NEUROPSYCHOPHARMACOLOGIC STUDIES OF MARIJUANA: SOME SYNTHETIC AND NATURAL THC DERIVATIVES IN ANIMALS AND MAN*

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INTRODUCTION

One of the tasks of a pharmacologist is the classification of drugs. Marijuana and its derivatives pose some problems. Legally marijuana has been classified as a narcotic, yet pharmacologically it is quite different. Mankind has known about the *Cannabis* plant from which marijuana and other products are obtained for more than 4,708 years. A considerable body of data is available to describe its gross effects. Over the centuries it has been called a depressant, euphoriant, inebriant, intoxicant, hallucinogen, psychotomimetic, sedative-hypnotic-anesthetic, and stimulant. Perhaps more than any other drug it is a social irritant that elicits rather remarkable behavior reactions in both users and nonusers. Moreau[1] in 1845, employed it to produce a model psychosis, thus initiating the fashion to study hallucinogens as a means of gaining insights into mental illness. Lewin[2] classified *Cannabis* as one of the "phantastica: hallucinating substances." One text on hallucinogens[3] does not even refer to *Cannabis*, but another does.[4]

There are some aspects of the subjective effects of both LSD-25 and *Cannabis* that are similar, yet the latter produces sedative effects, no significant sympathomimetic actions, and no cross-tolerance to LSD-25. Hollister[5] reviewed the recent findings on the effects in man of marijuana and its putative active ingredient Δ^9-THC, stressing that current investigators have, for the most part, confirmed the *Cannabis*-induced clinical syndromes long known to occur. New knowledge of the active ingredients, biotransformation, dosage, pharmacokinetics, tolerance, and cross-tolerance in animals and man is now rapidly accumulating, thanks to the medicinal chemists who have provided us with relatively pure compounds (see Mechoulam[6]).

Yet for all the reading one may do about marijuana, there is nothing like the experience of personal research. This paper describes some of those experiences done first in the 1950s and later in the 1960–70s in animals and, subsequently, in man. The issue of classification is implicit in the attempt to ask what marijuana or its pure active derivatives do to various brain functions. Where and how do they act, using those limited techniques available in one's own laboratory? This research is due to the efforts of many of my students or associates whose work has been or is being published in separate form in the near future.

ANIMAL STUDIES

Gross Behavioral Effects of Various THC Derivatives

In various animals, Δ^9-THC and the synthetic derivatives, DMHP, pyrahexyl, MOP, and NAP show predominant central nervous system depression.[7,8] The effects of the synthetic THC derivatives last from several hours to days, depend-

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ing upon the dose administered. During this period animals do not respond in a normal manner to painful stimuli. The degree of apparent analgesia is marked. Very small doses of the above drugs produce selective CNS depression of the dog or monkey free from signs of central excitation or analgesia. Yet, auditory and tactile stimuli can produce hyperreactivity. The animals appear to prefer to lie quietly, and passively resent being disturbed. Larger doses are required to produce a similar response in the monkey than in the dog. With increasing doses, a period of initial central nervous system stimulation is observed, followed by prolonged depression. The period of recovery is heralded by a return of the signs of stimulation. This sequence of events follows the classical pattern of induction and emergence from general anesthesia. The animals, however, are not anesthetized. They may show a reduction or absence of response to pain. However, it is almost always possible to arouse them from the depressed state through adequate sensory stimulation. The simple procedure of righting a dog or monkey and placing him in the normal position of locomotion usually produces arousal of short duration. This procedure may, in fact, induce a period of hyperexcitation and a very brief return of almost normal behavior. If the dose is sufficient to cause initial excitation characterized by tremors or convulsions, these may return when the animal is subjected to forced arousal during the period of marked central nervous system depression.

DMHP is the most potent of the synthetic THC derivatives we have tested.[7,8] MOP has an almost identical spectrum of activity and is nearly as potent as DMHP. NAP, the parent compound of this class, is considerably less potent. In adequate doses, however, it possesses all the qualitative actions of the other two drugs. As a group, these drugs possess several additional interesting properties. They are effective orally, and the duration of CNS depression is roughly proportional to the magnitude of the administered dose. After single large doses of DMHP (10 mg/kg, i.v.), dogs have been maintained in an unconscious state for five to six days and have recovered uneventfully. There is a marked reduction in body temperature, which is maintained during the phase of CNS depression. Elevating the body temperature by placing the dog in warm water can initiate behavioral arousal during the phase of CNS depression.

Effects of Δ^9-THC on Rat One-Way Avoidance Behavior

A total of 12 naïve adult male Holtzman rats were given 50 trials a day for two consecutive days to achieve a 90% avoidance criterion in a one-way jump box. The apparatus used was an adaptation of that by Caldwell and coworkers[9] described by Tenen.[10] The behavioral parameters used were as follows: The conditioned stimulus (CS) was a five-second presentation of four 7.5-watt red lights with the simultaneous presentation of an escape ledge. At the end of five seconds, the CS overlapped with a five-second unconditioned stimulus (US) that consisted of 1 ma 60 Hz electric shock delivered to the grid floor. When the subject jumped on the ledge, the sequence was terminated. A 30-second ledge rest period then ensued. If at the end of this time the animal still persisted in sitting on the ledge, he was automatically pushed off by an electromechanical moving wall. Random intertrial intervals were maintained with a mean of 30 seconds and a range of 15–60 seconds.

All drugs were given intraperitoneally (i.p.) following a 10-trial warmup session. After injection a five-minute rest period was allowed before the animals were given another 50 trials. The entire session lasted approximately one hour.

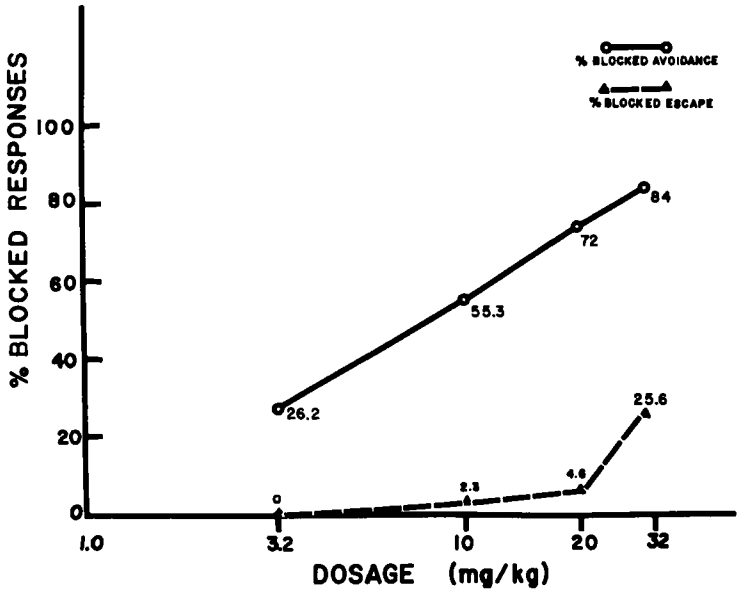

FIGURE 1. Dose-effect relation of Δ^9-THC on rat one-way avoidance and escape behavior. On the y-axis are plotted the mean blocked responses for 3 rats/point, 50 trials/rat. On the x-axis is plotted the dose of Δ^9-THC in mg/kg given i.p. Note that there is a marked separation of the blocked avoidance and escape responses.

Δ^9-THC was made with use of a 4% Tween-20 or -80 suspension from an original 99% ethyl alcohol solution. The method of preparation was as follows: The desired amount of Δ^9-THC in 95% ethyl alcohol was placed in a test tube. The ethyl alcohol was removed with a rotary evaporator (Buchler), with the water bath set at 45° C. When the ethyl alcohol evaporated, the THC remained in the bottom of the test tube as a thick, dark brown resin. The total time of evaporation usually was two to four minutes. Tween 20, 4% of the final volume, was then added and thoroughly mixed with the THC in a Vortex shaker. The desired volume of 0.9% NaCl was then added to the THC-Tween mixture with gentle heating and shaking. The final suspension looked milky white. It was used within 48 hours after placing in a refrigerator at 4° C. Before each injection, the suspension was again shaken to insure homogeneity. Very recently we have prepared Δ^9-THC via a new method developed by Stark.[11] The end product is an almost clear solution that seems to have a much smaller particle size. This method consists of dissolving 10 mg of THC in 20 ml of absolute ethyl alcohol. Then 20 ml of a 1% Tween-80-0.9% NaCl solution is added. The volume of this mixture is reduced by evaporation, as above, to a 20-ml solution containing 0.5 mg/ml. This new technique was not used in the work described below.

In FIGURE 1 are illustrated the dose-effect relations of Δ^9-THC on one-way rat avoidance behavior. On the y-axis is plotted the percentage of blocked responses, either avoidance or escape, while on the x-axis, the dose given i.p. Each point represents the mean of 50 trials for each of three rats. It can be noted that doses between 3.2 and 32 are quite effective in reducing avoidance behavior. By contrast, escape behavior is much less depressed, as is generally observed with narcotic analgesics and neuroleptics on various avoidance tasks.[12]

Effects of Various THC Derivatives on Schedule-Controlled Behavior in the Pigeon

In collaboration with Mrs. Mary Beth Black and Dr. James Woods, we have studied the effects of various THC derivatives on schedule-controlled behavior in the pigeon under a multiple fixed ratio 30 (FR 30) fixed-interval five minutes (FI 5) schedule of food reinforcement.[13] Twenty-seven male and female white Carneaux pigeons weighing between 450 and 600 g when given free access to food and water were used. They were deprived to 80% of the free-feeding weights and maintained at this level. The animals were conditioned with grain seed to peck a transilluminated response key.[14] Each pigeon was conditioned on an alternating multiple FR 30 FI 5 schedule, as has been used by many.[15] In the presence of a blue light, the 30th peck on the response key resulted in a five-second access to bird seed (FR 30). In the presence of a red light, the first response after five minutes also resulted in a similar access to seed (FI 5). A hold of 45 seconds regulated food presentations when no responding occurred. Session length was 40 food presentations or 40 limited holds, depending upon whether or not the animal responded.

All drugs were suspended as described above. They were given intramuscularly (i.m.) 30 minutes prior to the experimental session. Control injections consisted of an equal volume of Tween-saline. Mean rate of responding during the FR and FI periods were computed for the total session. Quarter-life was calculated for each session of the fixed-interval periods.

One of the first problems was to determine if the animals could be given drug injections at weekly intervals without any accumulative or tolerance effects. It was soon obvious that tolerance development was a marked problem (see below), and therefore all legitimate dose-effect studies had to involve naïve birds. The effects for three different animals for each dose are illustrated in FIGURE 2. On the y-axis is plotted the ratio of responding on the drug to vehicle control for FI, FR, and quarter life (QL) and on the x-axis dose. In general, the drug produces a dose-related depression of key pecking. We are now studying the effects of even lower doses of Δ^9-THC.

