the smoke of a standard dose of active marijuana. During the acute effects, there is generalized slower activity and sharp waves predominant in deep brain sites, most pronounced in the amygdala and septal region.

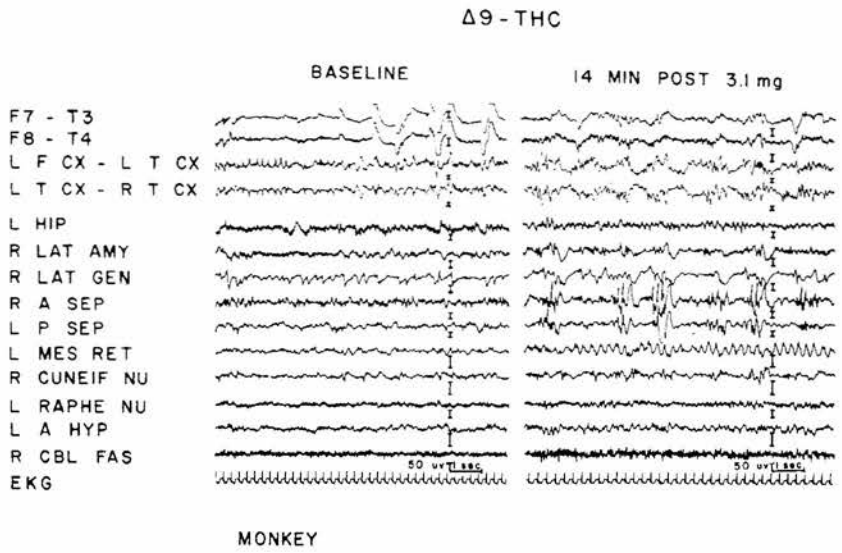

Fig. 6. Sample recordings obtained from an electrode Array #1 monkey (see Table I) before (base line) and 14 min after 3.1 mg iv administration of Δ-9-THC. Generalized slowing is evident in several leads. Associated with the monkey's catatonic behavior, there is theta activity predominantly in the mesencephalic reticulum concomitant with spike activity in septal leads (similar to EEG activity seen in psychotic patients).

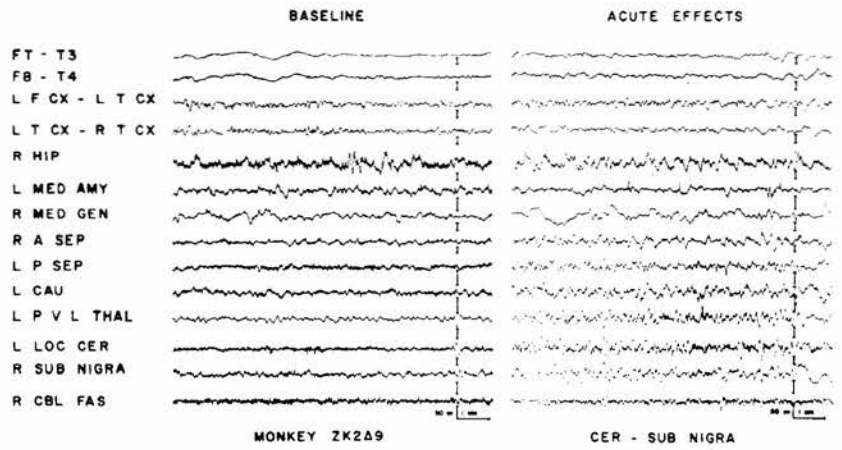

Fig. 7. Sample recordings obtained from an electrode Array #2 monkey before (base line) and 20 min after 0.69 mg/kg iv administration of Δ-9-THC. Distinct slowing is present in the medial geniculate and spiking activity is predominant in the locus ceruleus and substantia nigra. Recordings from cortical leads are not notably altered.

Δ-9-THC. The septal region, hippocampus, and amygdala were most profoundly affected, the changes consisting of increased spiking with slow-wave activity. In the moderate and heavy smokers of active marijuana, the chronic brain changes became apparent about 3 months after exposure began, and in the monkeys given injections of Δ-9-THC, the chronic changes were evident within 2 months. The recording changes outlasted the actute effects of the smoke or intravenous injection, persisting through the weekend when the monkeys were "rested."

No chronic changes appeared in the conventional scalp recordings of the two intact monkeys exposed to heavy smoking of active marijuana.

Monkeys classified as light smokers of *active* marijuana and those exposed to smoke of *inactive* marijuana showed no notable recording or behavioral alterations.

Postexposure Effects

After exposure, the monkeys were followed for 8 additional months. During this period, the electrodes of some of the monkeys had deteriorated so that meaningful deep recordings could no longer be made. In five monkeys, however, electrodes continued to function well, and recordings were obtained on a regular basis. Of this group, one monkey was a heavy smoker of *active* marijuana, two had received Δ-9-THC, intravenously, and two had been exposed to smoke of *inactive* marijuana. In the heavy-smoking monkey and in the two that had received intravenous Δ-9-THC, EEG alterations recorded at the end of the 6-months' exposure period persisted essentially unchanged throughout the entire 8 months' postexposure observation period (Figs. 8 and 9). The two monkeys exposed to *inactive* marijuana smoke continued to have normal recordings.

