Pharmacology Biochemistry and Behavior, Vol. 1, pp. 371–378. ANKHO International Inc., 1973. Printed in the U.S.A.

Inhalation of Cannabis Smoke in Rats[1]

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(Received 12 April 1973)

FRIED, P. A. AND G. W. NIEMAN. *Inhalation of cannabis smoke in rats.* PHARMAC. BIOCHEM. BEHAV. 1(4) 371–378, 1973.–A new inhalation apparatus for rats is described for administrating Cannabis smoke in a standardized, controlled fashion. Electroencephalographic data indicated that, 15 min after smoke administration, cortical and hippocampal electrical activity was similar to that seen 25 min after interperitoneal injections of Δ^9-tetrahydrocannabinol (THC). A comparison of 30 mg/kg THC given orally, 4 mg/kg THC injected interperitoneally, and an estimated 17.5 mg/kg THC inhaled in Cannabis smoke indicated that all three modes of drug administration resulted in a significant reduction of open-field activity with the injection route being the most effective.

Marihuana (Cannabis sativa) Δ^9-tetrahydrocannabinol Inhalation Modes of administration

MOST experiments on the behavioral, pharmacological, and neurological effects of marihuana and its extracts in animals have relied on the intraperitoneal (IP) route of administration of Δ^9-trans-tetrahydrocannabinol (THC) an active constituent of Cannabis [11]. Despite the popularity of the IP route, evidence has been accumulating that this mode of administration may not be the best. For example, Manning *et al.* [9] reported that rats receiving 30 IP injections of THC developed peritonitis and both Ho *et al.* [3] and Shannon and Fried [14] found that very little of tritiated THC, administered IP, reached brain tissue.

As marihuana and its extracts are consumed in man primarily by smoking, a desirable method to use in experimental situations would be inhalation [3, 6, 10]. Two studies by Ho and his co-workers [2,4] have examined the distribution and some pharmacological effects of inhaled THC in rats. In both these studies, the animals were exposed to the smoke by being placed on a desiccator plate in a large vacuum desiccator for a specified length of time with the ignited cigarette situated below the plate. Associated with this procedure are a number of problems which include the difficulty of quantifying and standardizing the amount of smoke inhaled and the fact that the entire animal is exposed to smoke. Pilot work in our laboratory has indicated that rats which were fully exposed to smoke in a small chamber manifested many signs of anxiety including defecation and urination and, later, neglected their grooming behavior.

The purpose of the present series of studies is to establish a procedure which permits the administration of marihuana smoke to the nostrils and mouth of rats with a considerable degree of standarization between animals without creating a highly stressful situation.

EXPERIMENT 1

The first experiment was designed to examine the efficacy of a new type of inhalation chamber in terms of the stress on the rat and a comparison of the effect of smoke from Cannabis sativa and Cannabis placebo on the animals when tested in an open field. This task was chosen as previous work [13] has shown that the open-field measurement is sensitive to the effects of IP injections of THC.

Method

Animals. Ten male rats of the Wistar strain averaging 200 g in weight at the beginning of the experiment were used. Upon their arrival in the laboratory the rats were placed upon a 20 g/day food regimen and housed individually with water available ad lib.

Apparatus. The inhalation chamber and the separate cigarette burner were constructed of clear Plexiglas material with an E & M (E & M Instrumentation Co. Inc., Houston, Texas) respirator providing the pressure and rate for generating Cannabis smoke and fresh air. All the connections between the respirator, cigarette burner, and inhalation chamber were made with polyethylene tubing. A schematic diagram of the apparatus is given in Fig. 1.

The cigarette burner consisted of a cylindrical chamber, 23 cm long x 8 cm dia., with an opening at one end in which a cigarette could be tightly inserted and an opening

[1] Based on part of an honours thesis for a B.A. in Psychology submitted by the second author to Carleton University. Supported by Medical Research Council Grant-MRC-DA-13 and Department of National Health and Welfare Grant 1215-5-54. All drugs were generously supplied by the Canadian Food and Drug Directorate. We thank the Carleton University Science Workshop for their assistance.

