EXPERIMENTAL NEUROLOGY 41, 357–365 (1973)

Effects of Tetrahydrocannabinol on Hippocampal Evoked Afterdischarges in Cats

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Received June 15, 1973; revision received July 13, 1973

The effects of repeated doses of the psychoactive component of marijuana, Δ^9-THC, on epileptiform EEG activity were examined. Hippocampal afterdischarges evoked at various stimulus intensities in chronically prepared cats were significantly changed in duration and threshold under repeated doses of several drug levels in comparison to predrug controls. Under Δ^9-THC, an elevation of threshold was indicated by a reduction in the probability of obtaining any afterdischarge to weak stimulus intensities. However, in response to strong hippocampal stimuli, a marked increase in the variance of afterdischarge duration was observed. This latter finding reflected an increase in the frequency of both long and short afterdischarges after administration of Δ^9-THC.

INTRODUCTION

Clinical reports of the medicinal qualities of marijuana (*Cannabis sativa*) have received little scientific attention. Among other effects, an anticonvulsant action has been attributed to the drug (16) and a few brief accounts have appeared exploring this hypothesis with tetrahydrocannabinal (THC), the psychoactive component of marijuana. Specifically, Loewe and Goodman (13) reported a reduction in the severity of the effects of electroconvulsive shock in rats after the administration of THC and two of its homologs. Similarly, Davis and Ramsey (4) described a remission of grand mal seizure activity in three institutionalized epileptics and complete abolition in two others after treatment with one of two isomers of a THC homolog.

[1] This work was supported by USPHS Grant NS 10469-01 to D. M. Feeney and the Δ^9-THC was furnished by D. Ferraro under HEW Grant RO3 MH20363-01. Address reprint requests to D. M. Feeney, Department of Psychology, University of New Mexico, Albuquerque, New Mexico, USA 87131. We thank Maura Cashion and Frank Rowe for technical assistance. M. C. McNamara is now at the University of North Carolina.

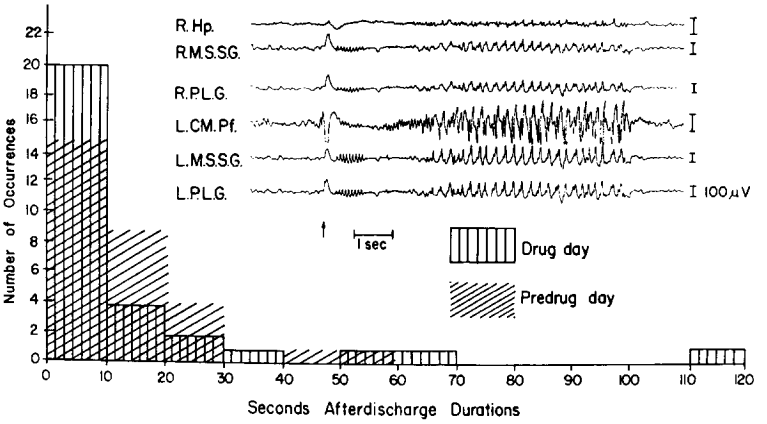

FIG. 1. Distributions of afterdischarge durations obtained for cat 1 on predrug days (vehicle only) and on drug days (Δ^6-THC). Note the increased variance on drug days. Insert depicts a representative afterdischarge evoked by stimulation of left hippocampus for cat 1 on a predrug day. A 1-sec stimulus was applied at arrow. Abbreviations are: R., right L., left; G., gyrus; M.S.S., mid-suprasylvian; P.L., posterior lateral; CM-Pf., centre medianum-parafasicularis; Hp., hypothalamus.

Garriott, Forney, Hughes, and Richards (5), while testing a group of eight THC homologs for pharmacologic effects consistent with those reported for marijuana, reported a reduction in electroconvulsive seizure duration in mice after the administration of two of the involved homologs. More recently, Killam and Killam (11) described a suppression of photomyoclonic seizures in baboons after the administration of Δ^9-THC.

The above findings suggest that THC and certain of its homologs might be expected to have an ameliorative influence on seizure activity—perhaps reflecting a drug-induced increment in seizure threshold. The present study was undertaken to test for such an affect using repeated dosages of Δ^9-THC in conjunction with evoked hippocampal afterdischarge in cats (6, 18). Selection of hippocampal evoked afterdischarge as a model of epilepsy was considered appropriate since much of human epilepsy is of temporal lobe origin and since Δ^9-THC has some affinity for the hippocampus (15).