All three agents, Δ^9-THC, pyrahexyl, and DMHP produced a marked decrease in rate of responding under both schedules, but FI behavior was somewhat more sensitive. Doses of 0.1–10 mg/kg, i.p., were used and showed primarily increasing depression.

Tolerance Development to the Behavioral Effects of THC Derivatives

It is widely believed that smoking marijuana does not produce tolerance. This belief was strengthened by Walton,[16] who reported that there was no tolerance to marijuana. In fact, hearsay reports from users suggest a sensitization upon repeated use. More recently, Seevers[17] and the AMA Committee on Alcoholism and Drug Dependence[18] agreed with the concept that no tolerance or physical dependence has been demonstrated with *Cannabis* preparations. Longo and coworkers[19] gave Δ^9-THC daily to rabbits in doses of 3 mg/kg, and they showed no EEG or behavioral tolerance, by contrast with LSD-25.

While there appears to be no good evidence of physical dependence on *Cannabis* preparations, there is now considerable evidence of tolerance development, especially to pure THC derivatives in both man and animals. Williams and associates[20] studied the effects of acute and chronic marijuana and pyrahexyl (Synhexyl) on six former opiate users who had previously smoked marijuana. Pyra-

hexyl was given in self-chosen intervals for 26–31 days. The daily dose range of 60–2,400 was taken orally in one to eight individual doses. About three hours after the initial dose of pyrahexyl, the subjects exhibited drowsiness, euphoria, dry mouth, injected sclera, increased laughter, and swollen eyelids. The patients became euphoric and garrulous and showed spontaneous laughter. Most reported that these effects were similar to marijuana but stronger. After four to six days the effects of the drug were less than on the first or second day. An increase in dosage of pyrahexyl caused a return of typical drug effects. During prolonged medication, the EEG showed a slowing of the dominant frequencies. Abrupt withdrawal showed no abstinence on the first two days. However, on the third day very mild effects were seen, such as restlessness, poor sleep, loss of appetite, and "hot flashes." These authors did not feel that physical dependence could definitely be established, although tolerance development was obvious.

Williams and colleagues[20] also studied the effects of chronic marijuana smoking of material of unknown resin content but described as "good weed" by the users. Six subjects were allowed to smoke marijuana *ad libitum* for 39 days. The rela-

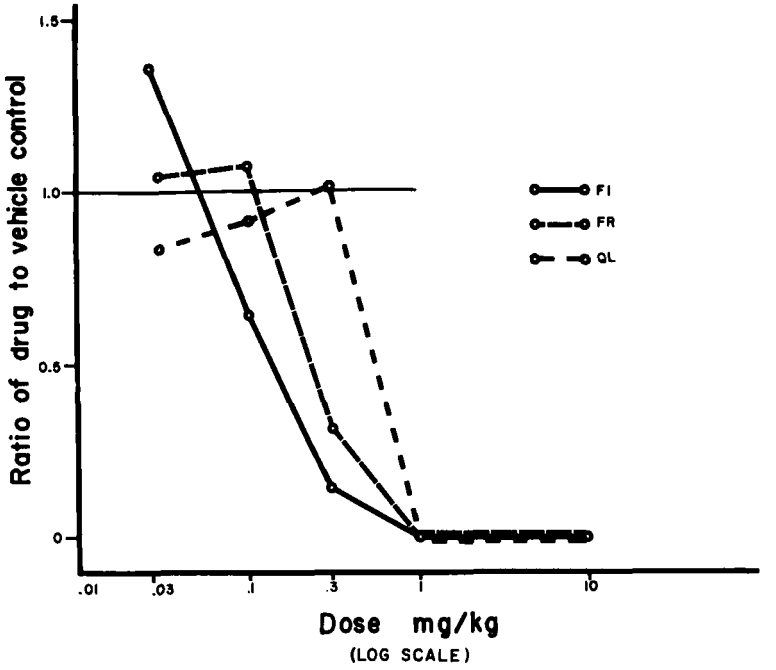

FIGURE 2. Dose-effect relation of Δ^9-THC on a multiple FI 5, FR 30 key pecking schedule in pigeons. The mean ratio of key pecking/2-hour period on the two schedules for drug vs. vehicle control are given on the y-axis and the dose on the x-axis. The drug was given to drug naïve pigeons, i.m. The effects for FI, FR, and FI quarter-life (QL) are shown. Note, in general, that the marked depressant effects with FI behavior are somewhat more sensitive than with FR behavior.

tively slight increase in the number of cigarettes smoked daily suggested that with these amounts rapid tolerance to marijuana does not develop. With both the pyrahexyl and marijuana the initial gaiety and loquaciousness lasted only a few days, but could be reelicited by increasing dosage. When cigarette smoking stopped suddenly, there were no objective signs of abstinence, but all patients reported that they were subjectively more "jittery." This study needs to be replicated with marijuana of known Δ^9-THC content.

Both Carlini[21] and Silva and coworkers[22] demonstrated tolerance development to repeated injections of 20–25 mg/kg of *Cannabis* extract and 10 mg/kg of Δ^9-THC in rats on various behavioral tasks. McMillan and colleagues[23] have also reported marked tolerance development of Δ^8- and Δ^9-THC when injected daily in increasing amounts to pigeons trained on a multiple schedule for food reinforcement.

Quite independently, our laboratory has been involved in similar studies that indicate marked tolerance development to both naturally occurring and synthetic THC derivatives given to animals. These studies first began in the mid-1950s when various synthetic THC derivatives were studied for the Army Chemical Center. Hardman and coworkers[7,8] and Domino and coworkers[24] have recently summarized this research, which had remained classified for some 15 years. Hardman and associates[25] observed that tolerance to the gross behavioral depressant effects of the potent synthetic THC derivative, DMHP, could be obtained in dogs and monkeys given repeated intravenous (i.v.) daily doses. After seven days of daily injections of DMHP, up to 1.0 mg/kg, surviving dogs showed minimal depressant effects, with only a slight reduction in motor activity and ataxia. No cross-tolerance to morphine (3.0 mg/kg, subcutaneously) was observed. After one week of daily DMHP, its abrupt cessation produced no obvious withdrawal symptoms. At the end of four weeks of abstinence, the response to 1 mg/kg, i.v., of DMHP was less profound than the initial response of the dogs to the drug.

A series of *Macaca mulatta* monkeys was studied in a similar manner and showed tolerance development and no obvious withdrawal symptoms within 24 hours after abrupt withdrawal one week after daily dosage. This study must be repeated with a longer dosage schedule and period of observation, in view of the Williams and coworkers report and the persistence of THC in the body. Monkeys showing gross behavioral tolerance also showed a diminished high-voltage, slow-wave EEG pattern as well. Tolerance to DMHP was partial, but also lasted for many weeks, just as in the dogs. DMHP in doses of 1.0–2.0 mg/kg, i.v., failed to substitute for morphine in chronically morphine-dependent monkeys, just as pyrahexyl failed to substitute for morphine dependence in man, as described by Himmelsbach.[26]

Because these observations were made with a synthetic THC derivative 15 years ago, we decided to pursue the problem further when Δ^9-THC became available. The Δ^9-THC was available in limited quantities so we decided to study its effects in small animals such as the rat and pigeon.

Tolerance Development to the Behavioral Effects of Δ^9-THC on Rat One-Way Avoidance Behavior

Six rats were trained in a one-way avoidance box as described above. When animals reached a 90% avoidance criterion they were then given 20 mg/kg, i.p., Δ^9-THC. As noted in FIGURE 3, this dose produced a marked decrease in avoidance behavior similar to that described previously (see FIGURE 1). Daily

administration of Δ^9-THC produced an obvious reduction of drug-induced behavioral depressant effects. Inasmuch as the animals were given daily injections and runs in the jump box, the issue of behavioral vs. pharmacologic tolerance to Δ^9-THC remains confounded. Currently, with Dr. L. M. Newman, we are attempting to determine which tolerance effect, behavioral or pharmacological, predominates.

Tolerance Development to the Effects of Various THC Derivatives on Schedule-Controlled Behavior in the Pigeon

McMillan and coworkers[23,27] reported that Δ^9-THC in a dose of 1.8 mg/kg, i.m., depressed the rate of key pecking in pigeons on a multiple FR 30, FI 5 schedule of food reinforcement. The rate of key pecking gradually returned to control levels after five to eight daily injections. Subsequently, tolerant pigeons given doses as large as 180 mg/kg three times per week continued to key peck at near-normal rates. These were lethal doses to nontolerant animals. Cross-tolerance to large doses (36 mg/kg, i.m.) of Δ^8 were also observed. Compo[28] has also reported tolerance development and cross-tolerance between azatetrahydrocannabinol and pyrahexyl that persisted for months.

In our own laboratory we were concerned with the dose-effect relations of DMHP, pyrahexyl, and Δ^9-THC on the same behavior as described above. Standard behavioral designs include one-week intervals of drug administration to the same bird. Much to our surprise, pigeons given various THC at weekly intervals showed tolerance as well as cross-tolerance. Δ^9-THC (10 mg/kg), DMHP

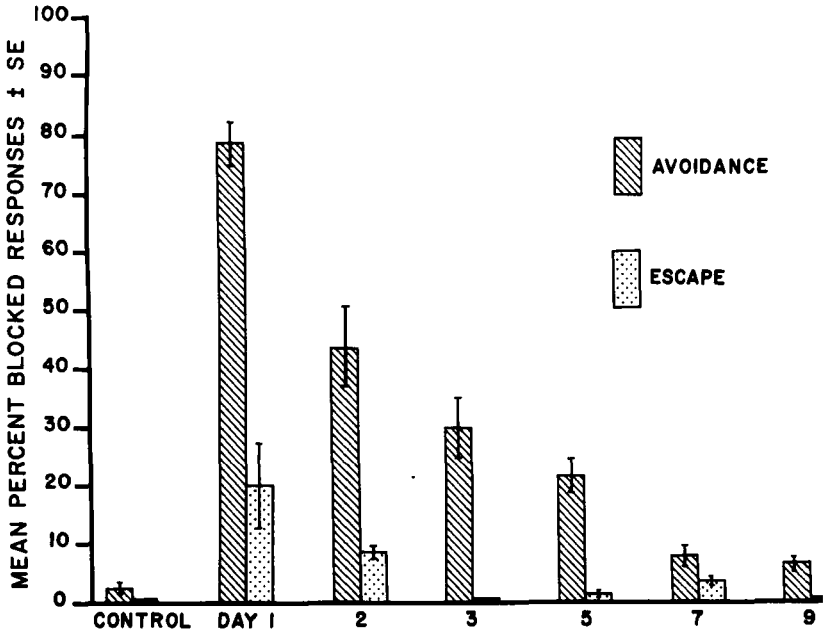

FIGURE 3. Tolerance development to the behavioral effects of Δ^9-THC on rat one-way avoidance. The height of each bar represents the mean blocked responses and the small vertical line ± S.E. of 6 rats, 50 trials/rat before and after daily Δ^9-THC. The drug was given i.p. in doses of 20 mg/kg for 9 days. Note the marked tolerance development.

