Pharmacology Biochemistry & Behavior, Vol. 9, pp. 753–758. Printed in the U.S.A.

Tolerance to Δ^9-Tetrahydrocannabinol in Adapted and Nonadapted Rabbits

PARTHENA MARTIN AND PAUL CONSROE

Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721

(Received 26 June 1978)

MARTIN, P. AND P. CONSROE. *Tolerance to Δ^9-tetrahydrocannabinol in adapted and nonadapted rabbits.* PHARMAC. BIOCHEM. BEHAV. 9(6) 753–758, 1978.—Two groups of New Zealand white rabbits, one which had been adapted to the testing chamber and one which had not been adapted to the testing chamber, were given Δ^9-tetrahydrocannabinol (Δ^9-THC; 0.5 mg/kg, IV) daily for 12 days. During vehicle control and on the first and last day of Δ^9-THC administration, electroencephalograms (EEG's) were recorded from the motor cortex and hippocampus, while standing, sprawling and behavioral activity were recorded concurrently. The results showed that tolerance to the behavioral and EEG effects of Δ^9-THC occurs in rabbits and that acute and chronic effects produced by Δ^9-THC are influenced by environmental factors.

Δ^9-Tetrahydrocannabinol   Tolerance   Rabbits   Behavior   EEG   Adaptation

IT IS WELL established that tolerance to various effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) develops in a wide range of species. Tolerance to impairment of avoidance and operant responses on various reinforcement schedules has been demonstrated in rats, pigeons, squirrel monkeys, mice, rhesus monkeys and pigs, while tolerance to hypothermia and other pharmacological effects such as ataxia and hyperexcitability has been shown in rats, chickens, dogs, squirrel monkeys, rhesus monkeys and frogs (for reviews, see [6,10]). Tolerance to some electroencephalographic (EEG) effects, but not others, have been found in rats. For example, rats became tolerant to low voltage cortical activity following administration of marijuana extract, but not to spindling activity which also follows administration of marijuana [11,12], while tolerance to cortical EEG synchronization following repeated administration of crude marijuana extract to rhesus monkeys is also reported [13]. However, 3 mg/kg of Δ^9-THC administered daily for 6 days to a rabbit produced no appreciable attenuation of the EEG or behavioral response to the drug [5]. Since tolerance to some effects of Δ^9-THC have been shown in many species, the purpose of the present study was to examine in more detail the behavioral and EEG changes following Δ^9-THC administration in rabbits over a period of 12 days. Since familiarity with the testing conditions has also been shown to be a factor in the animal's response to Δ^9-THC [1], tolerance to Δ^9-THC was studied in both rabbits who had previous exposure to the test conditions and rabbits who had not been previously exposed to the testing milieu.

METHOD

Animals

Animals were 7 New Zealand white rabbits ranging in weight from 2.3–3.0 kg. All animals were individually housed in a room maintained at constant temperature (25 ± 2°C) and under controlled lighting (12 hr light-dark). Animals were allowed access to food and water ad lib except during testing.

Electrode and Catheter Implants

Electrode implantation was stereotaxically performed in the motor cortex and right dorsal hippocampus under chlorpromazine pretreatment (25 mg/kg, intramuscular) and pentobarbital anesthesia (10 mg/kg intravenous). All electrodes were connected to an Amphenol connector anchored to the cranium with stainless steel screws and dental acrylic cement. At the same time, a catheter was implanted in the right external jugular vein so that drugs could be infused from outside the observation chamber without disturbing the ongoing behavior of the rabbit. All animals were allowed 7–10 days to recover from surgery.

Drugs

The Δ^9-THC was prepared for intravenous administration by incorporation into a 10% polysorbate (Tween) 81-saline solution.