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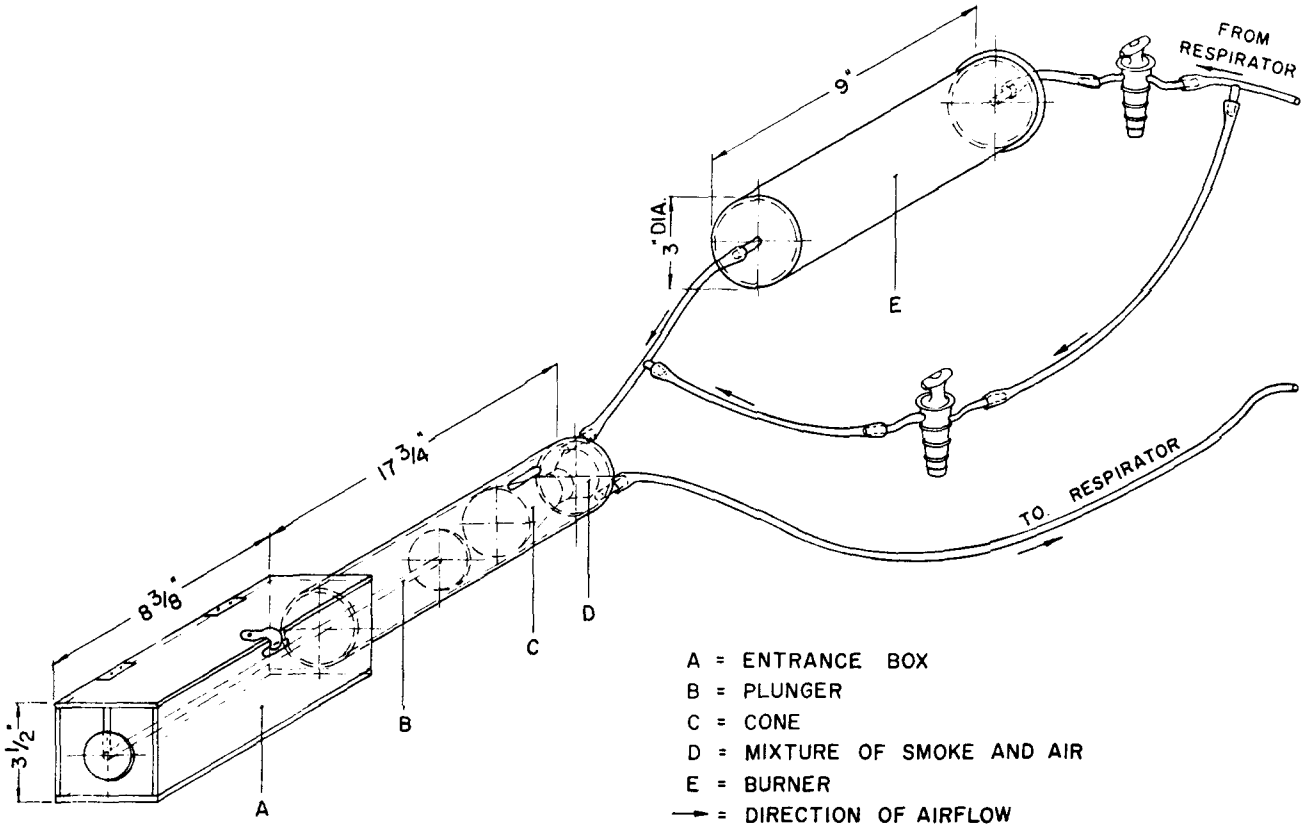

FIG. 1. Illustration of the inhalation apparatus.

at the other which was connected to the inhalation chamber. A polyethylene tube connected the inspiration terminal of the respirator to the cigarette.

The inhalation chamber, Fig. 1, consisted of three parts: a rectangular box, a long cylinder and a cone lying within one end of the cylinder. The box, an 8 3/8 in. x 3 1/2 in. rectangle, had a hinged lid which could be opened in order to place an animal in the apparatus. The cylinder, 17 3/4 in. long x 3 in. dia., was attached to one end of the box and served as a tunnel for the rat. In the last 11 cm of the cylinder a conical shaped piece of Plexiglas (Fig.2) was placed into which the animal could enter. A small hole (1.5 cm) was left at the tip of the cone into which the snout and mouth of the rat could be placed. This opening was lined with foam rubber, providing a nose cushion for the animal. The cone then widened again (much like an egg-timer on its side) to the full diameter of the cylinder, creating a small chamber into which the nose and mouth of the animal protruded. This chamber was tightly sealed with a removable cap, through which passed two hollow, metal tubes which could be connected to the inspiration and expiration outlets of the respirator. Into this small chamber, by the use of stop-corks, a mixture of smoke and fresh air (under equal pressure) could be pumped (Fig. 1) at a desired rate and volume. Extending the length of the inhalation apparatus was a movable plunger which served to hold the rat in place, once the nose and mouth were in position. A small aperature was cut in the cylinder, at the cone end, to allow electrode caps to protrude. Animals were removed from the inhalation apparatus by removing the smoke cone at the front of the cylinder.