Brain Recordings, Study II

Because of technical problems, long-term recordings were obtained from only two of the three monkeys with implanted electrodes. One monkey had two smokes per day 5 days per week, and the other, one smoke per day 5 days per week for 8 months. In both, acute effects were the same as those described for the Study I monkeys that received *active* marijuana smoke in heavy or moderate doses or intravenous Δ-9-THC. Persistent changes in recordings, as previously described, developed after 2-months' exposure in the monkey that had two smokes per day, remaining for 8 months until the electrodes became nonfunctional and recordings could no longer be made. The monkey exposed to one smoke per day developed lasting changes after 3 months in the form of spike and slow-wave activity, most pronounced at the same deep brain sites affected in Study I monkeys,

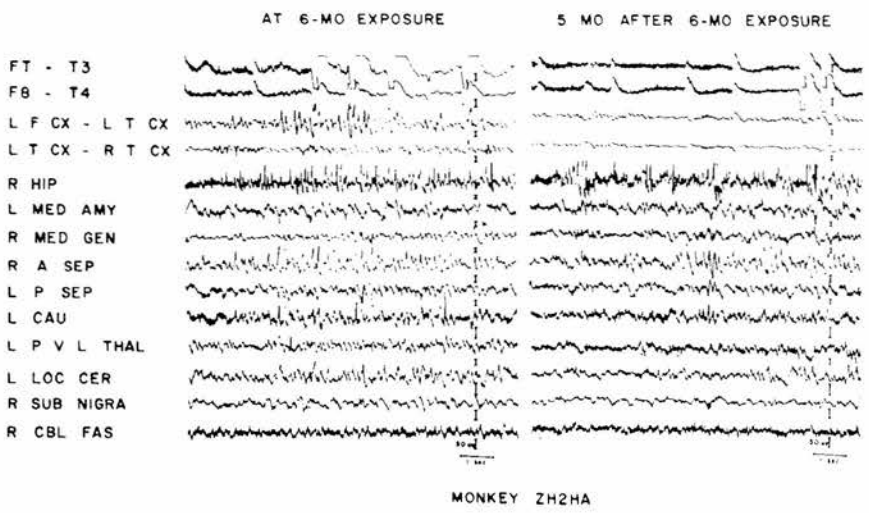

Fig. 8. Sample recordings illustrating long-term effects of exposure to active marijuana smoke. (See this Array #2 monkey's base-line recording in Fig. 5.) Left: Recordings obtained 2 days into the postexposure observation period after 6-months' exposure. The persistent effects, which began 3 months after exposure to smoke, are generalized slow-wave and spiking activity, most pronounced in the hippocampus, septal region, and amygdala. Right: Recordings obtained 5 months after the close of the 6 months' exposure period, the persistent changes most pronounced in the hippocampus, septal region, and amygdala.

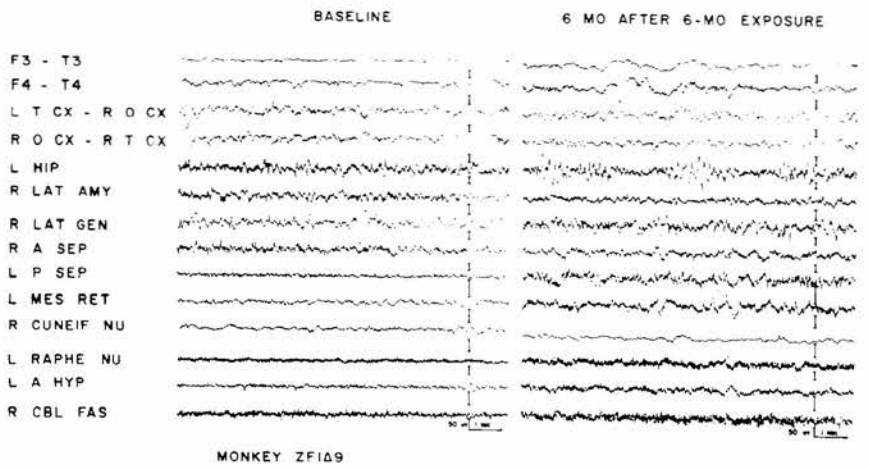

Fig. 9. Sample recordings from an Array #1 monkey illustrating long-term effects after 6-months' exposure to iv Δ-9-THC. In this sample, the most pronounced effects are the high-amplitude spiking in the hippocampus. In other recordings from this monkey, as well as other monkeys, alterations were more pronounced at other deep brain sites.

the changes persisting until the monkey's sacrifice 5 days after the last exposure.

Histopathologic Findings

No significant abnormalities were observed during light microscopic histopathologic studies on each of the six monkey brains.

Ultrastructural changes were consistent in the monkeys that showed lasting EEG changes after heavy or moderate smoking of *active* marijuana or to intravenous Δ-9-THC. In contrast, tissues of the monkey that heavily smoked *inactive* marijuana and those of the unexposed controls were normal. Pathologic changes were in (i) the structure of the synapse, (ii) the volume density and distribution of the rough endoplasmic reticulum, and (iii) the nuclei, characterized by the presence of a large number of intranuclear inclusions.

Changes were essentially the same at all sites studied, but the degree of change varied somewhat. Pathologic change was most pronounced in the septal region, next most notable in the hippocampus and amygdala, and least profound in the cortex.