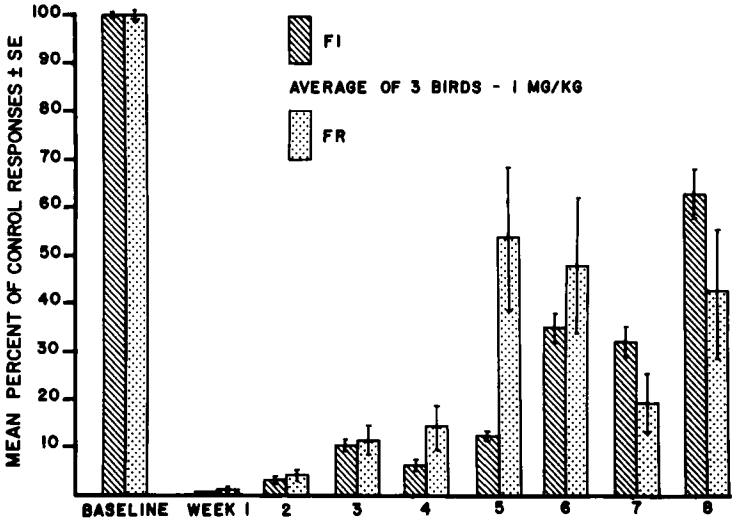

FIGURE 4. Bar graph showing tolerance development to the behavioral effects of weekly Δ^9-THC on multiple FI 5 FR 30 key pecking in the pigeon. The mean ± S.E. control rates of FR and FI key pecking for three different pigeons after Tween-20 saline alone are given as 100%. Δ^9-THC in a dose of 1.0 mg/kg, i.m. given once/day at weekly intervals caused a marked decrease in the percent rate of responding, as noted. After 1–8 weeks, tolerance development is progressively apparent.

(0.3 mg/kg), and pyrahexyl (10 mg/kg) were given i.m. once per week for seven weeks. All drugs produced a marked decrease in rate of responding on both the FR 30 and FI 5 schedule, as described above. Tolerance was observed with each successive drug administration. This is illustrated in FIGURE 4 for three pigeons given 1.0 mg/kg, i.m., of Δ^9-THC at weekly intervals. The mean control rate of key pecking on a FR and FI schedule after the vehicle control is given as 100%. Δ^9-THC given once the first week in a dose of 1 mg/kg markedly depressed FR and FI behavior. When given once per week on succeeding weeks (2–7), tolerance developed rapidly and seemed maximal by the eighth week under these circumstances. Cross-tolerance to the synthetic derivatives was tested on the eighth week in other animals. Pyrahexyl exhibited cross-tolerance with Δ^9-THC. Similarly, cross-tolerance was exhibited between DMHP and Δ^9-THC.

One very interesting effect was obtained when the dose-effect responses of naïve and tolerant pigeons were compared, as illustrated in FIGURE 5. The tolerant birds were given ascending or descending doses in sequence with a high dose of 10 mg/kg Δ^9-THC. As expected with tolerance development, there is a shift in the dose-effect curve to the right. Tolerance was more marked at the descending weekly series of injections than at the ascending series. This research is now in the process of being published elsewhere in more detail by Black and colleagues.[29]

Effects of Δ^9-THC on Brain Acetylcholine Content

In view of the fact that Δ^9-THC produces predominant sedation, it seemed of interest to determine its effects on brain acetylcholine (ACh). There is some data in the literature on its interactions with the peripheral cholinergic system.

Gill and coworkers[30] reported Δ^9-THC either to have no effect or to potentiate the action of ACh on the guinea pig ileum. Layman and Milton,[31] on the other hand, claimed that both Δ^9-THC and cannabidiol reduced the guinea pig ileum response to both ACh and histamine at 3.18×10^{-7} M, suggesting a general depressant effect. On the other hand, these compounds had no effect on the rat phrenic nerve diaphragm preparation, or on ACh-induced contractions of the frog rectus abdominis muscle. These THC derivatives given i.p. also had no effect on the pre- or postganglionically stimulated nictitating membrane. Thus, the nicotinic effects of ACh do not seem to be affected, but the muscarinic effects of ACh may be in the guinea pig ileum preparation.

Effects in Mice

Adult male pure-bred mice weighing from 20–26 g of the DBA/J2 and C3H/HeJ strain were obtained from the Jackson Laboratories, Bar Harbor, Me. The animals were on a 12 midnight–7 a.m. dark and 7 a.m.–12 midnight light cycle. They were given Δ^9-THC or Tween-80-saline vehicle, i.p., and sacrificed one-half hour later. The brain, minus the cerebellum, was removed and bioassayed for ACh with use of a modification of the method of Stone,[32] as described previously.[33]

As illustrated in TABLE 1, both strains of mice had similar control levels of brain ACh. The Tween-80-saline vehicle caused a slight but insignificant increase in brain ACh. Increasing doses of Δ^9-THC also caused a slight but progressive increase in brain ACh that was significantly above vehicle control levels ($P < .05$)

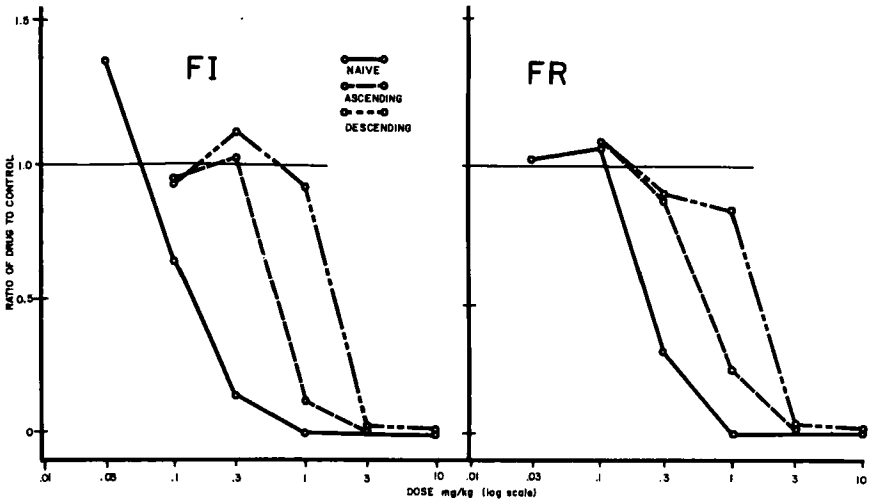

FIGURE 5. Dose-effect relations of Δ^9-THC on multiple FR 5 FR 30 key pecking schedule in drug-naïve and tolerant pigeons. The data are plotted similar to those in FIGURE 2 for the naïve animals. The tolerant birds were given ascending or descending doses in sequence with a high of 10 mg/kg of Δ^9-THC. Note the shift of the dose-effect curves to the right expected with tolerance development. Note that for the ascending or descending dose series the drug was given at weekly intervals for 7 weeks.

TABLE 1

EFFECTS OF Δ^9-THC ON THE CONTENT OF BRAIN ACETYLCHOLINE IN DBA/J2 AND C3H/HEJ MICE

Dose	Behavior	N	nMol ACh/gm ± S.E.			P Value
			DBA/J2	N	C3H/HeJ	
controls	active	8	18.1 ± 1.1	8	18.2 ± 1.1	—
tween-80 saline controls	active	14	20.8 ± 1.5	–	—	N.S.
5 mg/kg	active	9	19.4 ± 1.3	–	—	N.S.
10 mg/kg	depressed	11	23.2 ± 1.3	–	—	N.S.
50 mg/kg	comatose	6	25.5 ± 3.1	7	26.3 ± 2.8	$<.05$

when the mice lost their righting reflex and were comatose. It is well known that most central nervous system depressants in doses producing coma elevate brain ACh.[34] Thus the question of whether Δ^9-THC is producing these effects secondary to CNS depression becomes paramount.

Effects in Rats

An important measure is not steady state, but turnover of brain ACh. The turnover of any compound can be determined only if certain rigid criteria are fulfilled.[35,36] One technique is to use a drug to stop synthesis of the biogenic compound of interest. This effect must be immediate to obtain meaningful quantitative data. Such an approach is not really feasible with use of hemicholinium-3 (HC-3) as a cholinergic antisynthesis agent. Inasmuch as the mechanism of action of HC-3 is still debated, and it certainly does not act instantaneously, it cannot be used in measuring true brain ACh turnover. It can, however, be used as a tool to measure relative rates of ACh depletion if its rate of action is constant. We have studied the effects of Δ^9-THC on brain ACh depletion following intraventricular (i. vent.) HC-3 given to rats.

Young albino male Holtzman rats were used from 20–30 days of age. The animals were on a 12 p.m.–7 a.m. dark and 7 a.m.–12 p.m. light cycle. HC-3 bromide was given i. vent., with use of diethyl ether anesthesia. Δ^9-THC was given i.p. simultaneously with the injection of HC-3 i. vent. and termination of anesthesia. Animals were given various doses of HC-3 and equimolar NaBr and were sacrificed by guillotine. The brain, minus the cerebellum, was removed and bioassayed for ACh as described above. All drug dosage was calculated as base.

A dose of 20 μg total of HC-3 produced about a 60% depletion of brain ACh one-half hour after i. vent. injection, with a relatively low rate of mortality. Five minutes after this dose of HC-3, brain ACh dropped rapidly from a control postether mean ± S.E. of 18.9 ± 0.5 to 13.8 ± 0.3 nmoles/g for a depletion rate of 5.1/5 min or 1.2 nmoles/g/min. A second slower rate of ACh depletion occurred from 13.8 ± 0.3 to 10.1 ± 0.1 from 5 to 30 minutes postinjection with a rate of 3.7/25 or 0.15 nmoles/g/min. These data are in agreement with those of Hebb and coworkers,[37] for intracaudate and with Slater[38] for i. vent. injections of HC-3.

