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Δ^9-Tetrahydrocannabinol, EEG and Behavior: The Importance of Adaptation to the Testing Milieu

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CONSROE, P. F., B. C. JONES AND L. CHIN. Δ^9-*Tetrahydrocannabinol, EEG and behavior: the importance of adaptation to the testing milieu.* PHARMAC. BIOCHEM. BEHAV. 3(2) 173–177, 1975. – Δ^9-Tetrahydrocannabinol (Δ^9-THC) in doses of 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg, i.v. was administered to adult rabbits previously adapted to the testing chamber. Additionally, a group of rabbits not adapted to any part of the testing regimen was administered 1.0 mg/kg Δ^9-THC. Cortical and hippocampal electroencephalographs as well as postural and activity behaviors of the unrestrained animals were recorded. In the adapted rabbits, there were dose-related increases in cortical voltage output, disruption of hippocampal theta rhythm and cortical polyspike bursts. Behaviorally, there was a dose-related tendency for standing and exploration to decrease, and at 0.5 and 1.0 mg/kg, Δ^9-THC produced sprawling. In the nonadapted rabbits, administration of 1.0 mg/kg of the drug caused EEG and behavioral stimulation followed by depression of both. The results suggest that the behavioral actions of cannabinols are largely dependent upon the animal's existing state of arousal.

Δ^9-THC Cannabinols EEG Behavior Rabbit Adaptation

EFFECTS of marihuana and its derivatives on electroencephalogram (EEG) and behavior have been assessed in a variety of species. Delta-9-transtetrahydrocannabinol (Δ^9-THC), as well as other marihuana preparations or chemical analogs of THC (e.g., DMHP) increase high-voltage slow wave cortical EEG activity, indicative of sedation in macaques [11,16], cats [12], and dogs [7]. Squirrel monkeys demonstrate Δ^9-THC-induced EEG arousal (low voltage fast activity) followed by synchronization [1]. In rats, Δ^9-THC produces an increase in low voltage fast activity [19]. Disruption of hippocampal theta rhythms and/or induction of polyspike cortical activity have been consistently reported [8, 9, 15, 16, 17, 19].

At the level of gross behavior, investigators who reported desynchronized cortical EEG usually noted that their animals were excited, and those who reported that Δ^9-THC produced cortical synchronization noted that their animals appeared sedated. Additionally, Δ^9-THC treatment may result in a hypersensitivity to auditory or tactile stimulation [7,13].

Investigations of the effects of Δ^9-THC on the rabbit cortical EEG have yielded conflicting results. Δ^9-THC has been shown to produce low voltage fast activity [3,15] or predominately high voltage slow wave activity [5, 9, 14, 22]. Other have shown a temporally biphasic cortical EEG stimulation-sedation property of Δ^9-THC in doses of 0.5 and 1.0 mg/kg [8,23] or marihuana resin [4].

The original purpose of the dose-response part of the present study was to find a relatively nontoxic dose of Δ^9-THC suitable for our antagonism (of THC) studies, and to develop and test a quantitative behavioral observation method. As we administered additional doses of Δ^9-THC we obtained evidence that this drug produced dose-related sedative effects, without any initial stimulation whatever. Since these results appeared to conflict with the results obtained in other laboratories [3, 8, 15, 23] we hypothesized that the rabbits' state of adaptation, i.e., familiarity with the testing milieu might be an important factor in behavioral and EEG investigations involving Δ^9-THC.

Others have shown that familiarization of rats to the laboratory influences the activity of Δ^9-THC [2]. In the present investigation we tested our hypothesis by treating nonadapted rabbits with 1.0 mg/kg Δ^9-THC (the dose which previously produced the highest levels of sedation) and observing them under conditions identical to those in which we observed the adapted animals.

METHOD

Animals

Adult albino New Zealand rabbits were used. Five rabbits comprised the dose response (and adapted) group and 4 rabbits comprised the nonadapted group. Weights of the animals ranged from 2.5–3.6 kg.

Electrode Implantation

Electrode implantation was stereotaxically performed under pentobarbital sodium anesthesia and in the following loci: motor cortex and limbic cortex [5], and dorsal hippocampus [20]. All electrodes were connected to an Amphenol connector anchored to the cranium with stainless steel screws and dental acrylic cement.