Ultrastructure of the Synapse

At the synapse, the cleft was widened. An electron-opaque material was present in the cleft and in pre- and postsynaptic regions, and there was some clumping of the synaptic vesicles. Table IV shows a comparison of axodendritic synaptic width measurements in pyramidal cells of layer 5 of the septal region and CA III of the hippocampus. Tissues of monkeys exposed to active cannabis had a greater mean synaptic width (about 75 Å wider in the septal region and 70 Å wider in the hippocampus) than tissues of unexposed control monkeys or of the heavy-smoking monkey of inactive marijuana. The analysis of variance indicates the monkeys receiving the active materials did not differ significantly from one another, but had a significantly larger mean synaptic cleft width in the septal region (about 277 Å) and in the hippocampus (about 274 Å) than those not exposed to psychoactive material. On the other hand, no significant differences were observed between the two unexposed control monkeys or among unexposed controls and the monkey that smoked *inactive* marijuana heavily.

Figure 10 (left side) illustrates the neurophil of CA III of the hippocampal tissue of the monkey that heavily smoked *inactive* marijuana. Dendrites are evident in various planes of section, and an electronlucent intercellular space (100 Å—150 Å) separates all tissue components. Axodendritic synapses can also be seen, each characterized by a presynaptic bouton (B), with a complement of spherical synaptic vesicles, a distinct synaptic cleft, and a postsynaptic dendrite (D). The inset (left) of the

Table IV. Comparison of Synaptic Width Measurements

Monkey sample	Septal region				Hippocampus			
	n	Mean synaptic cleft (Å)	SD	SE	*n*	Mean synaptic cleft (Å)	SD	SE
Heavy smoker of active marijuana	120	275.93	32.72	2.99	100	271.67	26.54	2.86
Δ-9-THC iv	120	278.25	24.86	2.27	100	276.84	24.22	2.07
Heavy smoker of inactive marijuana	120	201.71	18.18	1.66	100	204.18	17.10	1.44
Unexposed control I	120	204.18	12.43	1.13	100	203.46	13.66	1.32
Unexposed control II	120	205.64	16.54	1.50	100	205.18	10.93	1.50

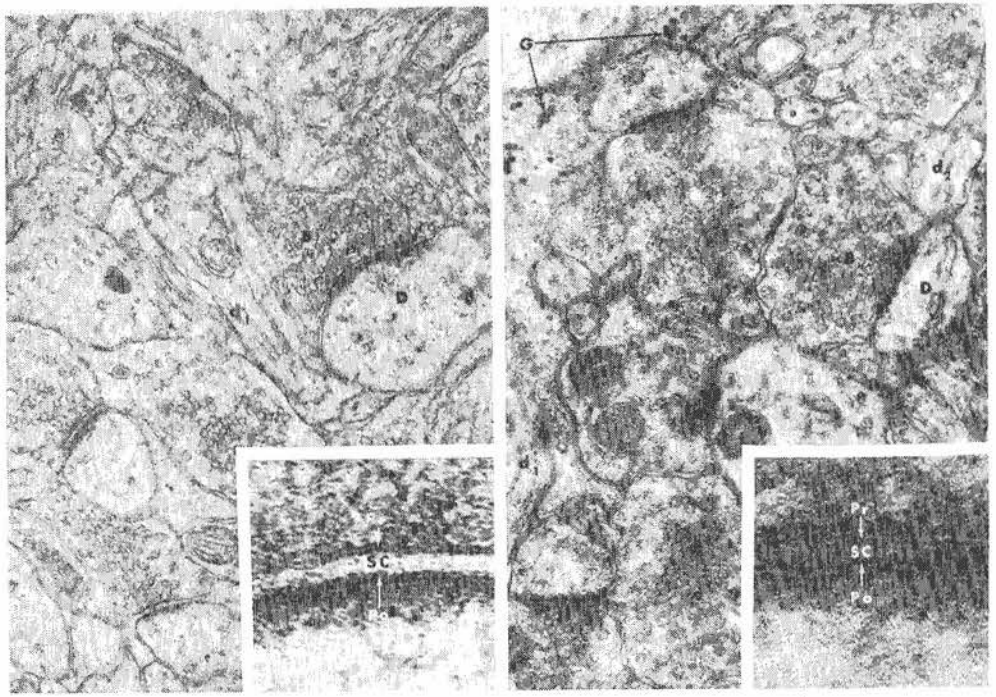

Fig. 10. Photomicrographs showing ultrastructure of CA III hippocampal tissue of a control monkey (left) and of a monkey exposed to smoke of active marijuana (right). 40,000 X. Inset, 120,000 X. B = presynaptic bouton; D = postsynaptic dendrite; d_1 and d_2 = dendrites in various planes of section; SC = synaptic cleft; Pr = presynaptic membrane; Po = postsynaptic membrane; G = glycogen particles. (Figure reduced 55% for reproduction.)

figure shows the synaptic cleft region of such a synapse at higher magnification. The presynaptic membrane (pr), the cleft region (sc) (about 200 Å in width), and the postsynaptic membrane (po) with its subsynaptic opacity are evident.