Apparatus

Freely moving animals were tested in a sound resistant test chamber measuring 82 cm square by 70 cm high. A one-way vision window permitted continuous observation of the animal's behavior. Shielded EEG cable connected the electrodes from the rabbits to a Grass Model 7B polygraph via a mercury cable coupler mounted on the test chamber. An EEG frequency analysis system (Med Associates, Inc.) consisting of an artifact detection circuit, 4 bandpass filters with corresponding amplitude integrator and digital counters and displays was connected to the EEG output of the polygraph. EEG signals were first rectified, then high voltage (>1,000 μv) artifacts (caused by the animal's movement) were excluded and the EEG signal was then separated into delta (0.5–4.0 Hz), theta (4.0–8.0 Hz), alpha (8.0–13.0 Hz) and beta (>13.0 Hz) frequency bands; the area (voltage output) under successive EEG waves of each band was integrated and the digital cumulative summation, i.e., the "energy spectrogram," was visually displayed.

In addition, the electrical activity occurring between the left and right motor cortical leads was integrated by means of the Grass Model 7P10B integrator system. In order to present relative changes in cortical voltage output, the mean frequency of integrator resets in each drug condition was plotted. Each integrator reset represents 70 μv seconds. In addition, hippocampal EEG tracings were visually assessed for frequency and voltage patterns.

A manually operated 10 channel digital event recorder was used to measure the corresponding behavior of the rabbits concurrently with the EEG.

Dependent Variables Measured

EEG. The bipolar electrical activity between the left and right motor cortex and from the dorsal hippocampus was assessed qualitatively; additionally, the energy spectrograms, calculated as percent delta, theta, alpha and beta EEG frequencies, and the mean frequency of integrator resets (cortical electrogenesis or voltage output) were quantitatively assessed.

Behavior. Frequency and duration of the following behaviors were measured:

1. Posture:
   a. Standing—weight of animal supported on the tarsals and front legs extended vertically; animal up on all 4 legs.
   b. Sprawling—weight of animal distributed along the ventral body surfaces, two or more legs extended laterally (splayed) and/or head touching floor chamber.
2. Activity:
   a. Grooming—licking or scratching directed toward the animal's body.
   b. Rearing—standing up with front paws off the floor of the chamber.
   c. Locomotion—movement of a distance equal to or greater than 1/2 the body length of the animal.
   d. Exploring—sniffing at the chamber or object in the chamber with extended head and vibrissae movements.

Procedure

Four rabbits were thoroughly adapted to the test chamber prior to experimentation, while 3 animals were not adapted to the chamber [1]. On the first day of the experiment, all rabbits were baseline tested for 20 min, then given intravenous (IV) injections of the vehicle (10% Tween 81-saline) and tested for 20 more minutes. During each 20 min period, the frequency and duration of posture and activity behaviors were recorded over 5 min intervals. Concurrently, EEG data were recorded over 5 min periods alternately from the motor cortex and the dorsal hippocampus. During each of the following 12 days, adapted rabbits were tested in the same way except that Δ^9-THC (0.5 mg/kg) was injected at the end of the 20 min baseline period. Nonadapted rabbits were injected in the same manner but were tested in the chamber only during baseline and on the first and last day of the Δ^9-THC injection.

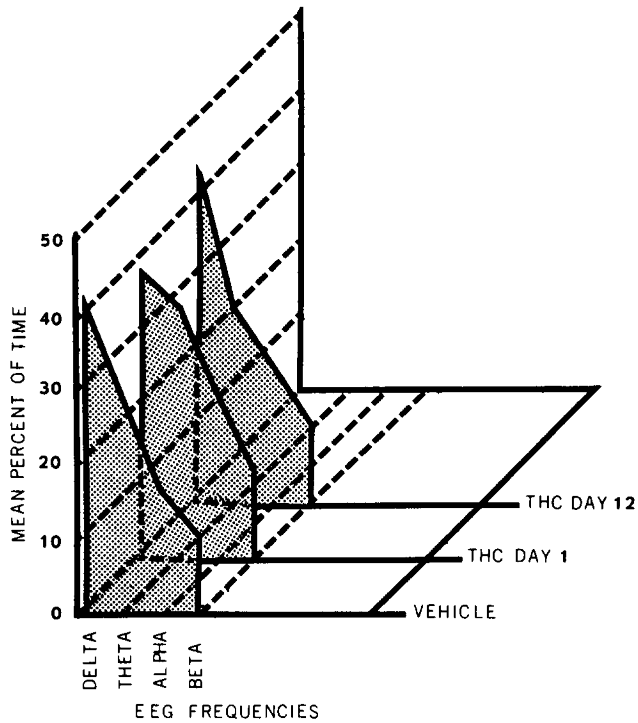

FIG. 1. Frequency spectrogram of the electrical activity in the motor cortex of rabbits adapted to the testing chamber. The EEG recorded during vehicle control and on the first and last day of administration of 0.5 mg/kg Δ^9-THC are shown. Frequency bands are shown on the abscissa and the mean percent of time occupied by the various frequencies is shown on the ordinant.