Drug

The Δ^9-THC was prepared for intravenous administration by incorporation into a 10% polysorbate (Tween) 80-saline solution [18]. Ten percent Tween 80 dissolved in normal saline was used as the control vehicle. All doses were calculated to provide equal volume per unit body weight (0.1 ml/kg).

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 animals' behavior. Shielded cable connected the electrodes from the rabbits to a Grass Model 7B recorder via a mercury cable coupler mounted on the test chamber. Behaviors were measured by one observer operating a 10-channel digital display event recorder designed to measure frequency and duration of several behaviors.

Dependent Variables

EEG. In all animals, the electrical activity occurring between the left motor and right limbic 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.

Behavior. Frequency and duration of the following behaviors were measured with a 10 channel digital event recorder: (1) *Standing.* Weight of animal on the tarsals and front legs extended vertically, or animal is up on all four legs. (2) *Sitting.* Weight of animal is distributed along the ventral body surface, head is up and front legs folded under body or legs parallel and rostrally extended. (3) *Sprawl.* Weight of animal is distributed ventrally, two or more legs extended laterally (splayed) and/or head touching floor chamber. (4) *Environmental Exploration.* Object sniffing or general sniffing of the chamber with extended head and movement of vibrissae.

Procedure

All animals were allowed 10 days to recover from surgery. Adapted animals were given 3 weekly 84-min exposures to the testing conditions with control vehicle treatment. The interval between drug treatments was 1 week. The design of the experiment consisted of 2 within-animals variables, treatments and periods (five 4-min time samples within each 80-min session).

Treatments were: (1) Tween-saline (control baseline); (2) 0.01 mg/kg Δ^9-THC; (3) 0.05 mg/kg; (4) 0.1 mg/kg; (5) 0.5 mg/kg; (6) 1.0 mg/kg; (7) Tween-saline (retest). Control baseline and retest determinations were performed in the order stated above as a test for trials effects. The first THC treatment was 1.0 mg/kg and the rest of the doses of Δ^9-THC were randomly distributed among test sessions. Within each treatment session, time sampling of the effects of the drug on behavior and EEG consisted of five 4-min periods at 0–4, 20–24, 40–44, 60–64, and 80–84 min following treatment. Additionally, a bell located within the chamber was sounded for 20 sec at 15 and 45 min after injection in order to assess the drug's effects on arousal. Testing of the nonadapted rabbits was identical to the procedure described above, except that they were treated with a single dose of Δ^9-THC (1.0 mg/kg) and tested once in the apparatus.

Data from the dose response part of the present investigation were evaluated by analyses of variance (ANOVA) for a 2 within-animals variables experiment. For each dose, temporal contrasts for linear and quadratic trend were analyzed. Data from the comparison of adapted and nonadapted rabbits (at 1.0 mg/kg Δ^9-THC) were analyzed by unweighted means ANOVA's for a one between and one within animals variables design [24]. Subsequent comparisons among means were made with the Newman-Keuls Multiple Comparisons Method [24].

RESULTS

The design employed permitted us to assess changes in behavior and EEG, within as well as across testing sessions. Adaptation is operationally defined here as intrasession decreases in behaviors and EEG patterns collected under the term arousal, viz., flattened EEG tracings, standing, environmental exploration. Throughout all testing conditions, the animals demonstrated higher levels of standing ($p<0.01$) and exploration (n. s.) and lowered electrogenesis ($p<0.01$) in the first period compared with subsequent periods within the 84-min treatment sessions.

EEG

The overall main effect for dose treatments was reliable ($p<0.001$). Subsequent comparisons between means revealed no difference between control and 0.01 mg/kg Δ^9-THC. The 0.05 and 0.1 mg/kg doses produced more cortical electrogenesis than did the 0.01 mg/kg or control treatments ($p<0.05$), but no reliable differences were seen between the 0.05 and 0.1 mg/kg doses. The 0.5 and 1.0 mg/kg doses produced the highest levels of electrogenesis, compared with control ($p<0.01$) and produced more electrogenesis than 0.05 and 0.1 mg/kg ($p<0.05$).

The significant treatments $\times$ periods interaction ($p<0.001$) is largely a result of the difference in trend among the doses used (Fig. 1). The two highest doses produced reliable quadratic trends ($p<0.05$, $p<0.05$). The function of both doses across periods was an inverted U, with the cortical electrogenesis of the 0.5 mg/kg dose peaking and declining more rapidly than the 1.0 mg/kg dose. The trends in the 0.05 and 0.1 mg/kg doses were not as clear. In the 0.1 mg/kg dose condition, a trend toward a quadratic contrast was seen ($p<0.1$), and in the 0.05 mg/kg dose treatment, neither linear nor quadratic contrasts approached significance (higher order trends were not tested).