In Fig. 10 (right side), an axodendritic synapse and astrocytic process (As) containing numerous glycogen granules (g) are seen in CA III neurophil from the monkey that was exposed to large quantities of *active* marijuana smoke (heavy smoker). When compared to control tissue, certain fine structural differences are disclosed. The synaptic cleft region and some of the extracellular space (at arrows) are electron-opaque. The inset (right side) shows the synaptic cleft region from a similar synapse at higher magnification. Electron-opaque granular material is seen to be accumulated in the cleft and in the pre- and postsynaptic regions. The distance between opposing synaptic membranes seems appreciably widened. In the monkeys that received psychoactive cannabis, the presynaptic boutons appeared swollen, containing aggregations of tightly packed synaptic vesicles (Harper *et al.*, 1977). These clumped vesicles usually occupy central regions of the bouton. Vesicles of this nature were not observed in brains of the three control monkeys that were studied.

Ultrastructure of the Organelles of Neurons

In exposed monkeys, there were distinct ultrastructural changes in organelles of neurons of all brain sites studied, specifically of pyramidal cells ranging from 20- to 24-μ diam. Volume density was reduced along with various degrees of fragmentation and disorganization of the rough endoplasmic reticulum. The volume density of the organized rough endoplasmic reticulum in the brains of the monkeys that heavily smoked *active*

Table V. Percentage Volume Density Cytoplasm Organized Rough Endoplasmic Reticulum

	Septal region			Hippocampus		
Monkey sample	Mean	Sum of means	SD	Mean	Sum of means	SD
Heavy smoker of active marijuana	18.17	20.12	± 2.74	18.79	20.29	± 2.22
Δ-9-THC iv	22.05			21.88		
Heavy smoker of inactive marijuana	41.08			39.86		
Unexposed control I	46.00	39.35	± 2.13	42.96	42.71	± 2.06
Unexposed control II	36.97			45.32		
				$F_{1,3}$ $p < 0.01$		

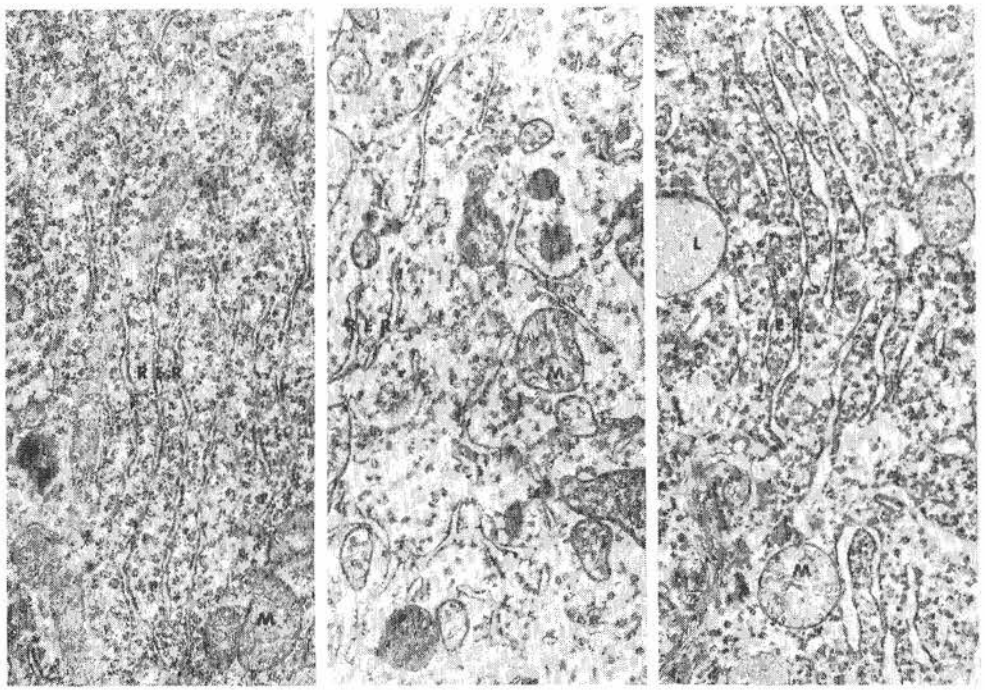

Fig. 11. Photomicrographs showing rough endoplasmic reticulum of CA III pyramidal cell of the hippocampus. Left: From the control monkey that was a heavy smoker of inactive marijuana. Note organized cisternal membranes and ribosomal attachment. Center: From the monkey that received iv Δ-9-THC. Note disorganization and fragmentation of cisternal membranes and loss of ribosomal attachment. Right: From a heavy-smoking monkey of active marijuana. Note swollen cisternal membrane. M = mitochondria; RER = rough endoplasmic reticulum. All panels, 28,000 X. (Figure reduced 55% for reproduction.)