RESULTS

Results of each dependent variable were analyzed using a mixed design analysis of variance; Duncan's New Multiple Range Test (NMRT) was used for post hoc comparisons of individual groups.

EEG

Frequency spectrograms of the electrical activity in the motor cortex of adapted and nonadapted rabbits during vehicle, Day 1 and Day 12 of Δ^9-THC are shown in Figs. 1 and 2. An analysis of variance for delta, theta, alpha and beta frequencies from the motor cortex showed that overall, adapted rabbits exhibited less beta, $F(1,5)=36.63$; $p<0.005$, from the motor cortex than did nonadapted rabbits. Adapted rabbits also tended to evince more cortical electrogenesis ($0.05<p<0.10$) as indicated by number of integrator resets than did nonadapted rabbits (Fig. 3).

There were no significant differences between the frequency distributions of the cortical EEG for vehicle control and the first and last day of Δ^9-THC administration, thus showing no overall tolerance effect. However, there were significant interactions between adaptation and day of Δ^9-THC administration for delta, $F(2,10)=4.128$; $p<0.05$, and theta, $F(2,10)=4.95$; $p<0.05$, in the motor cortex. Duncan's NMRT revealed an increase in delta from the motor cortex of nonadapted rabbits ($p<0.05$) following the first administration of Δ^9-THC, but not in adapted rabbits. This increase in delta tended to decline with repeated administra-

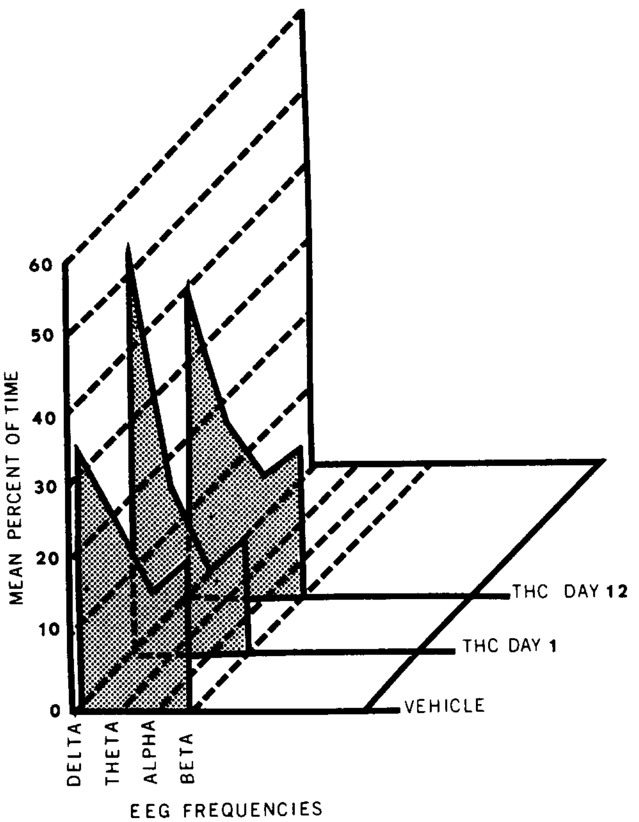

FIG. 2. Frequency spectrogram of the electrical activity in the motor cortex of rabbits not adapted to the testing chamber. EEG was recorded during vehicle control and on the first and last day of administration of 0.5 mg/kg Δ^9-THC. Frequency bands are shown on the abscissa and the mean percent of time occupied by the various frequencies is shown on the ordinate.