The open-field apparatus was a 40 in. white square bounded by 14 in. walls containing 25 8 in. painted squares.

Drugs. Cannabis sativa, provided by the Candian Food and Drug Directorate, assayed for a 1.35% Δ^9-THC content was used in the experimental conditions whereas Cannabis placebo was used in the control conditions. The placebo had all cannabinoids removed and then was rehydrated to the same consistency as the experimental drug. Both forms of Cannabis were used to make filtered tip cigarettes containing 0.7 g of the plant which were inserted into the cigarette holder in the burner, described previously.

Procedure. After pilot work which took into consideration such factors as the rate of burning of the cigarette, level of emotionality of the rat, and the general effectiveness of the procedure, the following parameters were chosen: (1) the rate of the respirator was set at 55/min; (2) the pressure was 20 cm of water; (3) the ratio of inspiration to expiration was 1:1 and; (4) a 50/50 mixture of air and smoke was delivered to the animal. The animal, once in position in the apparatus, was given forty inspiration–expiration cycles of the mixture of smoke and air followed by a 10 sec period, during which the plunger, which was restraining the animals, was withdrawn a few centimeters, thereby allowing the animal to change its position while still being exposed to the smoke. This regimen was repeated four times and took approximately four min. At the end of

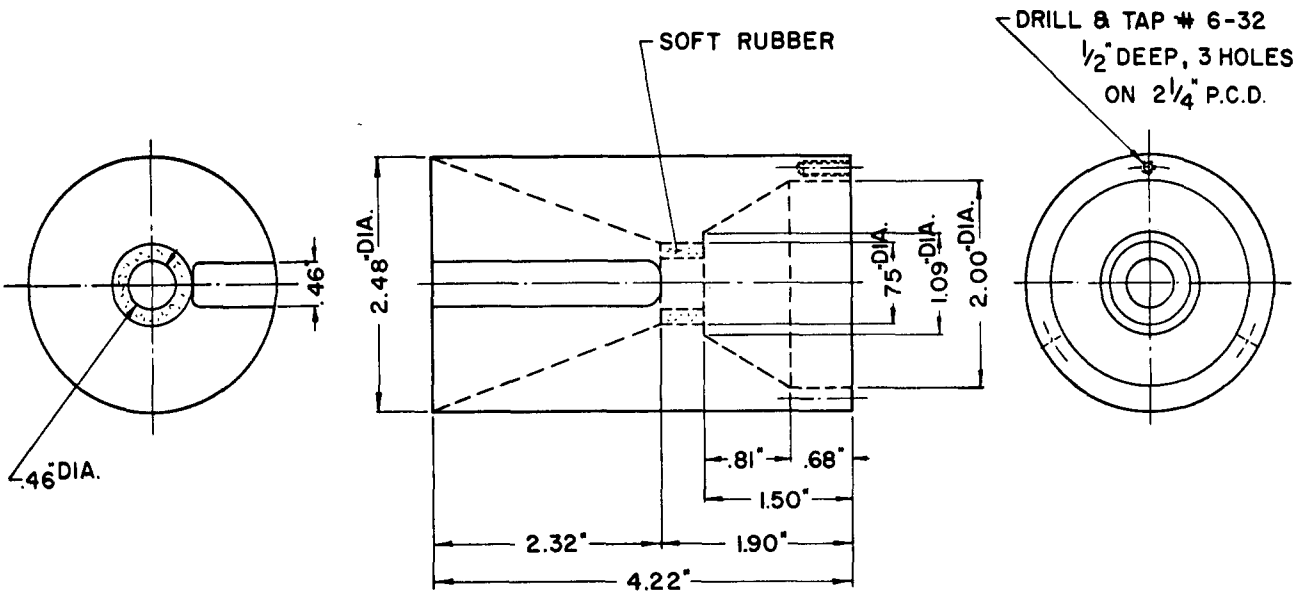

FIG. 2. Detail of the nose cone and smoke chamber.

this period, 75% of the cigarette was consumed.

The connection between the cigarette was such that the smoke did not pass through the filter portion of the cigarette but, rather was blown into the burner chamber and, from there, was drawn towards the rats' snout. As approximately 50% of the THC in the cigarette was actually delivered to the animal [15], the amount of THC each rat inhaled was approximately 3.5 mg (17.5 mg/kg). Although the quantification of the amount of THC received by an animal can only be approximated, there was a considerable degree of standardization between animals. Five rats received the Cannabis smoke containing THC and five animals received smoke from Cannabis placebo.