Groups of eight young rats were given i.p. Δ^9-THC alone and Δ^9-THC plus 20 μg i. vent. HC-3 and sacrificed 30 minutes later each morning between 8:30 and 9:30 a.m., and brain ACh was measured that day. The data obtained are plotted as a bar graph in FIGURE 6. The mean $\pm$ S.E. control level of brain ACh of 18.1 $\pm$ 1.3 nmoles/g is illustrated by the upper stippled bar and that following HC-3 alone (10.1 $\pm$ 0.1 nmoles/g) by the lower hatched horizontal bar. The open vertical bars represent the mean level of brain ACh alone, and the slanted vertical bars that after Δ^9-THC plus HC-3. A Tween-20 saline vehicle control given i.p. showed no significant change in brain ACh, compared with noninjected rats. The open vertical bars should be compared to the upper horizontal stippled bar for the effects of Δ^9-THC on steady state brain ACh, and the slanted vertical bars should be compared with the lower horizontal bar for the drug effect on HC-3-induced depletion. Group comparison student t tests were determined. The asterisks indicate the significance probabilities. It can be noted that only 32 mg/kg of Δ^9-THC given i.p. produced a slight but significant ($P < .01$) increase in steady state brain acetylcholine. This is a dose that produces marked depression of one-way avoidance behavior (see above). Smaller doses of Δ^9-THC alone have no significant effect on brain ACh. Following Δ^9-THC plus HC-3 in doses of 3.2 i.p. and 20 μg i. vent., no significant differences in brain ACh were observed from non-Δ^9-THC-injected animals. However, following 10 and 32 mg/kg of Δ^9-THC there was antagonism of the expected HC-3-induced reduction of brain ACh ($P < .01$). The effects at 10 mg/kg of Δ^9-THC are similar to those seen with morphine, whereas those at 32 mg/kg are similar to those seen with pentobarbital and chlordiazepoxide.

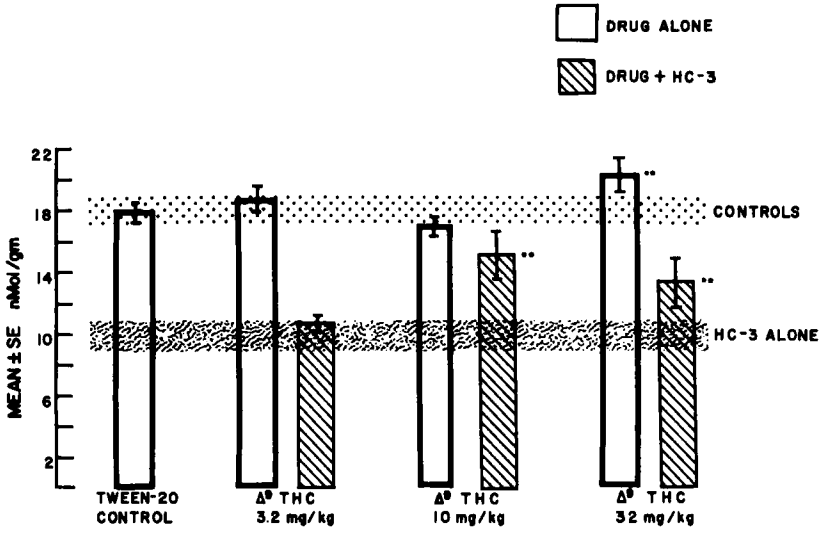

FIGURE 6. Effects of Δ^9-THC on brain acetylcholine depletion following i.vent. hemicholinium-3 in young rats. The mean $\pm$ S.E. brain ACh of control rats 25–30 days old is given by the upper horizontal bar and that after 20 μg i. vent. HC-3 by the lower horizontal bar. The open vertical bars represent the mean $\pm$ S.E. brain ACh ½ hr after Δ^9-THC given alone i.p., and the slanted vertical bars represent that after Δ^9-THC i.p. and HC-3 i. vent., as noted. All data are expressed as mean $\pm$ S.E. brain ACh in nMol/g. P values are ** $< .01$ student t test group comparison. The small vertical lines represent $\pm$ S.E. At least eight animals are in each group.

TABLE 2

EFFECT OF Δ^9-THC ON ACETYLCHOLINE RELEASE FROM CAT CEREBRAL CORTEX*

Dose mg/kg i.v.	ACh Output (ng/cm²/10 min)		Percent Variation
	Before Δ^9-THC (mean of 3 samples)	After Δ^9-THC (mean of 3 samples)	
0.5	15.9	22.5	+41%
0.5	24.6	31.4	+28%
0.5	28.4	25.2	−11%
mean	22.9	26.4	+19%
1.0	14.3	10.2	−28%
1.0	20.5	22.5	+10%
mean	17.4	16.3	− 9%
1.5	15.9	15.0	− 6%
1.5	24.6	22.6	− 8%
mean	20.2	18.8	− 7%
3.5	28.4	15.3	−46%
6.0	24.6	9.6	−61%
6.0	20.5	10.6	−48%
mean	22.5	10.1	−55%
11.0	14.3	5.3	−63%

* Data taken from Domino & Bartolini.[41]

Effects on Cortical Release of Acetylcholine

A considerable literature exists on the neocortical release of ACh with various physiologic and pharmacologic conditions. It is well known that amphetamine[39] and scopolamine[40] increase and pentobarbital decreases[40] neocortical release of ACh. Thus, it would be of interest to know what Δ^9-THC would do in view of its mixed stimulant and depressant effects. The cortical release of ACh therefore was studied in brainstem transected posthalothane anesthetized cats with use of the technique of Bartolini and Pepeu.[40] The ACh was bioassayed on the dorsal leech muscle. This research was done in collaboration with Dr. Bartolini and his wife, Rosalia. A total of six cats was used. Release of ACh was measured from the suprasylvian gyrus and bioassayed. The ACh output was expressed in ng/cm²/10 min. This research will be published in detail shortly.[41]

TABLE 2 lists the data obtained following the i.v. administration of Δ^9-THC in doses of 0.5–11 mg/kg. Small doses of Δ^9-THC of 0.5 mg/kg showed very marked individual variations in the percent release. Two animals showed a definite increase and one a slight decrease in ACh release, with a mean change for the group of +19%. Larger doses of Δ^9-THC showed progressive decreases in ACh release typical of central nervous system depressants such as pentobarbital or morphine. In addition, the neocortical EEG of these pretrigeminal brainstem-transected cats showed high-voltage slow waves after such large doses of Δ^9-THC.

The results of one experiment with an animal given Δ^9-THC and d-amphetamine as a possible antagonist on acetylcholine release are plotted in bar graph form in FIGURE 7. This was a post-halothane pretrigeminal brainstem transected cat who showed a high basal release of ACh in the order of 25–30 ng/cm²/10 minutes from the suprasylvian cortex. A dose of 0.5 mg/kg of Δ^9-THC caused a transient increase followed by a gradual decrease. ACh release was reduced

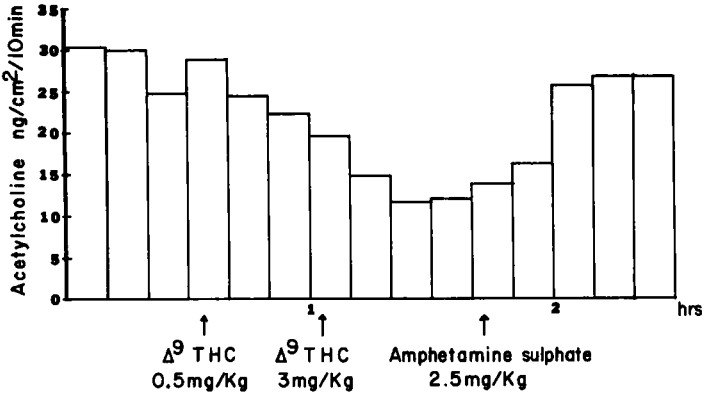

FIGURE 7. Effects of Δ^9-THC and d-amphetamine on neocortical release of acetylcholine. The height of each bar represents the ng/cm²/10 min of ACh released from the somatosensory cortex of a pretrigeminal brainstem-transected cat. In this cat, Δ^9-THC in a dose of 0.5 mg/kg, i.v. caused a negligible change in ACh release within experimental error. However, 3 mg/kg, i.v., produced a marked decrease, which was antagonized by 2.5 mg/kg of d-amphetamine, as noted.

even further by 3.0 mg/kg. At the same time, the neocortical EEG showed slow wave activity. d-Amphetamine in a dose of 2.5 mg/kg, i.v., caused EEG activation as well as a return of ACh release to control levels.

Electroencephalographic Studies in Animals

Depending upon dose and species, marijuana derivatives have a wide spectrum of electroencephalographic (EEG) effects. Bose and associates[42] reported that acute i.p. injections of *Cannabis* resin in rabbits caused an initial EEG activation followed by high-voltage sharp waves. Bicher and Mechoulam[43] observed that 8 mg/kg of racemic Δ^8- and Δ^9-THC caused restlessness, a lowering of arousal threshold to reticular stimulation, and EEG activation in rabbits with chronic indwelling brain electrodes. On the other hand, Longo and coworkers[19] and Lipparini and associates[44] showed in rabbits with chronic indwelling brain electrodes that pure (−) Δ^8- and Δ^9-THC abolished hippocampal θ-waves and gave rise to neocortical high voltage spikes and slow waves. While these results do not agree with those of Bicher and Mechoulam, they agree with those of Boyd and Merritt,[45] who showed that DMHP caused a decrease in EEG and behavioral arousal in the cat. Masur and Khazan[46] showed that in rats *Cannabis* extract and Δ^8-THC caused EEG polyspike discharges during both the awake state and REM sleep on both acute and chronic administration.

