Physostigmine-induced reversal of EEG and behavioral effects of Δ⁹-tetrahydrocannabinol

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The effects of Δ⁹-THC) in combination with physostigmine (PHYSO) on the electroencephalogram (EEG) and behavior were measured in adult unrestrained rabbits. The increase in mean cortical electrogenesis (voltage output) of the EEG induced by Δ⁹-THC was clearly reversed by PHYSO. Hippocampal theta rhythm disrupted by Δ⁹-THC was restored by PHYSO. Physostigmine also antagonized Δ⁹-THC-induced alteration of postural and activity behaviors. Except for very brief periods of retching, no behavioral indications of toxicity were observed.

Several investigators have suggested that the behavioral effects of Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the major psychoactive component of marihuana, may be one manifestation of its activity at central cholinergic pathways, especially in the limbic system. More specifically, Miller and Drew (1974) believe that Δ⁹-THC has anticholinergic (atropine-like) activity and that this anticholinergic activity is apparent especially in the hippocampus.

Alternatively, Δ⁹-THC did not block the cholinomimetic effects of tremorine in mice (Sofia, Kubena, and Barry, 1974) or physostigmine in rabbits (Consroe, 1973). Moreover, Δ⁹-THC has been shown to augment the cholinomimetic toxicity of physostigmine in rats (Rosenblatt, Janowsky, Davis, and El-Yousef, 1974). In humans, Freemon, Rosenblatt, and El-Yousef (1975) demonstrated that physostigmine may enhance the late effects of Δ⁹-THC, viz, lethargy and somnolence.

The present experiment was designed in order to investigate quantified EEG and behavioral changes in the presence of Δ⁹-THC, physostigmine, and their combination.

METHODS

Subjects
The subjects in the present experiment were nine New Zealand albino rabbits weighing between 3.0 and 4.5 kg. All subjects were housed individually in a room maintained at 25°C and under a 12 h light-dark cycle. All subjects had surgically implanted chronic recording electrodes; the implantation procedure is described elsewhere (Consroe, Jones, and Chin, 1975). The loci of the recording electrodes were motor cortex, parietal (limbic) cortex, and dorsal hippocampus.

Apparatus
Unrestrained rabbits were tested in a chamber measuring 82 cm square x 70 cm high. A one-way view side window permitted behavioral observation, and shielded cable connected the subject's recording electrodes to a Grass Model 7B recorder.

Drugs
Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) was prepared using a 10% polysorbate (Tween) 80-saline solution as a vehicle. Physostigmine salicylate (PHYSO) was dissolved in .85% saline. All drug dosages were calculated as the active base; drugs and vehicles were injected via the marginal ear vein and were administered in the volume of .1 ml/kg body weight. The dose of Δ⁹-THC employed in the present study was .5 mg/kg and was chosen on the basis of dose-response data (Consroe et al., 1975) that indicated that this dose produces characteristic EEG patterns without signs of behavioral toxicity, e.g., ataxia. Similarly, the dose of PHYSO (.05 mg/kg) was chosen for its consistent EEG changes and corresponding lack of toxicity, viz, fasciculations, drooling, etc. Drugs were given not more than once a week to any one animal.

EEG
Cortical EEGs were recorded from various bipolar leads and subsequently assessed qualitatively. In all animals, the electrical activity occurring between the left motor and right limbic cortical leads was integrated using the Grass Model 7P10B integrator system. The data reported here are mean frequency of integrator resets where each reset represents 70 microvolts. Additionally, hippocampal EEG tracings were assessed qualitatively for frequency and amplitude patterns.

Behavior
Postural and activity behaviors as described previously by Consroe et al. (1975) were measured.

Procedure and Testing Sequence
The subjects were allowed to recover from surgery for 10 days and then adapted to the testing apparatus/regimen as described previously (Consroe et al., 1975). The design of the present experiments permitted simultaneous recording of behavior and EEG for eight 4-min time samples spanning 54 min, i.e., 0-4, 5-9, 10-14, 20-24, 30-34, 35-39, 40-44, and 50-54 min. The treatment sequence was counterbalanced to control for sequence effects and consisted of: (1) Tween 80-saline (Tween 80-saline at time 0 followed 30 min later by saline). (2) THC-saline (Δ⁹-THC at time 0 followed 30 min later by saline). (3) Saline-PHYSO (saline at time 0 followed 30 min later by PHYSO). (4) THC-physostigmine (Δ⁹-THC at time 0 followed 30 min later

Table 1
Mean Frequency of Integrator Resets Observed for Cortical EEG and Mean Durations
(in Seconds) for Sprawling and Activity Behaviors

	Treatment Sequence						
	Tween → (0-24 min)	Saline (30-54 min)	Δ⁹-THC → (0-24 min)	Saline (30-54 min)	Tween → (0-24 min)	PHYSO (30-54 min)	Δ⁹-THC → (0-24 min)	PHYSO (30-54 min)
Mean Frequencies								
Integrator Resets	73.77[1] ±4.37	99.00[2] ±6.71	134.22[1] ±15.24	171.22[4] ±16.85	66.78 ±8.64	54.89[2] ±5.98	130.78[3] ±17.58	59.67[3,4] ±7.11
Mean Durations (Sec)								
Sprawl	0[1]	0	48.00[1,2] ±19.28	122.56[2,4] ±32.47	0	0	76.44[3] ±26.94	28.67[3,4] ±10.33
Activity	29.56[1] ±8.89	10.11[2] ±2.51	1.67[1] ±1.10	.22[3] ±.14	16.56 ±4.51	28.67[2] ±4.84	9.67 ±3.84	19.11[3] ±3.90