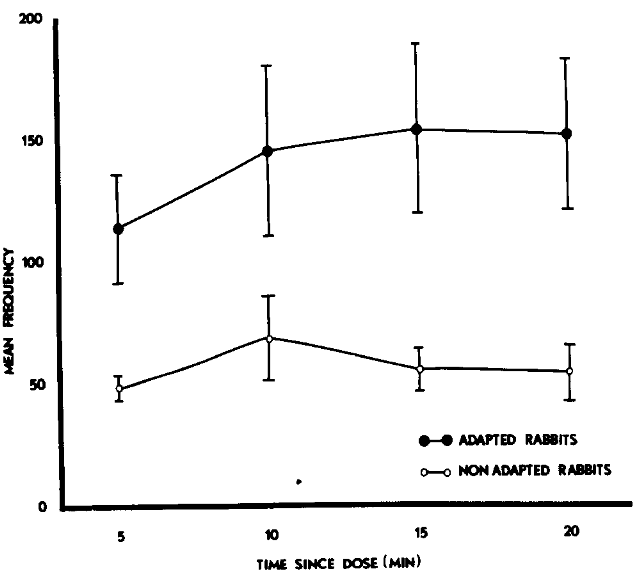

FIG. 3. Mean frequency of integrator resets (electrogenesis) from the left-right motor cortical leads in rabbits treated with 0.5 mg/kg Δ^9-THC. The upper line presents data from 4 animals adapted to the testing procedure and apparatus. The lower line presents data from 3 nonadapted rabbits.

tion of Δ^9-THC and returned almost to baseline by the last day of treatment ($0.05<p<0.10$). There was an increase in theta from the motor cortex of adapted rabbits following the first administration of Δ^9-THC ($p<0.05$) but not in the nonadapted rabbits and this increase persisted throughout the Δ^9-THC treatment. The analysis of variance did not reveal any significant changes in frequencies of the hippocampal EEG.

Examples of cortical and hippocampal EEG's from adapted and nonadapted rabbits under different drug conditions are shown in Figs. 4 and 5. Visual inspection of the EEG from the motor cortex of adapted rabbits showed that during vehicle control, the amplitude of the EEG increased over time and sleep spindles appeared in the recordings. Following the first administration of Δ^9-THC, there was an increase in the number of these high amplitude bursts which were primarily spindle shaped with frequencies of 10–14 Hz and amplitudes of 150–230 μV. Each burst was 1–3 sec in duration. Rabbits who were not adapted to the testing chamber did not exhibit these bursts of high voltage waves during vehicle control and only one burst of high amplitude activity occurred in one rabbit following the first injection of Δ^9-THC. There was, however, a small overall increase in the amplitude of the cortical EEG's of nonadapted rabbits following the first administration of Δ^9-THC. By the last day of treatment the high amplitude bursts of activity appeared only in one adapted rabbit but not in the other adapted or nonadapted rabbits. However, the amplitudes of the EEG's following the last administration of Δ^9-THC were overall higher and more irregular than vehicle control.

Although there were no significant effects of either adaptation or Δ^9-THC on the frequency distribution of the hippocampal EEG, the amplitude of the EEG became more irregular following administration of Δ^9-THC and increased from 50–100 μV to over 200 μV for both adapted and nonadapted rabbits. This increase in the variability of amplitude of waves continued through the last day of administration of Δ^9-THC. In general the hippocampal EEG from adapted rabbits was more irregular than that of nonadapted rabbits during vehicle control, which correlates with the findings that adapted rabbits are less active than nonadapted rabbits and spend more time sleeping as indicated by the cortical EEG.

Behavior

The effects of Δ^9-THC on standing, activity and sprawling in adapted and nonadapted rabbits are shown in Figs. 6, 7 and 8. Overall, adapted rabbits tended to be less active than nonadapted rabbits, $F(1,5)=6.523$; $0.05<p<0.10$. Both adapted and nonadapted rabbits spend less time standing following the first administration of Δ^9-THC, $F(2,10)=4.559$; $p<0.05$, but by the last day of Δ^9-THC treatment, standing had increased compared to the first day ($p<0.05$) and in fact rabbits tended to spend more time standing following the last injection of Δ^9-THC than during vehicle control ($0.05<p<0.10$).