Fifteen min after being removed from the inhalation apparatus, each rat was placed in the centre of the open field and its activity observed for five min.

Results

There were no apparent differences between the experimental and control animals while in the inhalation apparatus. None of the 10 aminals vocalized while receiving the smoke and only one out of five of the experimental and two out of five of the control animals urinated or defecated.

An analysis of variance performed on the total number of squares crossed by animals administered smoke from Cannabis containing THC as compared to those animals that received smoke from the placebo revealed that the experimental animals were significantly less active ($F = 5.65$, $df = 1/8$, $p = 0.043$). There were no differences between the groups in terms of the number of boli during the open-field test.

EXPERIMENT 2

Several authors [1, 8, 14] have reported that, following IP injections of THC, a general decrease in cortical electroencephalographic (EEG) frequency occurs with periodic bursts of spike-and-wave complexes followed by a cycling between slow (delta and theta) and fast (beta) wave activity. An additional EEG effect following IP THC administration is a suppression of hippocampal theta activity [1,8]. The present experiment compared the effects of inhaled marihuana smoke and IP injections of THC on cortical and hippocampal EEG traces.

Animals. Twelve male Wistar rats averaging 200 g in weight at the beginning of the study were used. Housing and feeding was as described in Experiment 1.

Drugs. Cannabis sativa containing 1.35% THC and Cannabis placebo, as described in Experiment 1 were used in the inhalation portion of this study. For the IP injections, synthetic THC was obtained from the Canadian Food and Drug Directorate. It was dissolved in absolute ethanol (0.2 g/ml) and was assayed by the above Directorate to be 93% pure. The THC was mixed with propylene glycol to produce a concentration of 4 mg THC/ml propylene glycol.

Apparatus. The inhalation chamber was the same as described in the first experiment.

All EEG records were obtained using surgically-implanted twisted bipolar electrodes constructed from Diamel insulated nickel-chromium wire of 0.127 mm dia. soldered to male connectors. The wires were recoated with Epoxylite and cut to length with the uninsulated tips separated by the thickness of the insulation. For EEG data collection, a 10-channel Beckman type CE polygraph recorder was used complemented by a multivibrator circuit and differential amplifier [6], and a Sony model TC 560 tape recorder. A PDP 8 computer and plotter were used to display the cortical EEG frequencies [14].

While EEG records were being taken, rats were placed in a 48 x 48 cm wooden recording box.

Surgery and histological procedures. Operations were performed under Equi-Thesin anesthesia (3.2 cc/kg). The electrodes were positioned in the dorsal hippocampus and area 4 of the cortex and secured to the skull with five stainless steel screws, one of which served as a ground, and a covering of dental acrylic. The male connectors were crimped into an electrode cap [12] which was held in place

by a further covering of dental acrylic.

Following the completion of this study, the animals were sacrificed, perfused through the heart with normal saline and then with 10% Formal saline. The brains were removed from the skull, frozen, sectioned at 40 μ and stained with cresyl violet.

Procedures. Following a two week recovery period six animals were randomly assigned to the inhalation condition and the remaining six animals were assigned to the IP condition. In the inhalation condition, each rat received smoke from Cannabis containing THC and from the placebo with a 48 hr temporal interval between the two conditions. Three animals received the placebo first and three rats received the Cannabis containing THC first. The animals in the IP condition were treated in an analogous fashion; three were injected with 4 mg/kg of THC followed 48 hr later by an injection of propylene glycol while the remaining three had the order reversed. One animal in this group died during the course of the study and thus data from five animals are included in the IP condition.

A baseline recording of 60 min was obtained for each animal. Twenty-four hr later and seventy-two hr later, the animal was administered the appropriate drug under the appropriate conditions, then placed in the recording box,