Our own research in this area was primarily in the acute and chronic dog and chronic monkey with brain electrodes. Most of our studies were with DMHP and fewer with MOP, and very recently with Δ^9-THC. Recently some of these studies have been reported.[24] The EEG effects in acute dogs were studied after i.v. administration. Mongrel dogs were immobilized by decamethonium or gallamine and placed on continuous artificial respiration. All surgery was performed under local anesthesia. Insulated steel nails were placed through the scalp into the calvarium for recording the electrical activity of the cerebrum. Care was taken to avoid passing through the posterior lamella. Electrical activity from the neocortex was recorded with a Grass electroencephalograph. The electrocardio-

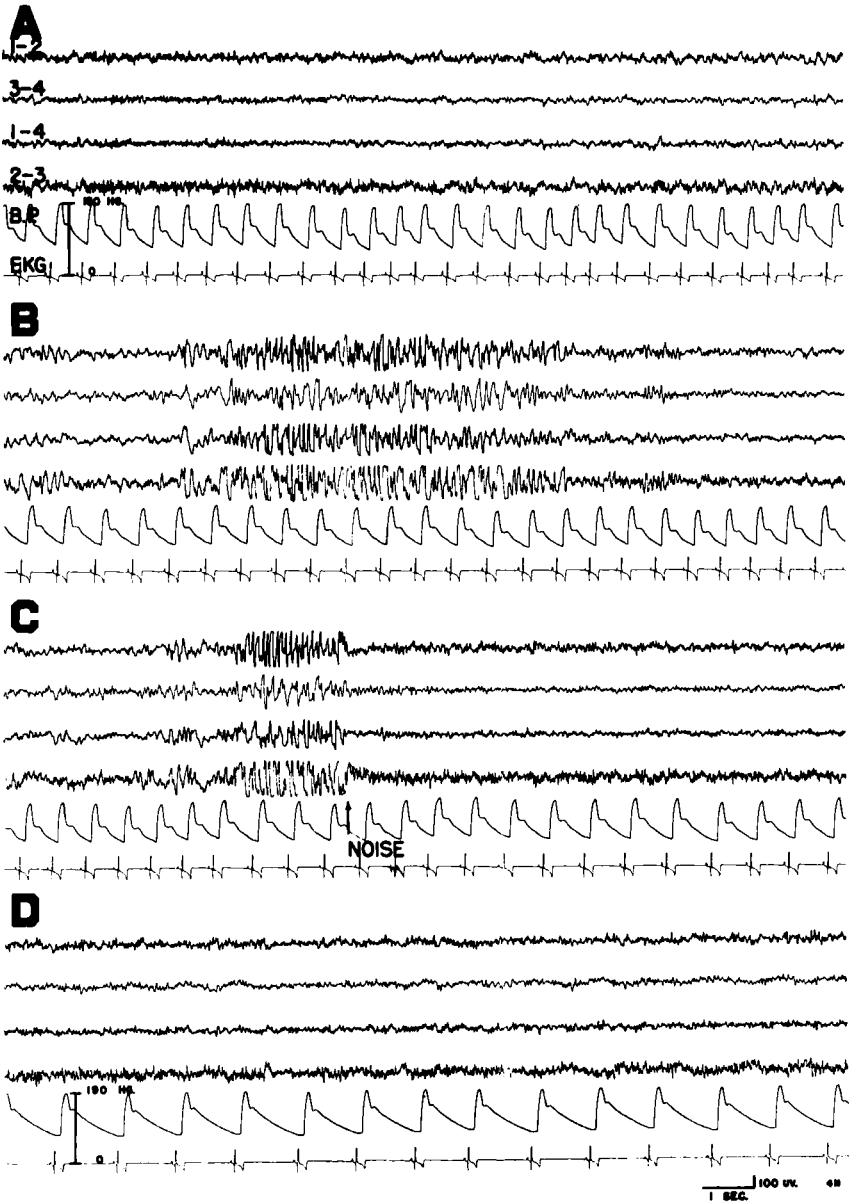

FIGURE 8. Effects of DMHP and d-amphetamine on the neocortical electroencephalogram of the dog. Bipolar EEG recordings were taken in a locally anesthetized, decamethonium immobilized dog on artificial ventilation. A = control. B = one hr after 100 μg/kg of DMHP, i.v. Note high-voltage, slow-wave bursts with spikes and bradycardia. C = same as B, but with a loud noise presented at the arrow (↑). D = After d-amphetamine, .1 mg/kg, i.v. 1 = left frontal, 2 = rt frontal, 3 = left parietal. 4 = rt parietal cortex. B.P. = arterial blood pressure in mm Hg. EKG lead II.

gram, lead I, and the femoral arterial blood pressure were recorded simultaneously. Seven dogs were utilized to obtain control EEG data. The dogs received either one ml of a 95% solution of ethyl alcohol or a comparable volume of isotonic sodium chloride solution and were observed for several hours. The usual EEG pattern observed consisted of low-voltage, fast-frequency waves characteristic of an awake, unanesthetized dog. Over a two-hour period, slow-wave bursts would occasionally appear. These were quickly converted by noise or other afferent stimuli to a low-voltage, fast-frequency EEG. Control animals, therefore, show electrical correlates of drowsiness or sleep that are quickly reversed to a state of wakefulness by certain afferent stimuli.

Seven dogs received varied doses of DMHP administered i.v. in 95% ethyl alcohol. With doses of 50 μg/kg, sleep spindles were occasionally observed. However, such spindling was also common in the control animals. After 100 μg/kg, the high-voltage slow waves were prolonged and were more frequent than in controls. The EEG record of one such experiment is shown in FIGURE 8. The upper tracing, A, represents the control EEG of the dog one hr after surgery. One hour after 100 μg/kg of DMHP, the high-voltage slow waves were prolonged and alternated frequently with low-voltage, fast-frequency activity (see B, FIGURE 8). The observed high-voltage, slow-wave bursts of twelve cycles per second could readily be converted to a low-voltage, fast-frequency EEG pattern by afferent stimuli such as noise (see C, FIGURE 8). Since increased afferent activity resulted in EEG activation, it was postulated that drugs that stimulate the brainstem-activating system would antagonize the EEG effects of DMHP. As shown (see D, FIGURE 8), d-amphetamine (100 μg/kg) does antagonize the slow-wave activity induced by DMHP. The EEG pattern obtained is almost indistinguishable from the control (compare A and D).

The effect of large doses of DMHP (1.0 mg/kg) is illustrated in FIGURE 9. Tracing A represents a normal low-voltage, fast-frequency control pattern. The frequency of spindling and slow waves increased about one-half hour after drug administration. One hour after the drug, generalized slow-wave activity with frequent spindle bursts were observed. Auditory stimuli were completely ineffective in producing EEG arousal during this period (see B, FIGURE 9). Exceedingly painful stimuli, however, such as pinching the testicles, resulted in a barely effective EEG arousal. High-voltage spike-like and slow waves are also seen (see C, FIGURE 9). Within a minute, the spontaneous electrical activity returned to a high-voltage, slow-wave pattern. DMHP-induced high voltage, slow wave and spikes could be partially reversed by psychomotor stimulants such as d-amphetamine in doses of .1–1.0 mg/kg, i.v. Although there was a definite EEG antagonism, the response after amphetamine was not the same as the very fast-frequency, low-voltage activity observed in the control tracing. Thus, the EEG antagonism was not complete.

Four dogs received MOP, i.v., in doses of 0.5–1.0 mg/kg. Occasionally, alpha-like slow waves were observed, but the predominant character of the EEG was a low-voltage, fast-frequency pattern. After an additional 1.0 mg/kg, marked high-voltage slow waves and spikes were seen. At this time, auditory stimuli were barely able to produce EEG activation. Fewer high-frequency waves were observed at this time than during the control tracing. In addition, there was a delay in the onset of arousal. After d-amphetamine (1.0 μg/kg), an incomplete antagonism of the slow-wave activity induced by MOP was observed. Thus, the acute EEG changes induced by MOP were very similar to DMHP but slightly less potent. Very recently, we studied Δ^9-THC in similar preparations.

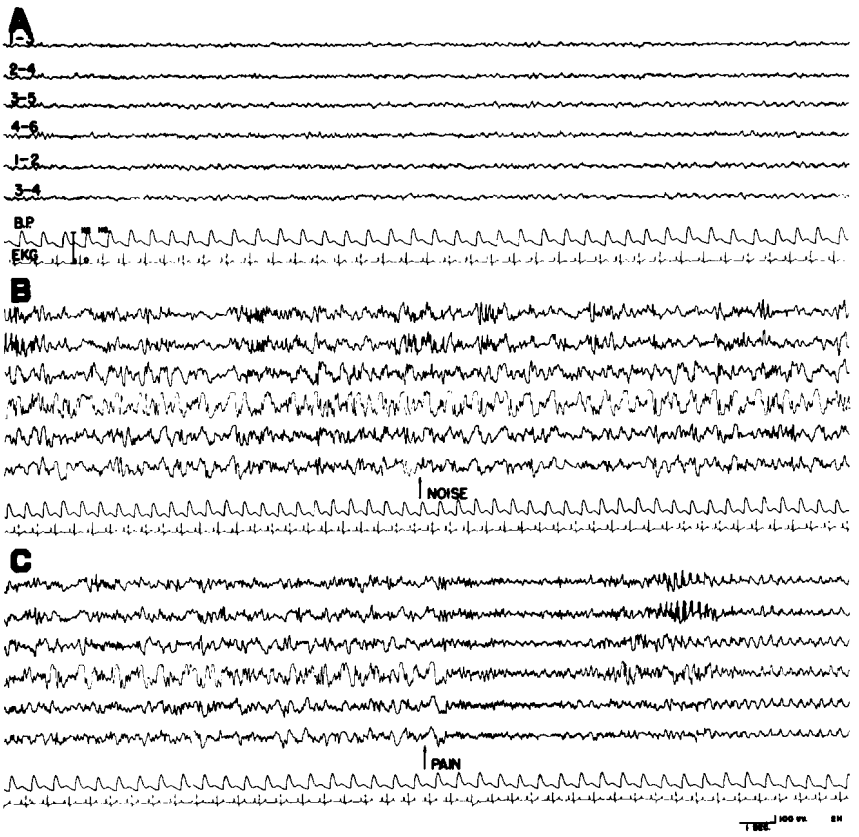

FIGURE 9. Effects of DMHP on the neocortical electroencephalogram of the dog. Recordings and symbols similar to those in FIGURE 8. A = Control. B = after 1 mg/kg DMHP. Intense auditory stimulus (↑) had no effect. C = as in B, but intense painful stimulus caused a transient and incomplete EEG activation with spike and slow waves as noted.

The EEG changes were quite similar, but the drug was less potent than these synthetic derivatives in doses of 0.5–10 mg/kg, i.v.

HUMAN STUDIES

Gross Behavioral and Neurologic Effects of Marijuana Smoking

To date, we have been involved in three different studies with marijuana smoking in normal volunteers over the past three years. Perhaps these studies emphasize more than anything else the basic pharmacologic principle of dose-effect. It is obvious that unless the concept of dose is constantly considered, marijuana effects vary from a very mild intoxication or reversible organic brain syndrome to frank hallucinations, some pleasant and others frightening enough to induce a panic reaction and, with large amounts, stupor and semicoma. Our first study was in collaboration with Dr. Caldwell and Dr. Myers and has been reported previously.[47-49] We were concerned with the effects of marijuana smoking to an endpoint of a "subjective high" on auditory and visual sensory thresh-

olds. Twenty experienced marijuana users were subdivided into a control and experimental group. The control group smoked alfalfa cigarettes in a pretest (C_1) and posttest (C_2), while the experimental group smoked alfalfa in the pretest (E_1) and marijuana in the posttest (E_2). A comparison of the mean changes in sensory thresholds between C_1 and C_2 was a measure of practice effect during testing. A comparison of the mean change in E_1 vs. E_2 and C_2 vs. E_2 was a measure of the effects of marijuana. All tests were conducted with the subject sitting in a totally dark, soundproof, air-conditioned room. All cigarettes contained 300 mg of material. The crude marijuana was obtained from Dr. Scigliano from NIMH and was said to contain 1.312% Δ^9-THC, but on reanalysis months later was found to contain .51% (courtesy of Dr. Wall) and 0.20% (courtesy of Dr. Forney). Our users reported it to be "good stuff," and were allowed to smoke on an *ad libitum* schedule until a subjective endpoint of a "high" was achieved. The results of this study were mostly negative, as noted in TABLE 3. There was no change in the visual brightness test at 2.4 and 4.8 foot candles, auditory amplitude threshold, auditory frequency threshold, and auditory decibel threshold. In only one aspect of the auditory amplitude difference threshold test did the marijuana smokers perform more poorly. The only consistent physiologic change was a slight increase in heart rate.

