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MONOGRAPH

Interactions of Δ^9-Tetrahydrocannabinol with d-Amphetamine, Cocaine, and Nicotine in Rats[1,2]

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PRYOR, G. T., F. F. LARSEN, S. HUSAIN AND M. C. BRAUDE. *Interactions of Δ^9-tetrahydrocannabinol with d-amphetamine, cocaine, and nicotine in rats.* PHARMAC. BIOCHEM. BEHAV. 8(3) 295–318, 1978. – The acute, reciprocal dose-response interactions between Δ^9-tetrahydrocannabinol (Δ^9-THC; 2.5, 5.0 and 10.0 mg/kg; IG) and each of three stimulants – d-amphetamine (dA; 1, 2 and 4 mg/kg; IP), cocaine (COC; 10, 20 and 30 mg/kg; IP), and nicotine (NIC; 0.25, 0.5 and 1.0 mg/kg; IP) were studied for their effects on performance of a conditioned avoidance response (CAR), photocell activity, heart rate, body temperature, and rotarod performance. Δ^9-THC impaired CAR and rotarod performance, depressed photocell activity, and decreased heart rate and body temperature. None of the three stimulants influenced CAR performance, but dA and COC increased the number of intertrial responses, and this latter effect was partially antagonized by Δ^9-THC. dA and COC, but not NIC, stimulated photocell activity. Δ^9-THC completely blocked this effect of dA, whereas there was mutual antagonism between Δ^9-THC and COC on this measure and NIC markedly potentiated the depression caused by Δ^9-THC. dA and COC tended to offset the impairment of rotarod performance caused by Δ^9-THC, whereas NIC augmented it. The bradycardia and hypothermia caused by Δ^9-THC tended to be augmented by these stimulants, especially NIC. The interactions were also studied after subacute treatment for six days with Δ^9-THC and/or each of the three stimulants. There was evidence for tolerance to the effects of Δ^9-THC on all measures and this tolerance generally resulted in less interactive effects between Δ^9-THC and the stimulants. Little or no tolerance was seen for the effects of the three stimulants or their interaction with Δ^9-THC. The time course of radioactivity derived from ^{14}C-Δ^9-THC and each of the radiolabelled stimulants was determined in plasma and brain. Only minor interactive effects were found and, in general, they could not account for the functional interactions.

Oral Δ^9-THC in rats d-Amphetamine Cocaine Nicotine Interactions between Δ^9-THC and stimulants Acute and subacute treatment CAR Photocell activity Heart rate Body temperature Rotarod Pharmacokinetics in plasma and brain

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THE widespread increase in the use of marihuana in recent years has been accompanied by a corresponding increase in multiple drug use [23]. The potential consequences of the combined use of marihuana and most other drugs are generally unknown in either animals or humans. Although a number of reports have appeared describing some of the interactions between cannabis or its constituents with several drugs (see [26] for selected references) no systematic attempts have been made thus far to characterize the interactions in terms of: (1) the doses and blood levels of the respective drugs; (2) the history of exposure to either or both drugs; and/or (3) the kinds of measures used to identify the interactions.

We have been engaged in such an evaluation of the possible pharmacological and metabolic interactions between a major psychoactive ingredient in marihuana – Δ^9-tetrahydrocannabinol (Δ^9-THC [1]) – and a number of other drugs from various pharmacological classes [25, 26, 27, 28]. In this paper we will describe some of the preclinical results obtained in rats with combinations of

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Δ^9-THC and each of three CNS stimulants – d-amphetamine (dA), cocaine (COC), and nicotine (NIC) – after acute administration and after subacute pretreatment with Δ^9-THC and/or each test drug. These three stimulants represent drugs that might be expected to be used together with, or in close temporal proximity to, the use of marihuana.

The experiments reported here were designed to determine: (1) the acute dose-response relationship of each of the three stimulants alone and in all combinations with three doses of Δ^9-THC for their effects on performance of a conditioned avoidance response (CAR), spontaneous locomotor activity, muscular coordination, heart rate, and body temperature; (2) the extent to which subacute pretreatment with Δ^9-THC and/or each drug for six days influenced these effects; and (3) the time course of levels of radioactivity in plasma and brain derived from administration of the radiolabelled drugs as they interacted acutely and after subacute pretreatment.

METHOD

Animals

Male rats of the inbred Fischer strain were used in all experiments. They were 55 to 60 days old (140 to 160 g) when received from Simonsen Laboratories, Gilroy, CA. They were housed singly in wire mesh hanging cages with food and water available at all times. The ambient temperature was 22° C and the lights in the room were turned on at 0700 hr and off at 1900 hr daily.