The interaction between adaptation and day of administration revealed that nonadapted rabbits tended to be more active, $F(1,5)=6.523$; $0.05<p<0.10$, following the first injection of Δ^9-THC than either adapted rabbits ($p<0.05$) during the same period or nonadapted rabbits during vehicle control ($p<0.05$). This activity decreased slightly with repeated Δ^9-THC treatment but did not reach baseline level by the last day. In contrast, 3 of the 4 adapted rabbits sprawled follow-

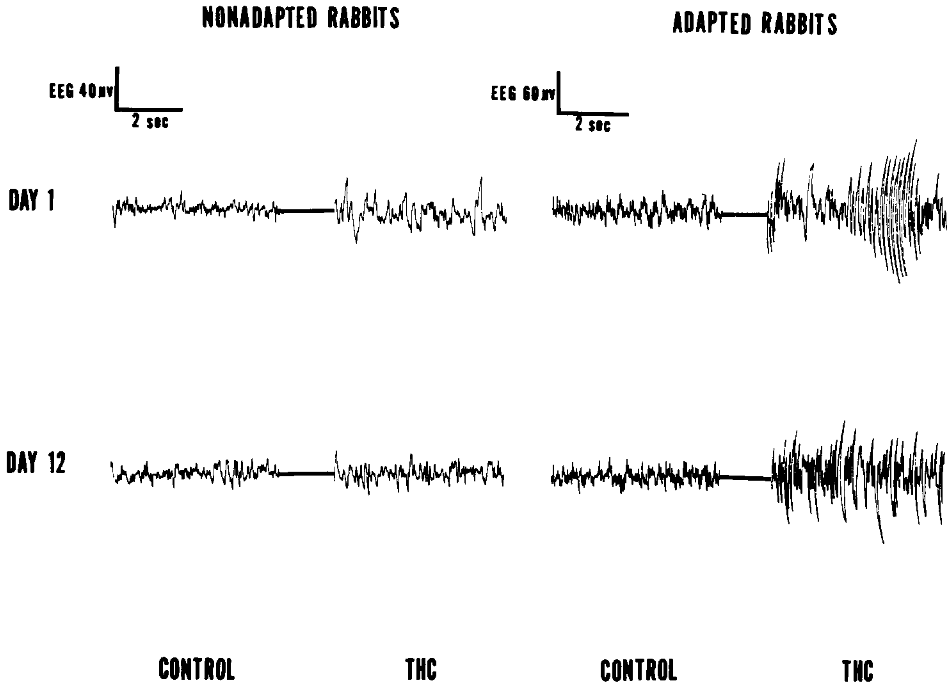

FIG. 4. Electroencephalograms from the motor cortex of adapted and nonadapted rabbits approximately 20 min after IV injection of 0.5 mg/kg Δ^9-THC. The upper trace is from the first day of administration while the lower trace is from the last day of administration.