The second study with the same batch of NIMH marijuana was conducted in collaboration with Dr. Rodin and Mr. Porzak and has also been reported.[50] Ten healthy freshmen medical students who had had previous experience with marijuana were allowed to smoke the 1.312% NIMH marijuana to their usual "high." On the average, two to three cigarettes were consumed per subject. On neurological examination, there was nothing significant noted except slight improvement of vibratory sense. Mental status examination showed a slight decrease in intellectual efficiency, some excess jocularity, a slight loosening of

TABLE 3

LACK OF SIGNIFICANT DIFFERENCES IN MEAN PERFORMANCE SCORES FOR ALFALFA AND MARIJUANA GROUPS FOR VISUAL AND AUDITORY DISCRIMINATION MEASUREMENTS*

Variables	First Test Session		Second Test Session	
	Control group (alfalfa)	Experimental group (alfalfa)	Control group (alfalfa)	Experimental group (marijuana)
Visual brightness test				
2.40 foot candle standard	2.25	2.51	2.21	2.46
4.80 foot candle standard	4.09	4.93	4.60	4.91
Auditory amplitude threshold test (decibels)				
difference threshold	1.46	1.71	0.94†	1.63†
point of subjective equality	14.56	14.40	14.59	14.41
constant error	−0.44	−0.66	−0.40	−0.63
Auditory frequency threshold test (cycles/sec)				
difference threshold	3.64	4.06	2.53	3.79
point of subjective equality	1001.67	1001.24	1000.88	1000.05
constant error	+1.67	+1.24	+0.88	+0.05
Auditory threshold test (decibels)	75.11	73.83	75.87	76.74

* Data taken from Caldwell *et al.*[49]

† $t = 3.25$, $P < .01$. Note that the threshold was increased for the second test session for the marijuana subjects when compared with the second test controls. All other test results were not significantly different.

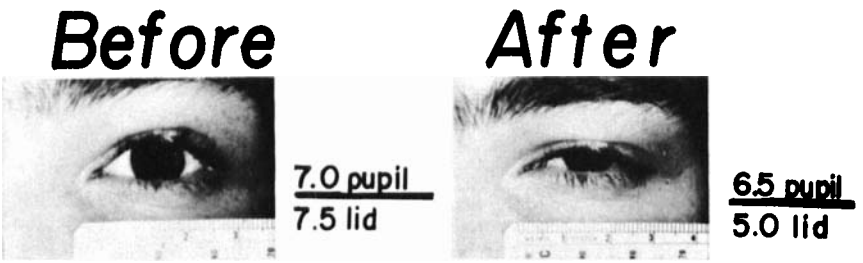

FIGURE 10. Photograph of the eye before and after marijuana smoking. The pupil size and distance between the lids are given. Note marked ptosis after subject is "stoned."

association, and a slight short-term memory loss, suggesting a mild organic brain syndrome that was fully reversible. Bender-Gestalt drawings were executed slightly more poorly after marijuana. Visually evoked cerebral responses were unchanged, but EEG α-rhythm showed a slight shift toward a lower frequency (see below). No hallucinations were observed with this particular batch of marijuana.

Subsequently, we obtained from Dr. Scigliano of NIMH marijuana that was chemically extracted so as to contain 0% Δ^9-THC, as well as batches containing 1.5 and 2.9% Δ^9-THC. We are conducting a series of behavioral and pharmacological studies with these materials and will report our data in the near future.[51-53] So far, about 16 student volunteers have been given the 2.9% Δ^9-THC marijuana and allowed to smoke to the point of refusal in order to obtain a more complete dose-effect curve. To date, we have had about four subjects who had visual and/or auditory hallucinations and one very severe panic reaction. Another two subjects smoked to the point of stupor and semicoma. Most of the remaining subjects found the marijuana experience most pleasant and reinforcing. Our research to date simply reaffirms what has long been known about the clinical descriptive pharmacology of marijuana as recently reviewed in comparison with other hallucinogens.[54,55] In spite of the fact that pharmacologically, LSD-25 and marijuana are quite different, we have observed a patient who had recurrent LSD-25 flashbacks triggered by marijuana smoking.[56]

Although conjunctival redness has long been mentioned as a consistent effect of marijuana smoking and was also observed in our studies, a more dramatic finding is a slight ptosis of the upper eyelid that characteristically occurs in the really "stoned" smoker. This is evident from the before and after photographs in FIGURE 10.

Cardiovascular Effects of Marijuana Smoking

Many investigators have observed that marijuana smoking or THC derivatives in man cause a significant increase in heart rate.[57-61] Manno and coworkers[60] and Renault and associates[61] showed that the heart rate increase is highly dose-related. We have just completed an analysis of our data from two studies in which a similar dose-related increase is highly significant.[62]

This study was performed in two parts. In 1969 ten subjects took part in a low-dose (assumed to be 0.5% Δ^9-THC as a median figure) marijuana study and then in a single-blind placebo (0% Δ^9-THC) study. In early 1971, 15 different subjects took part in a high dose (2.9% Δ^9-THC) marijuana study. All subjects were males between the ages of 21 and 33. All had smoked marijuana

previously, but only 4 of 10 in the low-dose study and 2 of 15 in the high-dose study were daily users. One of the subjects in the high-dose study was able to tolerate a large dose (30 mg Δ^9-THC) with little change in his outward behavior or conversation. The other 14 subjects reported that they were "as high or higher than ever before" during the high-dose marijuana study. All marijuana was administered as 300-mg cigarettes, which were smoked to the shortest possible butt. Butts were weighed to determine the dose smoked. In the low-dose study, subjects smoked from two to five cigarettes. In the high-dose study, subjects were instructed to smoke until they were as high as they had ever been on marijuana and felt they could not smoke any more. This required from one to four cigarettes. Subjects were told to inhale deeply and to let none of the smoke appear in the exhaled air. No corrections were made for pyrolysis, exhaled smoke, or cigarette smoke burned but not inhaled.

Blood pressure was measured at least three times before marijuana was smoked, in order to obtain a stable baseline. It was recorded three times again within one-half hour after smoking. Diastolic pressure was recorded at the muffling of the Korotkoff sound. The difference in means of the blood pressures before and after marijuana is reported as the change in blood pressure.

Pulse rate was recorded from a continuous polygraph record in those subjects who did not have complete EKG's. The change in pulse rate is the difference between a baseline rate and the highest rate obtained within one hour of smoking marijuana. Dose of Δ^9-THC was calculated as total available in the cigarettes smoked, and not as that inhaled. It is well known that pyrolysis reduces the maximum amount of Δ^9-THC available.

As shown in FIGURE 11, the increase in heart rate after smoking marijuana is dose-related. The coefficient of correlation between $\log_{10}$ dose and increase in

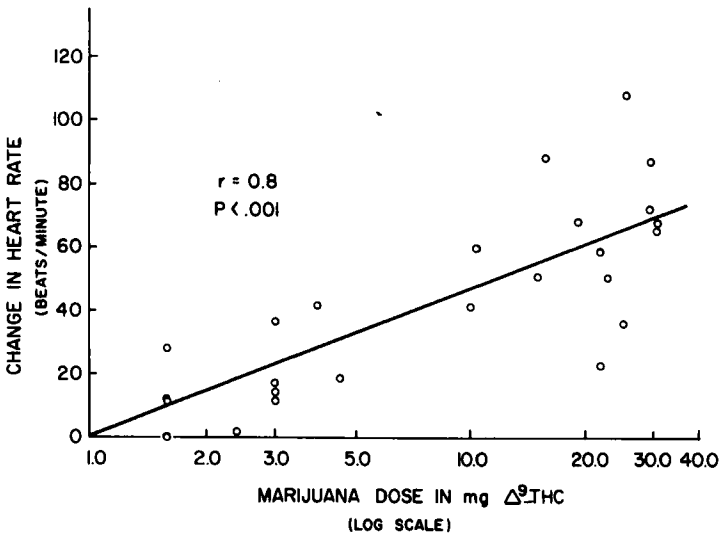

FIGURE 11. Dose-effect relations of marijuana smoking on heart rate. Adult male volunteers were given various amounts of marijuana of varying Δ^9-THC content. The maximal increase in heart rate above control is given on the y-axis, and the total amount of Δ^9-THC in the cigarettes on the x-axis. Each point represents an individual subject. The coefficient of correlation is 0.8 for which $P < .001$.

heart rate is 0.8, for which $P < 0.001$. One subject, a daily user of marijuana, seemed to be extremely tolerant of the psychic effects. However, his heart rate increased from 55 to 120 after a 30-mg dose.

The systolic blood pressure was significantly elevated in subjects receiving more than 10 mg marijuana, $P < 0.01$ on the Wilcoxin sign test for differences in related samples. The rise in systolic blood pressure appeared to be dose-related, although the coefficient of correlation was only 0.3 and the P value < 0.10. The diastolic blood pressure was also significantly elevated after marijuana smoking, $P < 0.02$ on the Wilcoxin test. For the diastolic pressure data, the coefficient of correlation of the $\log_{10}$ dose response curve was only 0.3.

The data from the single-blind placebo extract experiments showed no significant changes in heart rate or blood pressure. Calculation of the marijuana dosage on a mg/kg basis using the subjects' weights did not reduce the variation in the data. Two of our subjects showed premature ventricular contractions after marijuana smoking similar to those seen in susceptible nicotine tobacco and coffee users.

Electroencephalographic Effects of Marijuana and Derivatives

The first major study on the effects of acute and chronic pyrahexyl and marijuana in human users was that reported by Wikler and Lloyd[63] and Williams and coworkers. Single doses of oral 30 mg and 120 mg of pyrahexyl did not change the α-frequency significantly. In four of eight subjects the α-percentage decreased. On continuous daily medication there was a significant decrease in α-frequency in three of five subjects with variable changes in α-percentage. Two subjects showed Δ-wave changes that returned to normal four days after cessation of pyrahexyl administration. These same investigators studied the effects of marijuana smoking of unknown resin content under similar conditions. After acute marijuana smoking, no significant effect on α-frequency was observed, although α-percentage was usually decreased.