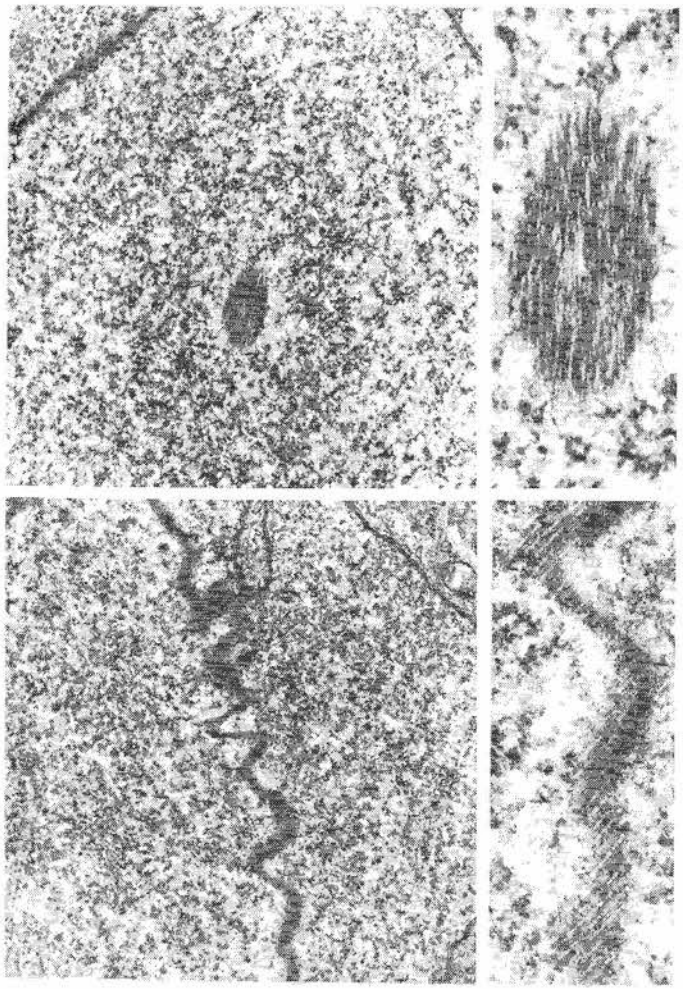

Fig. 12. Tissue from CA III pyramidal cell of the hippocampus. Left: Nuclear inclusion body, rodlike type; Top, 21,000 X; Bottom, 66,000 X. Right: Nuclear inclusion body, fibrillar or sheetlike type; Top, 21,000 X; Bottom, 66,000 X. (Figure reduced 55% for reproduction.)

Table VI. Intranuclear Inclusions in Nerve Cells: Monkey Brains

Brain site	Heavy smoker active[d] marijuana			Δ9 Injection iv			Heavy smoker active marijuana			Heavy smoker inactive marijuana			Control			Control		
	n[a]	I[b]	%[c]	*n*	I	%	*n*	I	%	*n*	I	%	*n*	I	%	*n*	I	%
Septal region	516	161	31.2	416	17	4.1	396	7	1.7	416	0	0	376	0	0	356	0	0
Posterior ventral lateral thalamus	320	82	25.6	236	7	2.9	340	2	0.5	350	0	0	410	0	0	340	0	0
Hippocampus	385	118	30.6	378	14	3.7	372	6	1.6	342	1	0.3	395	0	0	310	0	0
Amygdala	402	112	27.8	380	10	2.6	326	4	1.2	346	0	0	318	0	0	312	0	0
Motor cortex	368	96	26.0	272	7	2.5	320	2	0.6	328	0	0	324	0	0	316	0	0
Fastigial nucleus	246	2	0.8	220	1	0.45	245	0	0	206	0	0	220	0	0	244	0	0
Caudate nucleus	230	1	0.4	180	0	0	220	0	0	190	0	0	181	0	0	235	0	0
Totals	2467	572	23.1	2082	56	2.68	2219	21	0.94	2178	1	0.045	2224	0	0	2113	0	0

[a] Number of nuclei.
[b] Number of inclusions.
[c] Percentage of nuclei with inclusions.
[d] Monkey injected with Δ-9-THC 15 min before sacrifice.

Table VII. Types of Nuclear Inclusion Bodies: Monkey Brains

Brain site	Heavy smoker of active marijuana[d]			Δ-9-THC iv			Heavy smoker of active marijuana			Heavy smoker of inactive marijuana		
	I[a]	R[b]	S[c]	I	R	S	I	R	S	I	R	S
Septal region	161	99	62	17	9	8	7	4	3	0	0	0
Posterior ventral lateral thalamus	82	49	33	7	4	3	2	1	1	0	0	0
Hippocampus	118	72	46	14	9	5	6	3	3	1	1	0
Amygdala	112	71	41	10	6	4	4	3	1	0	0	0
Motor cortex	96	67	29	7	5	2	2	2	0	0	0	0
Fastigial nucleus	2	1	1	1	1	0	0	0	0	0	0	0
Caudate nucleus	1	0	1	0	0	0	0	0	0	0	0	0
Totals	572	359	213	56	34	22	21	13	8	1	1	0

[a] Number of inclusions.
[b] Number of rodlike inclusions.
[c] Number of sheetlike inclusions.
[d] Monkey injected with Δ-9-THC 15 min before sacrifice.

marijuana and those that received Δ-9-THC intravenously was substantially lower than that in the brains of the heavy *inactive* marijuana smoker and the two unexposed control monkeys. Values for the septal region and hippocampus are shown in Table V. Similar values were found in the amygdala. The golgi apparatus and the mitochondria varied little in volume densities when brains of the two groups of monkeys were compared (Myers and Heath, 1979).