Qualitatively, all doses of Δ^9-THC, except for 0.01 mg/kg produced more periods of high voltage slow wave activity from the neocortex and desynchronization of

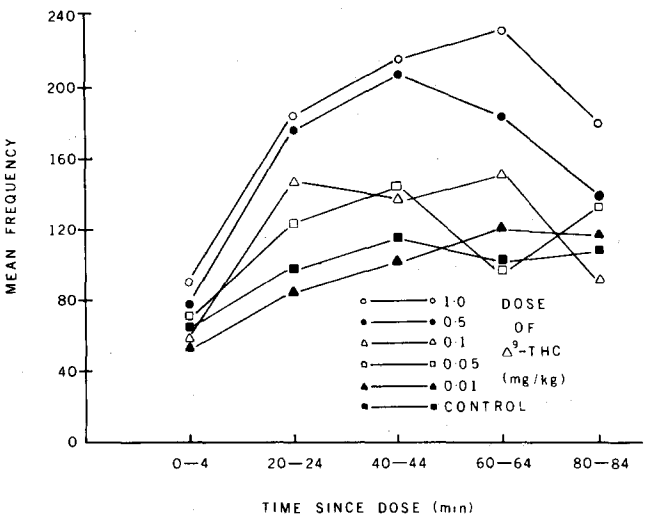

FIG. 1. Mean frequency of integrator resets (electrogenesis) from left motor – right limbic cortical leads in rabbits. Values presented are from adapted rabbits (n = 5).

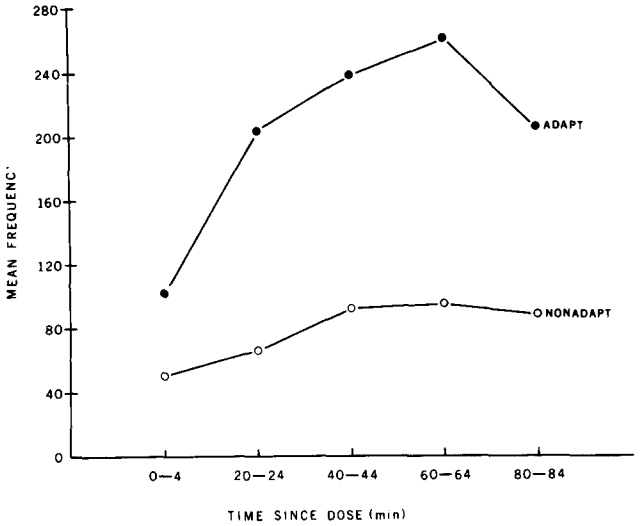

FIG. 2. Mean frequency of integrator resets (electrogenesis) from left motor – right limbic cortical leads in rabbits treated with 1.0 mg/kg Δ^9-THC. The upper line presents data from 5 animals adapted to the testing procedure and apparatus. The lower line presents data from nonadapted animals (n = 4).

theta rhythm from the hippocampus than was seen in control conditions. At all doses of Δ^9-THC, presentation of the bell always evoked low voltage fast activity from the cortex and also elicited the sinusoidal theta rhythm from the hippocampus.

We also noticed bursts of cortical polyspike activity (100–150 μv spikes at 10–14 Hz lasting 1–2 sec). At 0.05 mg/kg occasional polyspike episodes occurred during either low voltage fast activity or during high voltage slow wave activity. As dose increased, it appeared that more polyspike activity occurred and was superimposed on predominately slow wave high voltage activity.

Nonadapted animals evinced reliably less cortical electrogenesis than did the adapted animals ($p<0.001$). Figure 2 illustrates the significant Adaptation x Periods interaction ($p<0.01$). Subsequent comparisons between means revealed that with the exception of the 0–4 period, adapted animals treated with 1.0 mg/kg Δ^9-THC showed considerably higher cortical voltage output at each period ($p<0.05$ for 20–24, 40–44, 60–64, 80–84) than did the identically dosed nonadapted rabbits.