In all cases muscle artifact increased in the EEG recordings. After continued daily smoking of marijuana, four of six subjects showed a slight decrease in α-frequency that was marked in only one case. Alterations in α-percentage were also variable, with three of six showing an increase, two of six a decrease, and one no change. By contrast with the single study of smoking one to four marijuana cigarettes, muscle activity was not markedly increased during the continuous smoking experiments.

More recently, in collaboration with Dr. Rodin, we reexamined the effect of smoking marijuana cigarettes on the EEG of 10 freshman medical students who admitted to long-term use of marijuana.[50] After obtaining baseline controls, the subjects were given as many marijuana cigarettes as they desired to reach their usual "high." On the average, two or three 300-mg cigarettes were consumed (870 ± 80 mg). The marijuana used was initially assayed to contain 1.312% Δ^9-THC. However, as discussed above, independent assays by Dr. Wall (0.5%) and Dr. Forney (0.2%) done over a period of six months showed a progressive reduction in Δ^9-THC content of this marijuana kept in a safe at room temperature. Data acquisition for computer analysis was done immediately after the subject had reported he had achieved his "high." On visual inspection it was impossible to distinguish the premarijuana from the postmarijuana EEG, although there was a suggestion that after smoking there was a more persistent α-rhythm and possibly a slight slowing of the α-frequency. Power density

spectral analysis confirmed this impression, indicating a slight shift downward in the α-rhythm. This finding was statistically significant ($P < .05$).

In the past six months we have had an opportunity to replicate some of these findings in another study with Mr. Porzak and Dr. Kovacic. A total of five experienced marijuana smokers were given up to four 300 mg 2.9% Δ^9-THC-containing marijuana cigarettes or a comparable amount of THC-extracted material. Grass silver electrodes were placed in accordance with the 10–20 International Electrode System.[64] Electrical potentials were obtained from F_3 (frontal), C_3 (central), P_3 (parietal) and O_1 (occipital) to both ears, which served as a reference. All EEG recordings were made on the left side of the head. A Grass polygraph was used for amplification and recording. The data were recorded on analog tape, and selected portions were digitized for computer analysis. The procedure was to digitize one or more 60.5 second recordings at 128 samples/second for each subject/condition. With use of the standard Lafayette Clinic Computer Program,[65] power frequency spectra estimates were obtained with 0.25 Hz resolution. By using the half-height center as a measure of the α-peak frequency, the shifts shown in FIGURE 12 were obtained. For two subjects, α-peak frequency measures were obtained for extracted marijuana as well. EEG recordings were compared before and after smoking to the point of refusal of the marijuana. In FIGURE 12 are shown the average half-height α-center frequency shift in which the after-before difference in Hz is expressed. It can be seen that there is a very slight tendency for a 0.3–0.6 decrease in α-frequency in 4/5 subjects smoking active marijuana in contrast to extracted, which, on the Wilcoxin's one-tailed test, was marginally statistically significant ($P < .06$). While these are not very convincing data, the indications are that there is a slight α-shift associated with marijuana smoking. This shift cannot be visualized easily in the actual record. In FIGURE 12 also are plotted the EEG data of Williams and coworkers in subjects who smoked marijuana continuously. Their data show very similar trends to our computerized approach. One can conclude that an impartial computer analysis of EEG data offers information similar to that of scientists who very carefully "eyeball" their records.

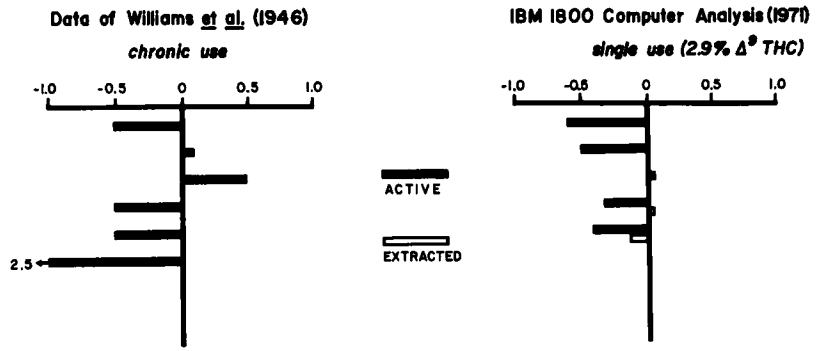

FIGURE 12. Comparison of α-frequency EEG shifts in marijuana smokers. The published data of Williams and coworkers[20] of 6 subjects who smoked marijuana daily to 5 of our subjects who smoked up to four 2.9% Δ^9-THC 300-mg marijuana cigarettes to the point of refusal. In both cases the shift in the α-center frequency is given as the difference between after and before marijuana smoking. A negative difference indicates a slowing in the basic α-frequency. The solid bars indicate active marijuana and the open bars two of the same subjects smoking the same number of extracted marijuana cigarettes. With use of a one-tailed Wilcoxin's test, that data are of borderline significance ($P < .06$).

TABLE 4

COMPARATIVE PHARMACOLOGICAL EFFECTS OF Δ^9-THC WITH VARIOUS PSYCHOACTIVE DRUGS

Pharmacological Parameters	Drug						
	Δ^9 THC	EtOH	Morphine	LSD-25	N_2O	Scopolamine	Phencyclidine
Euphoria	yes	yes	yes	yes	yes	no	yes
Panic reaction	yes	no	no	yes	yes	yes	yes
Stupor with large doses	yes	yes	yes	no	yes	yes	yes
Analgesia	yes (animals)	no	yes	yes	yes	no	yes
Hallucinations (acute dose)	yes	no	no	yes	yes	yes	yes
Auditory and visual sensations	↑	↓	–, ↓	↑	↑, ↓	–, ↓	↓
Tolerance	yes (animals)	yes	yes	yes	no	yes (animals)	?
Cross-tolerance to narcotic analgesics	no	no	yes	no	no	no	no
to sedatives	?	yes	no	no	yes	no	no
Physical dependence	no	yes	yes	no	no	no	?
Psychic dependence	yes	yes	yes	yes	yes	no	yes
Short-term memory loss	yes	no	no	no	yes	yes	yes
Antagonists							
Specific	none	none	Naloxone	none	none	none	none
Pharmacologic	amphetamines (poor)	amphetamines (poor)	–	CPZ Diazepam	none	physostigmine	none
Selective blockade conditioned avoidance	yes	no	yes	no	?	no	no
Brain ACh							
Content	↑	↑	↑	–	?	↓	–
Release	↓	↓	↓	–	?	↑	–, ↓
EEG Activity	slow	slow	slow	fast	slow	slow	slow-fast

COMMENT

After serving a pharmacologic smorgasbord, what can one conclude about *Cannabis* and its natural or synthetic THC derivatives? Perhaps what stands out most clearly is that these agents are primary depressants of central nervous system function. However, they possess a unique spectrum of pharmacological actions with only superficial relation to various other psychoactive drugs. The chemistry and pharmacology of marijuana are distinct from other central nervous system depressants, yet it shares some properties with ethyl alcohol, general anesthetics like nitrous oxide, and psychotomimetics like LSD-25. The recent report to the Congress on "Marijuana and Health" emphasizes this as well.[66] Dosage level is all-important! In low doses these drugs produce an intoxication that in some ways resembles that of ethyl alcohol or low concentrations of nitrous oxide. There are, however, definite subjective differences. In TABLE 4 are summarized some of the comparative aspects of Δ^9-THC, ethyl alcohol, morphine, LSD-25, nitrous oxide and scopolamine and phencyclidine on various pharmacological parameters. From a scientific point of view, there is great danger in presenting any such table because the question of dose and species is always a critical factor. If the reader accepts such limitations, it is obvious that Δ^9-THC has its own peculiar spectrum of pharmacology. Whether it has therapeutic merit is impossible to answer affirmatively as yet. We do have available a large variety of sedatives, analgesics, etc. Only further research will tell whether Δ^9-THC and related compounds will remain of primary social, rather than medical, consequence.

ACKNOWLEDGMENTS

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REFERENCES

1. MOREAU, J. 1845. Hashish and Mental Illness. G. Batnett, Trans. 1970. Raven Press. New York, N. Y.
2. LEWIN, L. 1964. Phantastica. Narcotic and Stimulating Drugs, their Use and Abuse. 335 pp. E. P. Dutton & Co., Inc. New York, N. Y.
3. HOFFER, A. & H. OSMOND. 1967. The Hallucinogens. 626 pp. Academic Press. New York, N. Y.
4. HOLLISTER, L. E. 1968. Chemical Psychoses, LSD and Related Drugs. 190 pp. C. C Thomas, Springfield, Ill.
5. HOLLISTER, L. E. 1971. Marijuana in man: Three years later. Science **172**: 21–29.
6. MECHOULAM, R. 1970. Marijuana chemistry. Science **168**: 1159–1166.
7. HARDMAN, H. F., E. F. DOMINO & M. H. SEEVERS. 1971. Structure activity relationships of Δ^3-tetrahydrocannabinols. Proc. Western Pharmacol. Soc. **14**: 14–20.
8. HARDMAN, H. F., E. F. DOMINO & M. H. SEEVERS. 1971. General pharmacological actions of some synthetic tetrahydrocannabinol derivatives. Pharmacol. Rev. In press.
9. CALDWELL, D. F., D. OBERLEAS, J. J. CLANCY & A. S. PRAASAD. 1970. Behavioral impairment in adult rats following acute zinc deficiency. Proc. Soc. Exp. Biol. Med. **133**: 1417–1421.
10. TENEN, S. S. 1966. An automated one-way avoidance box for the rat. Psychonomic Sci. **6**: 407–408.
11. STARK, P. 1971. Personal communication.