In addition to these measured differences, we also observed various degrees of fragmentation and disorganization of the rough endoplasmic reticulum patterns, with free ribosomal clusters throughout the cytoplasm, as well as swelling of the cisternal membranes (Fig. 11).

Ultrastructure of the Nuclei

Nuclear inclusions were observed in the neurons of the exposed monkeys in significantly greater quantity than in those of the monkey exposed to *inactive* marijuana or the unexposed controls. The inclusion bodies were of two principal types: longitudinal rodlike filaments and fibrillar or sheetlike bodies, both about 70-Å thick (Fig. 12). At each site, 180 to 516 cells were observed. Tissue of the monkey smoked heavily with the new apparatus and given Δ-9-THC intravenously 15 min before sacrifice had more (23%) inclusion bodies than tissues of the other exposed monkeys (Table VI). The inclusions were seen in 0.94% to 2.68% of the nuclei of monkeys exposed for long periods to Δ-9-THC and rested, and in 0.045% of nuclei of the monkey exposed to smoke of *inactive* marijuana and rested. None was present in tissues of the two unexposed control monkeys. Rodlike inclusion bodies were seen more frequently than the sheetlike type (Table VII).

DISCUSSION

The results of these studies indicate that irreversible changes in brain function and ultrastructure can be induced in monkeys by prolonged administration of the active ingredient(s) of cannabis sativa in quantities equivalent to those used by human smokers of marijuana. In monkeys, intravenous administration of the psychoactive ingredient, Δ-9-THC, induced the same brain changes as smoke of active marijuana containing numerous cannabis sativa derivatives, the data suggesting that Δ-9-THC is probably the major constituent of marijuana responsible for its effects on the brain. Our data are not sufficient to rule out the possibility that other cannabis sativa derivatives that may be present in smoke of active marijuana also contribute to the brain changes.

The brain sites at which changes occurred in function and where ultrastructural alterations were most notable are the same sites where activity has been correlated with changes in emotion in patients (Heath, 1975). One could speculate that the abnormalities observed at these brain sites of the monkeys are related to the disturbances in emotion (apathy, impaired motivation, increased irritability) and, in some instances, in psychotic behavior, reported in human smokers of marijuana (Hollister, 1971; Kolansky and Moore, 1972; Powelson, 1974; Jones, 1976).

Studies in monkeys offer an obvious advantage over human studies since rigid controls not possible in man can be applied. Earlier reports of deleterious effects of marijuana on behavior and brain function of human subjects were criticized on the bases that (i) the subjects had also used other psychoactive drugs or alcohol, or both, and (ii) the pathologic findings were probably endogenous and would have occurred without exposure to marijuana. These criticisms are invalid in the rigidly controlled monkey studies.

Nonetheless, studies in monkeys have disadvantages. Even though subhuman primates are phylogenetically close to man (and we had comparable data in monkey and man on the acute effects of marijuana) (Heath, 1972; 1973), the species differ physically in receptiveness and sensitivity to the agents tested. The monkey was an unwilling subject. Intravenously administered Δ-9-THC created no problem. But since man usually smokes marijuana, it was necessary to test this route in the monkey, and major methodologic problems occurred in establishing accurate dosage. Early in our studies, it became apparent that the quantity of starting marijuana to be smoked bore no relation to the ingredients absorbed by the monkey. Consideration of the usual comparable body weight and surface area to arrive at a reasonable amount of starting material for the monkey was shown to be useless when plasma levels of Δ-9-THC became available and provided meaningful information on the amount of active material being absorbed by the smoking monkeys. First, the loss of active ingredients with the original smoking apparatus was so great that the monkey was exposed to much smaller quantities of active material than we had estimated. Second, because the monkey did not inhale deeply, it absorbed a much smaller proportion of the ingredients to which it was exposed than man exposed to similar amounts of the smoke. During Study I, it also became obvious that the amount of smoke inhaled by the monkey varied notably from one exposure to another. Further, as the study progressed, the monkey inhaled less smoke. The plasma levels obtained in later studies indicated that the actual dose of active material inhaled by the Study I monkeys was usually less than 1 mg/kg of Δ-9-THC, despite the large amount of starting material. The irrelevance of the amount of starting material to true dosage was further underscored by the data of Study II,

where similar results were obtained in the monkeys even though the amount of starting marijuana was significantly reduced. From 250 mg of marijuana containing 1 to 2 mg/kg Δ-9-THC of the monkey's body weight, plasma levels obtained were comparable to those of a man feeling the effects of smoking a human-size joint of marijuana. The data reported here indicate that the monkey is at least as sensitive as the human subject to Δ-9-THC. The dose of 0.69 mg/kg iv used in Study I is low. Distinct recording changes were obtained when Δ-9-THC plasma levels were as low as 3 to 10 ng/ml.

In these monkey studies, nonspecific effects of exposure to smoke were carefully controlled. Blood gases indicated there was no anoxia. Further, control monkeys were exposed to the same amount of smoke devoid of psychoactive cannabis as those monkeys exposed maximally to smoke of active marijuana, the control group showing no EEG changes or brain ultrastructural changes. Moreover, the monkeys given Δ-9-THC intravenously showed permanent EEG changes and alterations in brain ultrastructure similar to those of the smoking monkeys. The results in these two control groups indicate that the altered brain function and ultrastructure were caused by the psychoactive components and not by nonspecific effects of exposure to smoke.