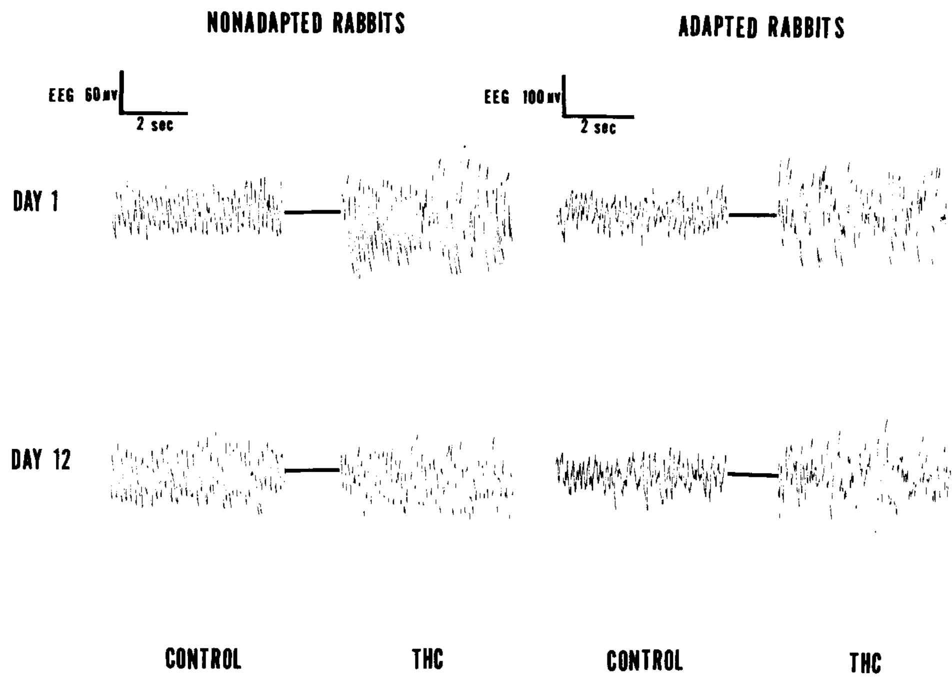

FIG. 5. Electroencephalograms from the hippocampus of adapted and nonadapted rabbits approximately 20 min after IV injection of 0.5 mg/kg Δ^9-THC. The upper trace is from the first day of administration while the lower trace is from the last day of administration.

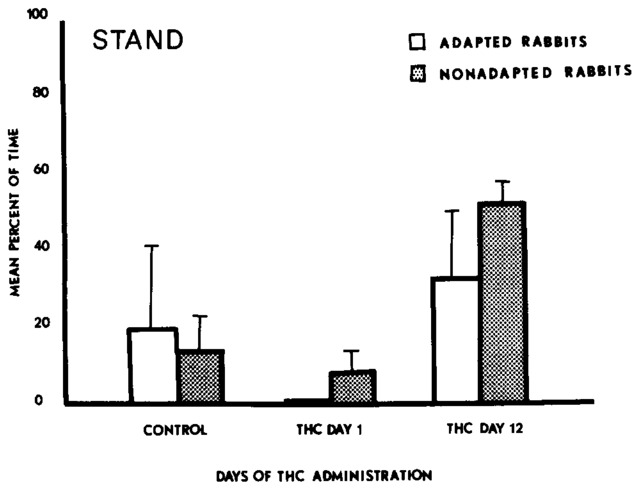

FIG. 6. Mean percent of time spent standing for adapted and nonadapted rabbits during a 20 min period following vehicle control and the first and last administration of 0.5 mg/kg Δ⁹-THC. Treatment groups are shown on the abscissa while mean percent of time is shown on the ordinate.

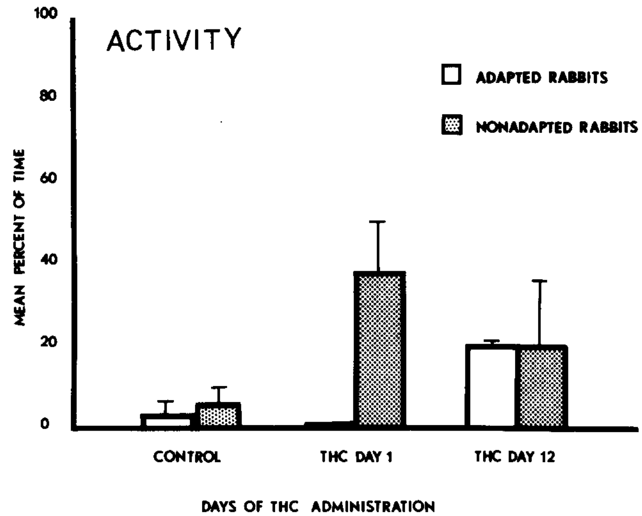

FIG. 7. Mean percent of behavioral activity for adapted and nonadapted rabbits during a 20 min period following vehicle control and the first and last administration of 0.5 mg/kg Δ⁹-THC. Treatment groups are shown on the abscissa while mean percent of time is shown on the ordinate.

ing Δ⁹-THC treatment. However, sprawling disappeared within the first 3 days of Δ⁹-THC treatment.