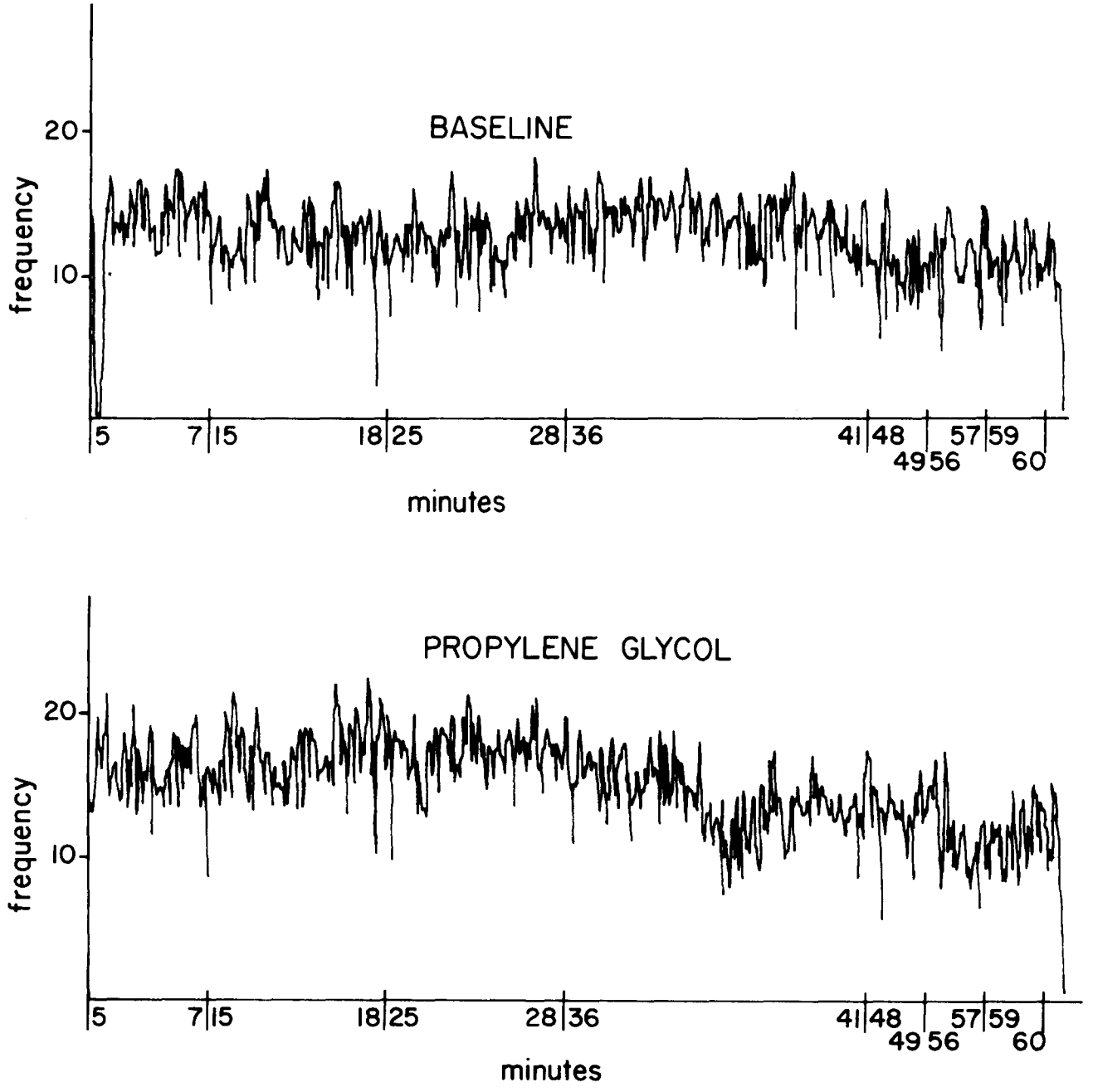

FIG. 3. Computer-produced histogram of cortical electroencephalographic activity during baseline (top) and propylene glycol conditions (bottom).

and a 60 min record taken.

Results

All cortical electodes were located in cortical area 4, and 10 of the 11 hippocampal electrodes were accurately placed in or near the apical dendrite layer of the dorsal hippocampal pyramid cells.

Cortical activity. In the baseline condition, the cortical records of all animals were quite similar. The dominant frequency for the first twenty min was in the Beta-one range (13–20 Hz) with most animals exhibiting between 90–100% of this fast activity. There was then a slight shift to lower frequencies and, towards the end of the 60 min half of the animals were exhibiting bursts of theta minutes activity (Fig. 3 top).

The cortical records of those animals that received propylene glycol differed slightly from those obtained during baseline. The initial waveform was of a slightly faster frequency than that seen during the baseline condition with the dominant waveform being between 16–20 Hz. This was followed, after approximately 36 min, by a marked shift to slow, high amplitude waveforms with four of the five animals exhibiting 30% alpha by the fortieth minute (Fig. 3 bottom).

Animals that inhaled Cannabis placebo smoke generally exhibited waveforms that were quite similar in later stages to those obtained from animals in the baseline condition. There was an initial predominance (95%) of fast frequency (20–30 Hz), low amplitude (10–20 μV) but, by the end of the recording session, all animals were exhibiting approximately 25% alpha activity.