The ultrastructural changes at the synapse correlate with the persistent EEG changes. Further studies are required to determine if the synaptic changes and changes in rough endoplasmic reticulum correlate with clinical changes being increasingly reported in human marijuana users.

Nuclear inclusion bodies of the type seen here have been described in numerous publications (Roncoroni, 1895; Cajal, 1909-1911, reprinted 1952). They have been noted in both pyramidal and glial cells of the central nervous system and in peripheral ganglia. They are present in many species, including the squirrel monkey (Seigesmund *et al.* 1964), but none has been described previously for the rhesus monkey. Their occurrence is rare in brain tissue, and their significance remains obscure. They have been reported to increase in number with aging (Field and Peat, 1971) and some investigators have speculated an association with viral disease. A notable increase in numbers in cells of the stellate ganglion has been reported following electrical stimulation (Seite *et al.,* 1971), but comparable studies have not been conducted on such alteration within cells of the central nervous system. Our data suggest a relationship between quantity of inclusion bodies and alteration in cell function and ultrastructure. Function was most altered at brain sites of exposed monkeys that showed the greatest numbers of inclusion bodies, the most profound increase appearing in the brain of the Study II monkey.

In the brain ultrastructural studies we have done thus far, the changes in Study I monkeys that were heavy smokers of active marijuana or received Δ-9-THC intravenously were essentially the same as in the Study II monkey

that was exposed to smoke of active marijuana. But, as indicated previously, there were some quantitative differences. Although the Study II monkey was exposed to only one smoke per day, 5 days per week, whereas those in Study I had three smokes per day, 5 days per week, plasma levels of Δ-9-THC induced in the Study II monkey were consistently higher because of the more efficient smoking apparatus used in Study II. Another variable was the duration of exposure to smoking, the Study II monkey being exposed for 8 months compared to 6 months for Study I monkeys. Further, the rest period of the Study II monkey following the last exposure was shorter (2 months *vs.* 8 months). Finally, the Study II monkey was given Δ-9-THC intravenously 15 min before sacrifice. Studies are currently under way to determine if any of these variables are relevant to the striking increase in numbers of nuclear inclusion bodies seen in the Study II monkey brain.

In summary, these studies indicate that the dosages we have used in the monkeys, if considered in the context of the plasma levels of the psychoactive component Δ-9-THC, have been within the range of levels reported in human marijuana smokers. Further, the physiologic changes we previously reported in monkeys exposed to marijuana correlate with the ultrastructural changes.

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REFERENCES

Barnes, C., and Fried, P. A. (1974). Tolerance to delta-9-THC in adult rats with differential delta-9-THC exposure when immature or during early adulthood. *Psychopharmacologia* 34: 181-190.

Berkowitz, L. R., Fiorello, O., Kruger, L., and Maxwell, D. S. (1968). Selective staining of nervous tissue for light microscopy following preparation for electron microscopy. *J. Histochem. Cytochem.* 16: 808-814.

Brecher, E. M., and Editors of Consumer Reports (1975a). Marijuana: The health questions. *Consumer Rep.* 40: 143-149.

Brecher, E. M. (1975b). Debunking the marijuana researchers. *Hosp. Physician* (December issue), pp. 12-15.

Cajal, S. R. (1909–1911). *Histologie du Systeme Nerveaux de l'Homme et des Vertebres,* Vols. 1 and 2, Maloine, Paris. (Reprinted Madrid Consejo Superior de Investigaciones Cienteficas, 1952.)

Campbell, A. M. G., Evans, M., Thompson, J. L. G., and Williams, M. J. (1971). Cerebral atrophy in young cannabis smokers. *Lancet* 2(7736): 1219-1224.

Co, B. T., Goodwin, D. W., Gado, M., Mikhael, M., and Hill, S. Y. (1977). Absence of cerebral atrophy in chronic cannabis users: Evaluation by computerized transaxial tomography. *J. Am. Med. Assoc.* 237: 1229-1230.

Colonnier, M. (1968). Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. *Brain Res.* 9: 268-287.

Field, E. J., and Peat, A. (1971). Intranuclear inclusions in neurones and glia. A study in the ageing mouse. *Gerontologia* 17: 129-138.

Fink, M., Volavka, J., Panagiotopoulos, C. P., and Stefanis, C. (1976). Quantitative EEG studies of marihuana, delta-9-THC, and hashish in man, in *Pharmacology of Marihuana,* Braude, M. C., and Szara, S. (eds.), Raven Press, New York, pp. 383-391.

Freireich, E. J., Gehan, E. A., Rail, D. P., Schmidt, L. H., and Skipper, H. E. (1966). Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemother. Rep.* 50(4): 219-224.

Goldblatt, P. J., and Trump, B. F. (1965). The application of Del Rio Hortega's silver method to epon-embedded tissue. *Stain Technol.* 40: 105-114.

Gray, I. G. (1959). Axo-somatic and axo-dendritic synapses of the cerebral cortex: An electron microscopic study. *J. Anat.* 93: 420-433.

Grinspoon, L. (1971). *Marihuana Reconsidered,* Harvard University Press, Cambridge.

Harper, J. W., Heath, R. G., and Myers, W. A. (1977). Effects of cannabis sativa on ultrastructure of the synapse in monkey brain. *J. Neurosci. Res.* 3: 87-93.