METHOD

Surgery was performed on three adult cats under barbiturate anesthesia (5.5 mg/kg of Promazine ip followed 30 min later by 23.3 mg/kg Nembutal, ip) using standard aseptic procedures. Bipolar concentric stimulating electrodes were chronically implanted unilaterally in the dorsal hippocampus. Recording was done through monopolar electrodes. Placements in various preparations were as follows: ventral hippocampus, contralateral dorsal hippocampus, hypothalamus, rostral thalamus, lateral geniculate body, and

the centrum medianum–parafasicularis complex. Skull screws were placed over the posterior lateral and posterior sigmoid gyri for cortical recording and above the anterior sinus as a reference in all animals.

Recordings were done on a Grass Model 78 polygraph. Stimuli were produced by a Nuclear Chicago constant current stimulator through isolation transformers and consisted of a 1.0-sec train of 0.5-msec duration square waves delivered at a frequency of 400/sec. Successive stimulations were separated by a 3-min interval in cat 1 and by 2-min intervals in cat 2 and cat 3. Interval timing was begun immediately after stimulation except in those cases in which stimulation produced an afterdischarge. In those latter cases, onset of timing followed the conclusion of the discharge. Variable intensities of stimulation were applied as discussed below. Hippocampal evoked afterdischarges have been described in detail (6, 18) and in the present experiments were characterized as large-amplitude synchronous rhythms of at least 2-sec duration often appearing in all leads (Fig. 1).

After recovery from surgery, a daily routine of preliminary stimulation was carried out to produce a reduction in seizure threshold toward some stable minimum level (18). For cat 1 and cat 2 this included an assessment of threshold followed by a step-wise increment in stimulus intensity from a starting point two units below threshold to a point two units above threshold (i.e., a total of five stimulus intensities for the entire sequence). Each sequence in this staircase method was repeated four times daily with the beginning intensity for each sequence being lowered accordingly as threshold decreased. The third cat received ten days of preliminary stimulation at a constant, above-threshold intensity (2.0 mamp) after which it too was switched to the staircase method. Stimulation for cat 1 and cat 3 was in 0.1-mamp increments while cat 2 received increments of 0.2 mamp. Stimulation intensities during drug evaluation ranged from 0.1 to 0.4 mamp for cat 1, 0.4–1.2 for cat 2, and 0.1–0.5 for cat 3. Each test session lasted approximately 1 hr.

In addition to threshold information, observations on behavioral effects of the stimulation and durations of evoked afterdischarges were recorded. The duration of each afterdischarge was measured from stimulus offset to the termination of high-voltage synchronous activity. Almost without exception, this activity terminated simultaneously at all recording sites.

Drug administration was begun after the establishment of a relatively stable threshold. This included a series of 0.25, 0.5, and 1.0 mg/kg dosage of Δ^9-THC for each cat. In cat 2, doses were increased to 0.5, 1.0, and 1.5 mg/kg during the third series. The order of dose administration was randomized within each series and experimenters were blind as to dose level. The dose series was repeated five times for each cat with 5-day intervals between drug administration. The Δ^9-THC was stored under nitrogen and refrigeration and prepared as a suspension in 0.5 ml of

sesame oil a few hours prior to administration. The drug was administered orally in a gelatin capsule 3 hr prior to electrophysiological testing. Cat 1 received a capsule containing only sesame oil 3 hr prior to testing on all nondrug days to control for possible effects of vehicle and/or capsule administration.

All subjects continued to receive daily stimulation using the staircase method throughout the period of drug administration. As there was a slight tendency toward further reduction of threshold during this period, all analyses were conducted over a comparison of effects for the day immediately preceding drug administration against the effects obtained on the day of dosing.

After five replications of the trilevel dose series, all animals were killed by barbiturate overdose and perfused with formalin. The brains were removed and subjected to histological examination using 80-μm sections stained with thionin.

RESULTS

Effects of Repeated Hippocampal Stimulation. Repeated hippocampal stimulation resulted in a rapid initial reduction of afterdischarge threshold. After approximately 2 weeks of daily stimulation a relatively stable threshold was attained.