The effects of IP injections of 4 mg/kg THC were quite consistent. Cortical traces were, initially like those obtained after propylene glycol injections. After approximately 20 min, however, in four of the five records, bursts of spike-and-wave complexes were interspersed between 70% delta and theta and 20% alpha frequencies (Fig. 4 top). In these records, a sudden shift from the slow wave activity to the fast beta-one occurred by the thirtieth min. Following this beta-shift the EEG returned slowly back to slow-wave activity, taking about 6 min to do so. These results are strikingly similar to those reported previously [14].

The effects of inhalation of active Cannabis smoke were not as pronounced as the effects of IP injections of THC although the trends were in the same direction. In four out of the six animals, a biphasic effect, similar although not as marked as that seen following injections, was noted. The records of the remaining two animals did not differ from the placebo condition. Of the four aniamls that did show consistent effects, the EEG characteristics were as follows: during the first 20 min there was a gradual shift from beta activity to alpha and then to theta. By the twentieth min, approximately 85% of the record was composed of frequencies between 6–10 Hz with a considerable amount of spindling interspersed between the slow wave activity. Between the 25th and 40th min, a shift to beta frequencies occurred followed by a gradual return to theta (Fig. 4 bottom).

Hippocampal activity. During the 60 min baseline condition, hippocampal activity was almost entirely a mixture of large-amplitude (150 μV), irregular activity (LIA) and rhythmical slow activity (RSA) or theta rhythm. The RSA was especially predominant at the beginning of the phase and LIA during the latter 30 min. With one exception, animals that inhaled Cannabis placebo smoke exhibited hippocampal electrical activity that was very similar to that seen during the baseline condition. The atypical animal demonstrated a marked suppression of theta rhythm. The injections of propylene glycol had little effect on the hippocampal electrical activity.

Following injections of 4 mg/kg THC or inhalation of Cannabis smoke containing THC, the electrical activity of the dorsal hippocampus did not differ from the control conditions until approximately ten to fifteen minutes after the drug administration. At this time, an almost total suppression of RSA was apparent with the EEG activity consisting of low amplitude (20 μV), fast frequency waveforms. At approximately the thirty-five minute mark in the inhalation condition and the 45 min point in the IP condition some RSA was noted and by the last 10 min of the record, the EEG activity was quite similar to the control conditions as considerable LIA was observed.

Combining the cortical and hippocampal data it is apparent that the maximum drug effect when administered by inhalation was after 15 min and when administered by IP injections was after 25 min. There was no differential effect of whether an animal received the active or placebo (vehicle) drug first.

EXPERIMENT 3

The results of the first two studies suggest that the method of inhalation of Cannabis smoke described, may be a useful experimental procedure. In order to examine this hypothesis in greater detail, a comparison of the effects of three different methods of administering THC of Cannabis on open-field behavior was undertaken.

Method

Animals. Thirty, naive male Wistar rats, averaging 200 g were used. They were housed and fed as in the previous two studies.

Apparatus. The inhalation apparatus and the open field were the same as those described in the first study.

Drugs. Cannabis sativa containing 1.35% THC and Cannabis placebo, as described in Experiment 1 were used in the inhalation portion of this study, and a mixture of propylene glycol and THC or just propylene glycol, as in Experiment 2, was used in the IP condition of this experiment. A mixture of THC and sesame seed oil was prepared (7.50 mg THC/cc sesame oil) for oral administration.

Procedure. The thirty animals were randomly assigned to one of six treatments; Cannabis with THC inhalation, Cannabis placebo inhalation, IP injections of 4 mg/kg THC, IP injection of a volume of propylene glycol needed to serve as a vehicle for 4 mg THC/kg, stomach loading of 30 mg/kg THC mixed in sesame oil, and stomach loading of the volume of oil needed to serve as a vehicle for 30 mg/kg.

The rats in the two IP conditions were tested 25 min after administration, the rats in the inhalation groups were tested 15 min after administration and the rats in the oral groups were tested 3 hr after receiving the appropriate treatment. These times were determined on the basis of the results of the second study and on unpublished pilot work. The procedures and the dependent measures used in the open field task were the same as described in Experiment 1.