Heath, R. G. (1954). Definition of the septal region, in *Studies in Schizophrenia,* Heath, R. G., and the Tulane University Department of Psychiatry and Neurology (eds.), Harvard University Press, Cambridge, pp. 3-5.

Heath, R. G. (1972). Marihuana: Effects on deep and surface electroencephalograms of man. *Arch. Gen. Psychiat.* 26: 577-584.

Heath, R. G. (1973). Marihuana: Effects on deep and surface electroencephalograms of rhesus monkeys. *Neuropsychopharmacology* 12: 1-4.

Heath, R. G. (1975). Brain function and behavior: 1. Emotion and sensory phenomena in psychotic patients and in experimental animals. *J. Nervous Mental Disease* 160: 159-175.

Heath, R. G. (1976a). Marihuana and delta-9-tetrahydrocannabinol: Acute and chronic effects on brain function of monkeys, in *Pharmacology of Marihuana,* Braude, M. C., and Szara, S. (eds.), Raven Press, New York, pp. 345-356.

Heath, R. G. (1976b). Cannabis sativa derivatives: Effects on brain function of monkeys, in *Marihuana: Chemistry, Biochemistry, and Cellular Effects,* Nahas, G. G. (ed.), Springer-Verlag, New York, pp. 507-519.

Heath, R. G. (1976c). Correlation of brain function with emotional behavior. *Biol. Psychiat.* 11: 463-480.

Heath, R. G., and Harper, J. W. (1976). Descending projections of the rostral septal region: An electrophysiological-histological study in the cat. *Exptl. Neurol.* 50: 536-560.

Heath, R. G., Fitzjarrell, A. T., Garey, R. E., and Myers, W. A. (1979). Chronic marihuana smoking: Its effects on function and structure of the primate brain, in *Marihuana: Biological Effects,* Nahas, G. G., and Paton, W. D. M. (eds.), Pergamon Press, Oxford, pp. 713-730.

Hollister, L. E. (1971). Marihuana in man: Three years later. *Science* 172: 21-24.

Huber, J. D., Parker, F., and Odland, G. F. (1968). A basic fuchsin and alkalinized methylene blue rapid stain for epoxy-embedded tissue. *Stain Technol.* 43: 83-87.

Jones, H. B. (1976). What the practicing physician should know about marijuana. *Private Practice* (January 1976), pp. 34-40.

Kolanksy, H., and Moore, W. T. (1972). Toxic effects of chronic marihuana use. *J. Am. Med. Assoc.* 222: 35-41.

Lane, B., and Europa, D. L. (1965). Differential staining of thin sections of epon embedded tissue for light microscopy. *J. Histochem. Cytochem.* 13: 579-582.

Loud, A. V. (1968). A quantitative sterological description of the ultastructure of normal rat liver parenchymal cells. *J. Cell Biol.* 37: 27-46.

Myers, W. A., and Heath, R. G. (1979). Cannabis sativa: Ultrastructural changes in organelles of neurons in brain septal region of monkeys. *J. Neurosci. Res.* 4: 9-17.

Møllgaard, K., Diamond, M. C., Bennett, E. L., Rosenzweig, M. R., and Linder, B. (1971). Quantitative synaptic changes with differential experience in rat brain. *Intern. J. Neurosci.* 2: 113-127.

Powelson, D. H. (1974). Marijuana: More dangerous than you know. *Reader's Digest* (December 1974), pp. 95-99.

Renault, P. F., Schuster, C. R., Heinrich, R., and Freedman, D. X. (1971). Marihuana: Standardized smoke administration and dose effect curves on heart rate in humans. *Science* 174: 589-591.

Roncoroni, L. (1895). Su un nuovo reperto nel nucleodelle cellule nervose. *Arch. Psichiati.* 16: 447-451.

Rosiwal, A. (1898). Ueber geometrische gesteinanalysen. Ein enfacher weg zur ziffermassigen feststellung des quantitatsverhaltnisses der mineralbestandteile gemengter gesteine. *Verhandl. Kaiserl. Konigl. Geol. Reichsanstalt* 1: 143-175.

Rubin, V., and Comitas, L. (1975). *Ganja in Jamaica: A Medical Anthropological Study of Chronic Marihuana Use,* Mouton Press, The Hague.

Siegesmund, K. A., Dutta, C. R., and Fox, C. A. (1964). The ultrastructure of the intranuclear rodlet in certain nerve cells. *J. Anat.* 98: 93-97.

Siete, R., Mei, N., and Couineau, S. (1971). Modification quantitative des batonnets intranucleaires des neurones sympathiques sous l'influence de la stimulation electrique. *Brain Res.* 34: 277-290.

Snider, R. S., and Lee, J. C. (1961). *A Stereotaxic Atlas of the Monkey Brain* (*Macaca Mulatta*), University of Chicago Press, Chicago.

Tennant, F. S., and Groesbeck, C. J. (1972). Psychiatric effects of hashish. *Arch. Gen. psychiat.* 27: 133-136.

Weibel, E. R., Kistler, G. S., and Scherle, W. F. (1966). Practical stereologic method for morphometric cytology. *J. Cell Biol.* 30: 23-38.