DISCUSSION

The major findings of this experiment were first, rabbits do become tolerant to Δ⁹-THC and, second, the response to Δ⁹-THC depends on the animal's familiarity with the testing environment.

Tolerance to Δ⁹-THC was shown most clearly in the behavior of the rabbits. Adapted rabbits sprawled following the first administration of Δ⁹-THC and both adapted and nonadapted rabbits showed a marked reduction in standing. These behavioral effects disappeared by the last day of administration of Δ⁹-THC and in fact, percent of time standing increased beyond control by the last day of administration of Δ⁹-THC. The EEG, although more subtle, also indicated tolerance to Δ⁹-THC. The increase in cortical delta observed in nonadapted rabbits returned almost to vehicle control levels by the last day of the experiment, although the increase in theta in adapted rabbits did not. However, the occurrence of cortical high voltage bursts of polyspikes observed in adapted rabbits during the first day of Δ⁹-THC also decreased in frequency with repeated administration, indicating tolerance. The finding that rabbits do become tolerant to the behavioral and electroencephalographic effects of Δ⁹-THC is consistent with the reported tolerance to the convulsant effect of Δ⁹-THC in a specially-bred population of New Zealand White rabbits [8], and with other reports of behavioral and/or electroencephalographic tolerance to Δ⁹-THC in other species including mice, rats, pigeons and monkeys. Although no tolerance to Δ⁹-THC was found in one rabbit following 6 days of daily treatment with Δ⁹-THC [5], neither the experimental conditions of testing (e.g., adaptation, etc.) nor the use of quantitative methods to interpret effects were reported. The assessment of quantified EEG and behavioral responses in a larger group of rabbits given Δ⁹-THC over a longer interval in the present study did yield signs of tolerance and, hence, these experimental factors may explain the discrepancy between the two studies.

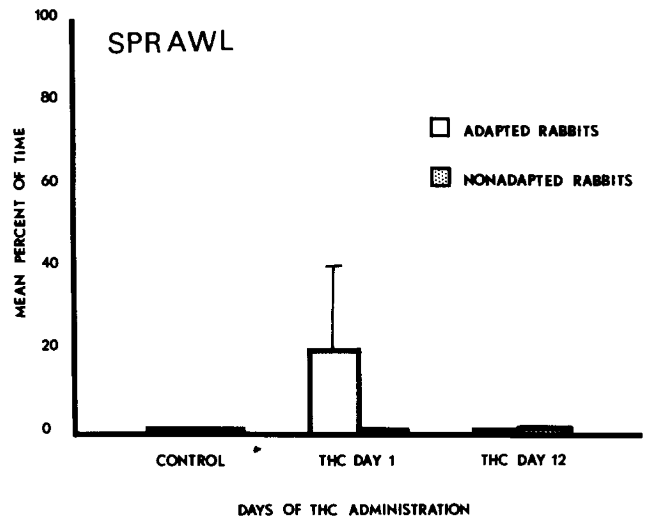

FIG. 8. Mean percent of time spent sprawling for adapted and nonadapted rabbits during a 20 min period following vehicle control and the first and last administration of 0.5 mg/kg Δ⁹-THC. Treatment groups are shown on the abscissa while mean percent of time is shown on the ordinate.

The finding that rabbits who are familiar with the testing environment respond different to both acute and chronic Δ⁹-THC than rabbits who are not familiar with the environment confirms and extends the finding that adapted rabbits showed behavioral depression and an increase in electrogenesis following acute Δ⁹-THC, while nonadapted rabbits showed EEG and behavioral stimulation followed by depression of both [1]. The increase in electrogenesis observed in adapted rabbits is probably due to the high amplitude of the cortical EEG following Δ⁹-THC since both adapted and nonadapted rabbits evinced a shift toward slow voltage waves following Δ⁹-THC treatment.