Results

Those animals that had received THC or Cannabis

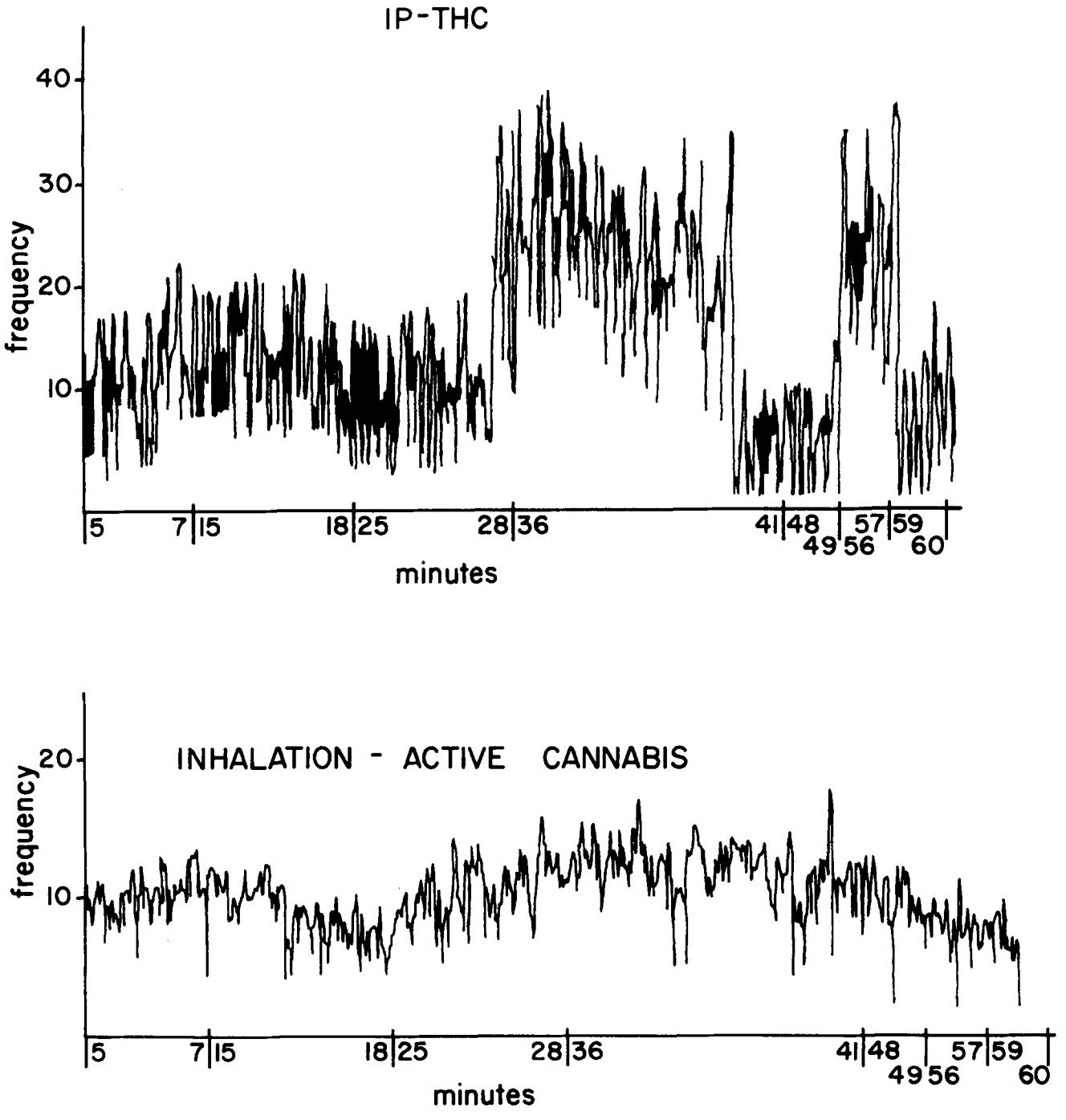

FIG. 4. Computer-produced histogram of cortical electroencephalographic activity following an injection of 4 mg/kg Δ^9-THC (top) and inhalation of Cannabis smoke containing Δ^9-THC (bottom).

containing THC crossed significantly fewer squares than those animals in the control conditions ($F = 32.83$, $df = 1/24$, $p<0.001$) and there was a differential drug effect as a function of which method of administration was utilized ($F = 3.27$, $df = 2/24$, $p<0.05$). A simple main effects analysis indicated that the greatest difference between experimental and control conditions occurred under the IP treatment ($F = 29.53$, df 1/24, $p<0.001$), followed by the oral administration ($F = 8.98$, df 1/24, $p<0.01$), and then inhalation ($F = 4.49$, df 1/24, $p<0.05$). A Newman-Keul's Test was used to examine the experimental and control groups in detail. Although none of the three experimental groups differed from one another, the propylene glycol, IP control group crossed significantly more squares than animals in the other two control treatments ($p<0.05$). These results are graphically displayed in Fig. 5.