Note—Each mean (± SE) is the average of the values obtained from nine rabbits observed for four 4-min time samples, i.e., 0-4, 5-9, 10-14, and 20-24 min for Tween or Δ⁹-THC; 30-34, 35-39, 40-44, and 50-54 min for saline or PHYSO. Matching superscripts, e.g., 1-1, 2-2, etc., indicate reliable contrasts (p < .05).

by PHYSO). Within each treatment period, drugs were given in the order indicated above. Earlier research in our laboratory (Consroe et al., 1975) revealed that the EEG and behavioral manifestations of .5 mg/kg Δ⁹-THC peaked at 30 min and persisted fully until at least 1 h after administration. Therefore, PHYSO, a drug which is a rapidly acting CNS and behavioral stimulant was administered during the peak of Δ⁹-THC activity.

Data Analysis

Analyses of variance for two within-subjects variables were employed (Winer, 1971); each subject served as its own control. The Newman-Keuls multiple comparisons methods was used to evaluate differences between means. In order to facilitate data presentation, data were lumped into two segments, i.e., Time Samples 0-4 to 20-25 min (Treatment 1) and Samples 30-34 to 50-54 min (Treatment 2).

RESULTS

Table 1 presents the data. The integrated cortical activity showed a reliable amplitude increase after Δ⁹-THC treatment (p < .05). Additionally, PHYSO reliably reversed the Δ⁹-THC-induced increase in cortical EEG amplitude (p < .05). As expected, Δ⁹-THC disrupted the sinusoidal hippocampal theta rhythm. As with the cortical EEG, PHYSO reversed the Δ⁹-THC-induced change in the hippocampal trace, i.e., restored theta.

Behaviorally, Δ⁹THC appeared to sedate the subject. Posturally, Δ⁹-THC tended to decrease the amount of standing, however, the large variance in this behavior prevented even the large changes in the mean durations from being statistically reliable.

In contrast, the mean duration of sprawling (defined as the subject's head on the floor of the cage and limbs splayed laterally) occurred only following administration of Δ⁹-THC (p < .05). As with the EEG, PHYSO reliably reduced the Δ⁹-THC induced sprawling (p > .05). The sprawling observed in the PHYSO condition following Δ⁹-THC pretreatment was observed exclusively during the first 4 min following PHYSO administration.

Activity behaviors, grooming, locomotion, rearing and exploration showed similar changes throughout the experiment, and their composite is presented in Table 1. Δ⁹-THC depressed (p < .05), whereas PHYSO increased the mean duration of activity (p < .05). The intrasession trend toward PHYSO reversing the Δ⁹-THC-induced depression of activity approached significance (p < .10), and the intersession PHYSO-induced reversal of the Δ⁹-THC depressed activity was reliable (p < .05).

Additionally, three of the rabbits evinced brief bouts of retching without vomiting.

DISCUSSION

The results of this experiment show unequivocally that at the present dose levels, the sedative effects of Δ⁹-THC on EEG and behavior can be reversed by physostigmine. Further, with the exception of the brief periods of retching, we observed no unique interaction effects of the combination of the two drugs.

While our results may be considered as supportive of Miller's and Drew's (1974) proposal that Δ⁹-THC has anticholinergic properties, our results may have been due to nonspecific stimulant properties of physostigmine rather than direct antagonism. Indeed, physostigmine has been shown to reverse physiological depression induced by a variety of agents not considered to have specific anticholinergic effects. For example, physostigmine has been shown to reverse the depressant effects of haloperidol, chlorpromazine (Bernards, 1973; Consroe & White, 1972), pentobarbital, phenoglycodol, and meprobamate (White & Boyajy, 1959). In humans, coma resulting from tricyclic antidepressant poisoning has been reversed successfully with physostigmine (Slovis, Ott, Teitelbaum, & Lipscomb, 1971).

Recently, physostigmine has been applied in treating humans self- or experimentally dosed with marihuana (El-Yousef, Janowsky, Davis, and Rosenblatt, 1973) or Δ⁹-THC (Freemon et al., 1975). In both cases, physostigmine reversed the Δ⁹-THC-induced tachycardia and conjunctival injection, while in the former case, precipitating quasipathological depression and in the latter case producing lethargy and drowsiness.

In conclusion, Δ⁹-THC administration produces EEG and behavioral changes similar to the anticholinergics, atropine and scopolamine (Miller & Drew, 1974). Further, these changes can be reversed by physostigmine. However, in light of the results of Consroe (1973) and Sofia et al. (1974), who failed to demonstrate reversal of cholinomimetic activity by treatment

with Δ^9-THC, it is difficult to ascribe specifically anticholinergic properties to Δ^9-THC.

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