The question of whether the bursts of high voltage activity following administration of Δ^9-THC in adapted rabbits are representative of the polyspike activity described by Lipparini *et al.* [5] and others, or are more similar to sleep spindles should be considered. Sleep spindles are usually described as having frequencies of 6–14 Hz with amplitudes of 100–500 μV [3, 4, 7] while Δ^9-THC produced polyspikes have been described as bursts of cortical activity with frequencies ranging from 6–14 Hz and amplitudes up to 600 μV [1, 9, 13]. Additionally, one study [14] in rats has reported that surgical implantation of cortical and subcortical electrodes caused polyspikes which were exacerbated by subsequent Δ^9-THC. Since the frequency and amplitude of these two events are so similar it is possible that what are frequently reported as polyspikes are really sleep spindles. This is especially true in studies such as the present one where these high voltage bursts of activity were observed primarily in rabbits who were adapted to the observation chamber and who were behaviorally inactive, but not in nonadapted rabbits who were behaviorally alert and active. It is possible that animals become drowsy in the familiar environment and the depressant effect of Δ^9-THC is added to this.

There is some evidence that Δ^9-THC does indeed produce spikes which are distinct from sleep spindles, since cortical spiking activity against a background of activated EEG has been reported [2,5]. However, a clear distinction should be made between cortical spikes elicited by THC and an increase in high voltage activity representative of a behaviorally depressed, sleepy state. To do this, the behavior of the animal must be taken into account when interpreting the EEG.

ACKNOWLEDGEMENTS

This research was supported by Grant No. DA 01448 from the National Institute of Drug Abuse (NIDA) of the Alcohol, Drug Abuse and Mental Health Administration. The authors thank NIDA for the generous supply of cannabinoids.

REFERENCES

1. Consroe, P., B. Jones and L. Chin. Δ^9-Tetrahydrocannabinol, EEG and behavior, the importance of adaptation to the testing milieu. *Pharmac. Biochem. Behav.* **3:** 173–177, 1975.
2. Fujimori, M. and H. Himwich. Δ^9-Tetrahydrocannabinol and the sleep-wakefulness cycle in rabbits. *Physiol. Behav.* **11:** 291–295, 1973.
3. Khazan, N. and C. Sawyer. Mechanisms of paradoxical sleep as revealed by neurophysiologic and pharmacologic approaches in the rabbit. *Psychopharmacologia* **5:** 457–466, 1964.
4. Klemm, W. *Animal Electroencephalography*. New York: Academic Press, 1969.
5. Lipparini, F., A. Scotti de Carolis and V. G. Longo. A neuropharmacological investigation of some trans-tetrahydrocannabinol derivatives. *Physiol. Behav.* **4:** 527–532, 1969.
6. Lemberger, L. *Physiologic Disposition of Drugs of Abuse*. New York: Spectrum Publications, 1976.
7. Longo, V. *Electroencephalographic Atlas for Pharmacological Research*. New York: Elsevier Publishing Company, 1962.
8. Martin, P. and P. Consroe. Cannabinoid induced behavioral convulsions in rabbits. *Science* **194:** 965–967, 1976.
9. Masur, J. and N. Khazan. Induction by cannabis sativa (Marijuana) of rhythmic spike discharges overriding REM sleep electrocorticogram in the rat. *Life Sci.* **9:** 1275–1280, 1970.
10. McMillan, D. and W. Dewey. On the mechanism of tolerance to Δ^9-THC. In: *Current Research in Marijuana*, edited by M. F. Lewis. New York: Academic Press, 1972.
11. Pirch, J., R. Cohn, P. Barnes and E. Barratt. Effects of acute and chronic administration of marijuana extract on the rat electrocorticogram. *Neuropharmacology* **11:** 231–240, 1972.
12. Pirch, J. H., K. Osterholm, R. Cohn and E. Barratt. Studies on EEG tolerance to marijuana in the rat. *Arch. int. Pharmac. Ther.* **203:** 213–220, 1973.
13. Stadnicki, S., U. Schaeppi, H. Rosenkrantz and M. Braude. Crude marihuana extract: EEG and behavioral effects of chronic oral administration in rhesus monkeys. *Psychopharmacologia* **37:** 225–233, 1974.
14. Stadnicki, S., U. Schaeppi, H. Rosenkrantz and M. Braude. Δ^9-Tetrahydrocannabinol: subcortical spike bursts and motor manifestations in a Fischer rat treated oraly for 109 days. *Life Sci.* **14:** 463–472, 1974.