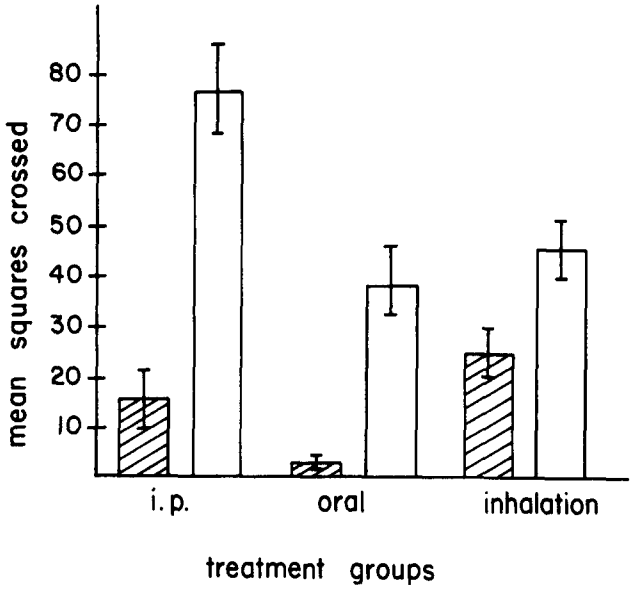

FIG. 5. Mean number and standard error of squares traversed within 5 min by each group. The open rectangles are the control group means in each condition and the hatched rectangles are the experimental means in each condition.

Following the five minute test period, when the rats were lightly stroked and touched, those animals in the three experimental conditions exhibited considerable vocalization. When the animals were picked up, the rats in the IP and oral experimental conditions appeared hypersensitive. There were no differences between any of the six groups in terms of boli eliminated during testing.

DISCUSSION

The principal findings of the present series of experiments are that, utilizing the apparatus described, Cannabis smoke can be administered safely to rats in a relatively standard and controlled manner and that the effects, at least in terms of EEG and open field activity, are comparable to the effects of THC administered by either the IP or oral route.

In the first study, the inhalation apparatus was found to be a reliable and safe method of administering Cannabis smoke at a designated rate and volume. The degree of standardization can be partially assessed by the similarity in the behavior of the animals in the experimental group when tested in the open field (S.D. = 7.96) as compared to the control group (S.D. = 22.89). The general behavior of the animals during the administration of smoke did not give any indication of undue stress as there was no vocalization, little defecation, and the animals in both the control and experimental treatments were easy to handle after the inhalation.

The EEG data of the second study is, substantially, the same as that reported elsewhere [1, 8, 14]. Following an IP administration of 4 mg/kg THC suspended in propylene glycol, the cortical EEG cycled between beta and slow wave (delta and theta) with bursts of spike and wave complexes. This polymorphic effect was also seen, but to a lesser extent, following the inhalation of active Cannabis smoke. A similar parallelism between IP injections and inhalation was the suppression of hippocampal RSA observed in the two experimental groups.

Although the cortical EEG was similar in general trends under the two experimental treatments, the shift to slow wave activity was not as pronounced in the inhalation condition with virtually no delta activity occurring. There is evidence [1,8] that the cortical EEG effects may be dose dependent with delta waveforms occurring at higher drug levels. Although this dose-dependency data is derived from IP injections it would appear likely that the paucity of six cycles/sec or less in the experimental inhalation condition may be attributable to the dose level. This interpretation is supported by the open-field results in the third study in which, of the three experimental groups, the inhalation animals were the least effected. The time point of testing following the inhalation procedure may also have contributed to the lesser effect and needs further investigation.

The results of the second and third study also suggest that propylene glycol may act as somewhat of a stimulant as a considerable amount of beta activity was observed in the EEG of those animals receiving the vehicle and as the IP control group was significantly more active on the open field when compared to the other control groups.

The reduced activity of all three experimental groups in the open field is similar to that found following IP injections reported elsewhere [13] and the times of the maximum drug effects under the three modes of administration is consistent with that reported in studies with humans [7] with the delay of onset of the oral administration presumably due to the time required for the THC to be absorbed through the gastrointestinal tract. The relatively high dose needed to produce an effect when the drug was administered orally is also consistent with the human literature [5] as subjects judged an oral dose of THC that was twice as great as one administered by inhalation as being subjectively equal.

The combined results of the three experiments reported above, demonstrate that inhalation can be a useful and controllable experimental procedure for the investigation of Cannabis effects in the animals model.

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