The experimental procedures were apparently not aversive to the cats as they did not resist placement in the experimental chamber and often slept during testing. At intensities at or above afterdischarge threshold, the cats initially showed pupillary dilatation; with continued stimulation the behavior of the subjects during the afterdischarge became uniform and stereotyped. During initial stages of experimentation, they failed to demonstrate any motor responses during periods of afterdischarge but were often unresponsive to sensory stimulation. In the ensuing weeks all subjects began to show blinking, salivation, and gagging during the afterdischarge followed by vocalizations and grooming upon its termination. At some point during the second to third week, gagging became interspersed with clonic head movements and, occasionally, contraversive circling. Between the fourth to seventh weeks all subjects displayed tonic–clonic "grand mal" seizures in response to stimulation. Grand mal responses occurred daily throughout the remainded of the experiment with the two females (cat 2 and cat 3) occasionally displaying spontaneous grand mal seizures while in their home cages during the final weeks of testing. Additionally, both of these cats exhibited striking episodes of transient personality change. On two separate occasions, both prior to the administration of drug or stimulation, these normally friendly and docile cats became vicious to the

TABLE 1

PROBABILITY OF AFTERDISCHARGES AFTER STIMULATION WITH AN INTENSITY JUST ABOVE THRESHOLD

	Dosage of Δ^9-THC[a]		
	Low	Medium	High
Predrug	0.92	0.87	0.85
Drug	0.63	0.75	0.70

[a] Dosages were given in five series of 0.25, 0.5, and 1.0 mg/kg for cats 1 and 3. For cat 2 the dosages during the 4th and 5th series were increased to 0.5, 1.0, and 1.5 mg/kg.

point of resisting even the simplest handling. This behavior regressed after a few hours and was not again observed.

Effects of Δ^9-THC. After the administration of 0.5–1.5 mg/kg doses of Δ^9-THC, all animals typically showed some ataxia, vocalization, and mild sedation. In exception to this syndrome cat 1, after a 0.5 mg/kg dose during the second series, became markedly hyperactive. This episode lasted for a period of several hours during which the animal appeared to be highly excited. At no time throughout this period could afterdischarges be evoked within the normal range of stimulating intensities. The reaction was not seen again and did not appear in the other subjects.

The effects of Δ^9-THC on afterdischarge threshold are presented in Table 1 which compares the probability of obtaining an afterdischarge for predrug and drug days for the stimulus intensity just above threshold. There was a tendency for a reduction of the probability of obtaining an afterdischarge on drug days and this change was statistically reliable (sign test using normal approximation to binomial: $z = 2.755$; $P < 0.01$).

An analysis of variance over the durations of afterdischarge demonstrated statistical significance only between levels of stimulus intensity ($F(2,4) = 7.46$; $P < 0.05$). This difference reflects an expected increase in duration of the afterdischarge with an increase in the strength of the eliciting stimulus. These data are presented in Table 2 showing the durations of afterdischarge on predrug and drug day as a function of stimulus intensity and drug level. The mean durations evoked by weak stimuli decreased under the drug condition whereas those evoked by strong stimuli increased from predrug to drug day. While suggestive of a drug by intensity interaction, these results were not statistically reliable ($F(2,4) = 5.92$; $P > 0.05$). However, an *ad hoc* specific comparison indicated that for the high intensity stimulation a statistically significant increase in duration was obtained under high dose levels ($t(14) = 1.762$; $P < 0.05$).

More importantly, Table 2 shows a marked change in the variance of

TABLE 2

MEAN AND VARIANCE OF AFTERDISCHARGE DURATION IN SECONDS

Stimulus intensity[a]		Dosage of Δ^9-THC in Mg/Kg					
		Low		Med.		High	
		Pre	Drug	Pre	Drug	Pre	Drug
Low	$\bar{X}$ =	3.9	2.7	7.5	2.7	2.0	1.7
	S^2 =	25.5	10.8	99.0	11.2	5.4	7.6
Med.	$\bar{X}$ =	14.2	17.8	14.5	12.4	13.7	7.3
	S^2 =	118.8	424.4	185.0	176.9	141.6	66.1
High	$\bar{X}$ =	27.4	29.5	31.1	41.5	23.1	31.0
	S^2 =	432.6	590.5	492.8	1075.8	424.4	615.0

[a] The three above-threshold intensities for each cat are presented in milliamperes.