In contrast to the high voltage slow wave cortical activity characteristic of the adapted rabbits' tracings, the nonadapted rabbits' tracings showed predominately low voltage fast activity. Bursts of cortical polyspike activity were noted in both groups of rabbits. Hippocampal theta rhythms in nonadapted animals appeared to be impaired, however the large amount of movement artifact made visual assessment difficult.

Postural and Activity Behaviors

Changes in standing, sprawling and environmental exploration are illustrated in Fig. 3. High levels of inter-animal variability rendered standing (and sitting) less sensitive to dose treatments than the other dependent variables that were measured. In the 0.01 mg/kg dose condition, the amount of standing was comparable with that seen in the control condition. The 0.05 mg/kg dose produced an increase (n. s.) in standing; 0.1 and 0.5 mg/kg both showed a trend toward reducing the amount of standing ($p<0.10$, $p<0.10$). The dose of 1.0 mg/kg was accompanied by a reliable decrease (from baseline levels) in standing ($p<0.05$). Sprawling was a sensitive indicator of the two highest doses of Δ^9-THC. The dose of 1.0 mg/kg produced higher levels of sprawling than did 0.5 mg/kg ($p<0.05$), and 0.5 mg/kg was the lowest dose of Δ^9-THC at which sprawling appeared ($p<0.05$).

At dose levels of 0.01 and 0.05 mg/kg, decreases in environmental exploration which were not statistically reliable occurred. At 0.1, 0.5, and 1.0 mg/kg environmental exploration was virtually abolished ($p<0.05$, $p<0.05$, and $p<0.01$, respectively, compared with control levels).

The shaded bars of Fig. 3 show that the nonadapted rabbits treated with 1.0 mg/kg Δ^9-THC stood and explored more ($p<0.05$, $p<0.01$) and sprawled less ($p<0.05$) than their identically dosed, but adapted cohorts (open bars). Quite often, the nonadapted rabbits assumed frozen standing postures which alternated with quickly executed orienting movements. Additionally, the nonadapted animals evinced exophthalmus, mydriasis, opisthotonus, nystagmus, hindlimb ataxia and pleurothotonus. None of these behaviors was observed in the adapted animals.

Incidentally, an unexpected behavioral pattern was detected in a few rabbits. We necessarily had to exclude 3 of our original animals (data not included in the present study), because within 3 min following administration of as little as 0.05 mg/kg or more THC, each of the 3 (all littermates) exhibited clonic convulsions. Administration of the same dose of the drug to a nonimplanted littermate produced identical convulsions. The THC seizure susceptibility appears to be strain related because the only rabbits in which we have observed this phenomenon are descendants of a particular doe.

DISCUSSION

In the present investigation, several findings were salient.