12. DOMINO, E. F. 1968. Substituted phenothiazine antipsychotics. *In* Psychopharmacology – A Review of Progress 1957–1967. D. H. Efron, Ed. : 1045–1056. PHS Publication 1836. U. S. Gov't. Printing Office. Washington, D. C.
13. BLACK, M. B., J. H. WOODS & E. F. DOMINO. 1970. Some effects of (−)Δ^9-trans-tetrahydrocannabinol and other cannabis derivatives on schedule controlled behavior. Pharmacologist **12**: 258.
14. FERSTER, C. B. & B. F. SKINNER. 1957. Schedules of Reinforcement. 741 pp. Appleton-Century-Crofts, New York, N. Y.
15. KELLEHER, R. T. & W. H. MORSE. 1968. Determinants of the specificity of behavioral effects of drugs. Ergebenisse der Physiologie **60**: 1–56.
16. WALTON, R. P. 1938. Marijuana–America's New Drug Problem. 223 pp. J. B. Lippincott Co. Philadelphia, Pa.
17. SEEVERS, M. H. 1962. Medical perspectives on habituation and addiction. JAMA **181**: 92–98.
18. COUNCIL ON MENTAL HEALTH AND COMMITTEE ON ALCOHOLISM AND DRUG DEPENDENCE. 1967. Dependence on cannabis (marijuana). JAMA **201**: 368–371.
19. LONGO, V. G., F. LIPPARINI & A. SCOTTI DE CAROLIS. 1969. A neuropharmacological investigation on the new hallucinogens, DOM, Δ^9 and Δ^8 trans tetrahydrocannabinol and muscimol. Presented at the Int. Symp. on Psychodysleptics and Pharmacopsychosis. Laval Univ. Quebec, Canada.
20. WILLIAMS, E. G., C. K. HIMMELSBACH, A. WIKLER, D. C. RUBLE & B. J. LLOYD. 1946. Studies on Marijuana and Pyrahexyl Compound. Reprint 2732, Public Health Reports. **61**(29): 1059–1083. U. S. Gov't. Printing Office. Washington, D. C.
21. CARLINI, E. A. 1968. Tolerance to chronic administration of *Cannabis sativa* (marijuana) in rats. Pharmacology (Basel) **1**: 135–142.
22. SILVA, M. T. A., E. A. CARLINI, U. CLAUSSEN & F. KORRE. 1968. Lack of cross-tolerance in rats among (−)Δ^9-trans-tetrahydrocannabinol (Δ^9-THC), cannabis extract, mescaline and lysergic acid diethylamide (LSD-25). Psychopharmacologia (Berl.) **13**: 332–340.
23. MCMILLAN, D. E., L. S. HARRIS, J. M. FRANKENHEIM & J. S. KENNEDY. 1970. 1-Δ^9-trans-tetrahydrocannabinol in pigeons: tolerance to the behavioral effect. Science **169**: 501–503.
24. DOMINO, E. F., H. F. HARDMAN & M. H. SEEVERS. 1971. Central nervous system actions of some synthetic tetrahydrocannabinol derivatives. Pharmacol. Rev. In press.
25. HARDMAN, H. F., E. F. DOMINO & M. H. SEEVERS. 1957. Chemical Warfare Laboratories Technical Memo 27-2.
26. HIMMELSBACH, C. K. 1944. Treatment of the morphine abstinence syndrome with a synthetic cannabis-like compound. Southern Med. J. **37**: 26–29.
27. MCMILLAN, D. E., L. S. HARRIS, R. F. TURK & J. S. KENNEDY. 1970. Development of marked behavioral tolerance to 1-Δ^9-tetrahydrocannabinol (Δ^9-THC) and cross tolerance to 1-Δ^8-tetrahydrocannabinol (Δ^8-THC) in the pigeon. Pharmacologist **12**(2): 258.
28. COMPO, R. 1970. Development of tolerance to behavioral effects of a synthetic tetrahydrocannabinol derivative in pigeons. Abstracts of the CINP VII Congress **1**: 73. Prague, Czechoslovakia.
29. BLACK, M. B., J. H. WOODS & E. E. DOMINO. 1971. Some effects of (−)Δ^9-trans-tetrahydrocannabinol and other cannabis derivatives on schedule controlled behavior. J. Pharmacol. Exper. Ther. Submitted for publication.
30. GILL, E. W., W. D. M. PATON & R. G. PERTWEE. 1970. Preliminary experiments on the chemistry and pharmacology of cannabis. Nature (London) **228**: 134–136.
31. LAYMAN, J. M. & A. S. MILTON. 1971. Some actions of Δ^1-tetrahydrocannabinol and cannabidiol at cholinergic junctions. Brit. J. Pharmacol. **41**: 379P–380P.
32. STONE, W. E. 1955. Acetylcholine in the brain. I. "Free," "bound" and total acetylcholine. Arch. Biochem. Biophys. **59**: 181–192.
33. DREN, A. T. & E. F. DOMINO. 1968. Effects of hemicholinium (HC-3) on EEG activation and brain acetylcholine in the dog. J. Pharmacol. Exper. Ther. **161**: 141–154.
34. GIARMAN, N. J. & G. PEPEU. 1962. Drug-induced changes in brain acetylcholine. Brit. J. Pharmacol. Chemother. **19**: 226–234.
35. NEFF, N. H., R. C. LIN, S. H. NGAI & E. COSTA. 1969. Turnover rate measurements of brain serotonin in unanesthetized rats. *In* Advances in Biochemical Psychopharmacology. E. Costa & P. Greengard, Eds. **1**: 91–109. Raven Press. New York, N. Y.
36. NEFF, N. H., P. F. SPANO, A. GROPPETTI, C. T. WANG & E. COSTA. 1971. A simple procedure for calculating the synthesis rate of norepinephrine, dopamine and serotonin in the brain. J. Pharmacol. Exper. Ther. **176**: 701–710.
37. HEBB, C. O., G. M. LING, E. G. MCGEER, P. L. MCGEER & D. PERKINS. 1964. Effect of locally applied hemicholinium on the acetylcholine content of the caudate nucleus. Nature (London) **204**: 1309–1311.

38. SLATER, P. 1968. The effects of triethylcholine and hemicholinium-3 on the acetylcholine content of rat brain. Int. J. Neuropharmacol. **7**: 421–427.
39. PEPEU, G. & A. BARTOLINI. 1968. Effect of psychoactive drugs on the output of acetylcholine from the cerebral cortex of the cat. Europ. J. Pharmacol. **4**: 254–263.
40. BARTOLINI, A. & G. PEPEU. 1967. Investigations into the acetylcholine output from the cerebral cortex of the cat in the presence of hyosine. Brit. J. Pharmacol. Chemother. **31**: 66–73.
41. DOMINO, E. F. & A. BARTOLINI. 1971. Effects of various hallucinogens on neocortical release of acetylcholine. In preparation.
42. BOSE, G. C., A. Q. SAIFI & A. W. BHAGWAT. 1964. Observation on the pharmacological actions of *Cannabis indica* (Linn). Arch. Int. Pharmacodyn. **147**: 285–290.
43. BICHER, H. I. & R. MECHOULAM. 1968. Pharmacological effects of two active constituents of marijuana. Arch. Int. Pharmacodyn. **172**: 24–31.
44. LIPPARINI, F., A. DE CAROLIS & V. A. LONGO. 1969. Neuropharmacological investigation of some trans-tetrahydrocannabinol derivatives. Physiology and Behavior **4**: 527–532.
45. BOYD, E. S. & D. A. MERRITT. 1965. Effects of thiopental and a tetrahydrocannabinol derivative on arousal and recruiting in the cat. J. Pharmacol. Exper. Ther. **149**: 138–145.
46. MASUR, J. & N. KHAZAN. 1970. Induction by *Cannabis sativa* (marijuana) of rhythmic spike discharges overriding REM sleep electrocorticogram in the rat. Life Sci. **9**: 1275–1280.
47. CALDWELL, D. F., S. A. MYERS, E. F. DOMINO & P. E. MERRIAM. 1969. Auditory and visual threshold effects of marijuana in man. Perception and Motor Skills **29**: 755–759.
48. CALDWELL, D. F., S. A. MYERS, E. F. DOMINO & P. E. MERRIAM. 1969. Auditory and visual threshold effects of marijuana in man: Addendum. Perception and Motor Skills **29**: 922.
49. CALDWELL, D. F., S. A. MYERS & E. F. DOMINO. 1970. Effects of marijuana smoking on sensory thresholds in man. *In* Psychotomimetic Drugs. D. H. Efron, Ed. : 299–321. Workshop Series of Pharmacology Section, National Institute of Mental Health. Raven Press. New York, N. Y.
50. RODIN, E., E. F. DOMINO & J. PORZAK. 1970. The marijuana-induced "social high" – neurological and electroencephalographic concomitants. JAMA **213**: 1300–1302.
51. DOMINO, E. F., P. RENNICK & J. PEARL. 1971. Pharmacological effects of marijuana smoking in normal volunteers. In preparation.
52. RENNICK, P., E. F. DOMINO & J. PEARL. 1971. Neuropsychological effects of marijuana smoking in normal volunteers. In preparation.
53. PEARL, J., E. F. DOMINO & P. RENNICK. 1971. Some effects of marijuana smoking on higher cognative functions. In preparation.
54. DOMINO, E. F. 1971. Clinical pharmacology of marijuana and its derivatives. Mich. Med. Center J. **37**: 1–6.
55. DOMINO, E. F. 1971. Pharmacology of madness – the hallucinogens. International Symposium on Drug Abuse. C. J. D. Zarafonetis, Ed. In press. Lea and Febinger. Philadelphia, Pa.
56. FAVAZZA, A. & E. F. DOMINO. 1969. Recurrent LSD experience (flashbacks) triggered by marijuana. Michigan Med. Center. J. **35**: 214–216.
57. MAYOR'S COMMISSION ON MARIJUANA. 1944. The problem of marijuana in the city of New York. Jacques Cattell Press. Lancaster, Pa.
58. ISBELL, H., C. W. GORODETSKY, D. JASINSKI, U. CLAUSSEN, F. VON SPULAK & F. KORTE. 1967. Effects of $(-)\Delta^9$-trans-tetrahydrocannabinol in man. Psychopharmacologia (Berlin) **11**: 184–188.
59. HOLLISTER, L., R. K. RICHARDS & M. K. GILLESPIE. 1968. Comparison of tetrahydrocannabinol and synhexyl in man. Clin. Pharmacol. Ther. **9**: 783–791.
60. MANNO, J. E., G. F. KIPLINGER, S. H. HAINE, I. F. BENNETT & R. B. FORNEY. 1970. Comparative effects of smoking marijuana or placebo on human motor and mental performance. Clin. Pharmacol. Ther. **11**: 808–815.
61. RENAULT, P. F., C. R. SCHUSTER, R. HEINRICH & D. X. FREEDMAN. 1971. Marijuana: Standardization of smoke administration and dose effect curves on heart rate in humans. Presented at National Research Council Committee on the Problems of Drug Dependence. Toronto, Canada.
62. JOHNSON, S. & E. F. DOMINO. 1971. Some cardiovascular effects of marijuana smoking in normal volunteers. Clin. Pharmacol. Therap. **12**: 762–768.
63. WIKLER, A. & B. J. LLOYD, JR. 1945. Effect of smoking marijuana cigarettes on cortical electrical activity. Fed. Proc. **4**: 141–142.
64. JASPER, H. H. 1958. The ten-twenty electrode system of the International Federation. Electroenceph. Clin. Neurophysiol. **10**: 371–375.

65. HOVEY, G. W. Lafayette Clinic Computing Laboratory Technical Manual no. 6. Lafayette Clinic. 951 E. Lafayette, Detroit, Mich. 48207.
66. A REPORT TO CONGRESS FROM THE SECRETARY, DEPARTMENT OF HEALTH, EDUCATION AND WELFARE. 1971. Marijuana and Health. 176 pp. National Institute of Mental Health. U. S. Government Printing Office, Washington, D. C.