	Low	Med.	High
Cat 1	0.2	0.3	0.4
Cat 2	0.8	1.0	1.2
Cat 3	0.3	0.4	0.5

the afterdischarge durations under Δ^9-THC. The pattern of the variance change is similar to the change in mean durations but is of considerably greater magnitude. Pooling the data across stimulus intensity and drug dosage and comparing the change in variance of afterdischarge from the predrug to drug day by a test of the equality and symmetry of covariance matrices (20), indicated a statistically significant ($\chi^2(1) = 8.70$, $P < 0.005$) increase in variance under Δ^9-THC. It is apparent from inspection of Table 2 that increased variance was obtained consistently at high levels of stimulation across all dose levels.

Figure 1 depicts the distribution of all afterdischarge durations for cat 1. This figure illustrates the increase in the frequency of both very short and long afterdischarges under Δ^9-THC. This effect was clearly seen in two of the three cats. Cat 2 did not show an increase in the frequency of long duration afterdischarges but did show the increase in frequency of short duration afterdischarges.

Histological analysis indicated that for cats 1 and 3 the stimulating electrodes were in the dorsal hippocampus at approximately A 5.0, and for cat 2 the stimulating electrode was immediately above the hippocampus. Since the behavioral and electrophysiological responses to stimulation were similar in all cats, and similar to that described previously for hippocampal afterdischarges, it is most likely that the afterdischarges of the present

study were all of hippocampal origin. Other electrode placements were accurate.

DISCUSSION

The initial expectation of an anticonvulsant action of Δ^9-THC was only partially supported by the results of the present experiment. An increase in threshold for hippocampal evoked afterdischarge was indicated by the reduction in the probability of obtaining an afterdischarge to just-above threshold stimulation under Δ^9-THC. However, the drug produced an increase in the frequency of long-duration afterdischarges. These combined mechanisms resulted in an overall significant increase in the variance of the durations of the afterdischarges under Δ^9-THC.

Thus, in cats under Δ^9-THC, hippocampal evoked seizures are not only more difficult to initiate but, once initiated, are of longer duration. This interpretation of the data as a combination of two mechanisms of action for Δ^9-THC predicts complex effects on seizure discharges depending on the strength of eliciting conditions. With near-threshold stimuli, a reduction of seizure likelihood under Δ^9-THC obtains through an elevation of threshold. Conversely, with stimuli well above threshold an exacerbation of seizures follows from the increased probability of obtaining seizures of inordinately long durations.

Interestingly, Ishakawa *et al.* (8) have reported both increases and decreases in hippocampal afterdischarges after the administration of scopolamine. Similarities between the effects of scopolamine and the findings of the present study support recent suggestions proposing anticholinergic-like properties for Δ^9-THC (3, 7). This interpretation is consistent with the dense cholinergic innervation of the hippocampus (12) and proposals relating epileptiform activity to a cholinergic mechanism (9). The administration of the anticholinergic drug, atropine, has been reported to specifically activate temporal lobe foci in human epileptics (17).

While the data of the present study only partially agree with the animal research on the effects of THC on seizure discharges, disparities may be attributed to species or procedural differences. Prior experiments reporting reduction of the duration of electroconvulsive seizures in rodents (5, 13) measured the duration of tonic hindleg extension. This peripheral measure may be differentially affected as both diencephalic and spinal motor pathways are reportedly depressed by a tetrahydrocannabinal derivative, DMPH (1). More in accord with the present results are the recent electrophysiological data from primates which suggest that tetrahydrocannabinols markedly affect response variance. These data indicate that corticocortical evoked responses in the cerveau isolé squirrel monkey undergo a dramatic increase in variability under both Δ^8-THC and Δ^9-THC (see Figs. 1 and 2 in Ref. 2).

Reports of potentiation of seizures by THC are not without precedent. Lowe and Goodman (13) reported a synergistic action for a THC homolog with metrazol convulsions in rats. Davis and Ramsey (4), while reporting anticonvulsant effects obtained from one of two isomers of THC, observed an exacerbation of seizures in one patient upon transfer to a second homolog. Keeler and Reifler (10), in an anecdotal account, describe a resumption of grand mal seizures in a 20-year-old patient after marijuana use. And in recent work similar to the present study, Segal and Barak (19) described a facilitation of the propagation of electrically evoked cortical afterdischarge in the cat by Δ^1-THC and a Δ^6-THC potentiation of ouabain-induced seizure activity in the hippocampus of the rabbit. Finally, the appearance of epileptiform EEG discharges after administration of Δ^9-THC discharge has been reported in primates (2, 14).

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