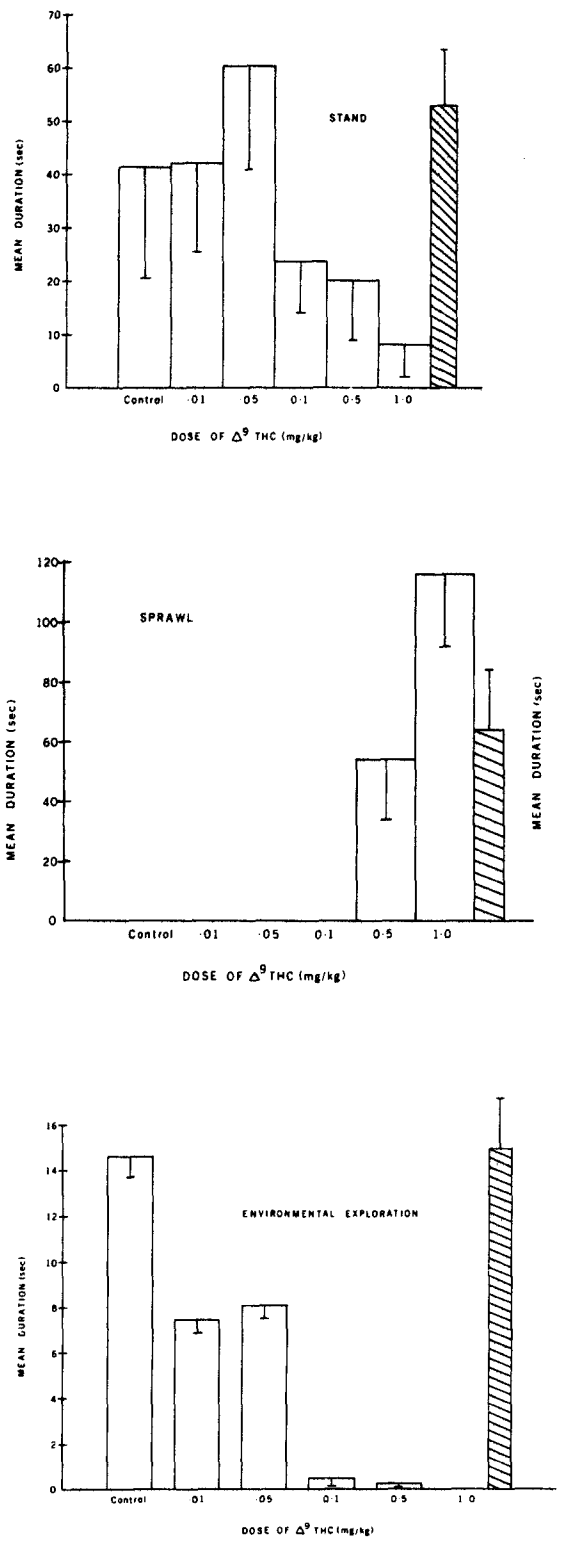

FIG. 3. Mean duration of standing, sprawling, and environmental exploration. Open bars represent measurements obtained from animals adapted to the testing milieu and regimen (n = 5). The shaded bar at 1.0 mg/kg represents measurements obtained from nonadapted animals (n = 4). Vertical lines are standard errors.

First, in a very broad sense, EEG and behavior in both experiments were congruent. Behavioral and postural components of arousal accompanied fast low voltage cortical tracings, and inactivity accompanied high voltage slow cortical activity. However, at a finer level of inspection, the three general dependent measures were differentially sensitive, dose-wise, to Δ^9-THC. EEG was the most sensitive measure, with reliable changes occurring at 0.05 mg/kg. Next in sensitivity was exploratory activity, which was reliably decreased at 0.1 mg/kg. Standing was the least sensitive measure, and this was probably due to the large amounts of interanimal variability; sprawling occurred only at the two highest doses.

The Δ^9-THC induced seizures at 0.05 mg/kg in three of our original animals and their unoperated littermate came as quite a surprise to us. Only one other study has reported THC-induced convulsions [21]. Rats treated with high doses of Δ^9-THC convulsed after chronic administration in excess of 100 days.

The present demonstration that the experimental setting is a crucial element in Δ^9-THC studies is in accord with results obtained by Barry and Kubena [2] and Adams and Barratt [1]. In the former case, rats given one day to adjust to a laboratory were more active when treated with Δ^9-THC, 4 mg/kg, i.v., than were their cohorts given an equal amount of drug, but allowed 5–7 days adaptation. In the latter case, squirrel monkeys treated with Δ^9-THC, p.o., 0.342–2.736 mg/kg (as marihuana extract distillate at 17% Δ^9-THC) evinced cortical potentials appropriate for contingency demands in an operant task. During periods of availability of response-contingent reinforcement (and subsequent responding) the EEG tracings of monkeys showed fast, low voltage activity, and during the post session period the tracing showed high voltage slow activity. Our findings and other evidence cited herein support the hypothesis that behavioral effects of tetrahydrocannabinols can be expected to vary with the setting of their use or investigation. The observation of Willinsky and his colleagues [23] that Δ^9-THC in rabbits caused stimulation followed by sedation is similar to our observation in similarly treated nonadapted rabbits, but they did not clearly specify testing conditions. However, these authors did mention that when objects were presented to rabbits treated with a synthetic THC, the rabbits responded in an aggressive manner.

In short, it appears that any situation which is arousing to an animal will affect its response to Δ^9-THC either by: (1) overcoming the sedative action of Δ^9-THC or (2) actually intensifying the behavioral arousal as seen when amphetamines are given in combination with Δ^9-THC [6,10]. The former hypothesis, that arousal resulting from environmental novelty or situational demands may overcome the sedative effects of Δ^9-THC, seems appropriate in the present study. Although the appropriate data were not available for comparison, it is our belief that the EEG and quantified behavioral components of arousal in the present nonadapted drugged rabbits did not differ in a predictable manner from our adapted rabbits given no drug during their first exposure to the testing environment.

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