Apparatus

Avoidance chambers. Each avoidance chamber consisted of a 30 x 36 x 40 cm wooden box housed inside a sound-attenuated, ventilated cabinet. Scrambled, constant current 1.0 mA shock applied to 0.32-cm dia. brass rods spaced 1.27 cm apart served as the unconditioned stimulus (UCS). Downward displacement (0.16 cm) of a 1.27-cm-dia. aluminum pole suspended from the center of the ceiling served as the operant response. A 7.5-W light and a 11.4-cm-dia. loudspeaker provided ambient light (0.44-ft-candles measured at floor level) and an ambient 4-kHz tone (8 dB above background, which was 50 dB measured at the center of the floor using a General Radio Co. Type 1551–C sound-level meter set for A weighting). A pulsating increase (2.5 times per sec) in intensity of either the light (to 0.88 ft-candles) or the tone (to 63 dB), or the application of a low intensity, nonaversive current (120 μA) to the floor served as conditioned stimuli (CS). Twelve such chambers were interfaced with a Digital Equipment Corporation PDP 8/F computer (located in an adjoining room) that provided automatic stimulus presentation and data collection.

Photocell activity chamber. For measuring spontaneous motor activity, a single rat was placed in a black, cylindrical chamber 30 cm in dia and 28 cm high. Six photocells positioned 1.3 cm above the floor and oriented at 60° around the periphery recorded the animal's movements on a digital counter. The chamber was housed inside a sound-attenuated, ventilated cubicle equipped with a 7.5-W light located above the center of the chamber.

Heart rate. Heart rate was measured by attaching subdural wire electrodes to both sides of the thorax under light anesthesia. Clip-on leads were connected through an EKG preamplifier into a signal detector. The width of the detection window was set for each animal to exclude noise and movement artifacts. The EKG was converted to rate by a spike-interval analyzer and recorded continuously on a strip-chart recorder as interbeat intervals. The interbeat intervals were averaged visually – by drawing a best-fit line through the graph over a distance corresponding to 2 min and converted to beats per min (bpm). Heart rate was recorded in the photocell chamber.

Body temperature. A lubricated rectal probe attached to a Yellow Springs telethermometer was used to measure body temperature to the nearest tenth degree C.

Rotarod. The rotarod was an 8.9-cm-dia. wooden rod measuring 91-cm long and suspended 46 cm above the test surface. The surface of the rod was covered with emory cloth to provide footing. Its rate of rotation was controlled by a variable speed motor.

Procedure

Two groups of rats were used to evaluate each drug, drug combination, or placebo condition. The first group was pretrained in a single 30-trial session to escape footshock (1.0 mA) by pulling a 20-cm pole. Each trial lasted 30 sec unless the animal responded sooner. The intertrial interval was variable and averaged 60 sec (15 to 120 sec). After pretraining, this group was given three daily 60-trial sessions in which to learn to avoid the footshock by pulling a 13-cm pole in the presence of each CS that preceded the UCS by 10 sec. The CS and UCS remained on together for 30 sec unless the trial was terminated earlier by a pole-displacement response. The three CS (tone, light, or nonaversive footshock) were presented randomly for 20 trials each. The intertrial interval was variable, averaging 1.5 min (15 sec to 3 min). The entire 60-trial session required 2 to 2.5 hr. Response latencies and intertrial responses (ITR) were recorded on punched paper tape for processing on a CDC 6400 computer. Rats that failed to learn the escape response were discarded (with the Fischer strain, less than 5% fail to meet the criterion). Performance is typically 80% avoidance or better to all three CS after this training phase. The test session following acute or subacute drug treatment was conducted in the same way as the training sessions. No appreciable loss of the avoidance response has been found following intervals of up to 14 days between training and testing in control animals.

The second group of rats was used for measuring photocell activity, heart rate, body temperature, and rotarod performance. Before receiving any drug treatment, each rat was given a 5-min pretest in the photocell activity chamber. Based on its score, the rat was ranked and assigned to a control or drug treatment group so that all group means were about equal. After the photocell activity pretest, each rat was given up to four practice trials to learn to stay on the rotarod for 120 sec at 6.25 rpm. Over 90% of the rats met this criterion; rotarod data from rats that failed to meet this criterion were not used. Then the wire electrodes were implanted for subsequent EKG measurement. On the test day, photocell activity was measured for 10 min. Heart rate was then monitored in the same chamber for the next 2 min. The rat was removed from the chamber and body temperature was recorded after a 1-min equilibration period. The rat was then placed on the stationary rotarod and the rotation was gradually increased to 11 rpm. The amount of time that the rat was able to remain on the rod – up to 120 sec – was recorded.

Experimental Design

After pretraining or pretesting each rat was assigned to 1 of 25 groups. For the next six days each rat was intubated with sesame oil (SO, 2 ml/kg) or 10 mg/kg Δ^9-THC dissolved in SO, or injected IP with a selected dose of the test drug. No further training or testing occurred during this subacute treatment phase to ensure that any observed tolerance or cumulative effects of the drugs could be interpreted simply and would not be influenced by the test procedures. On the seventh day each rat was intubated with SO (2 ml/kg) or one of three doses of Δ^9-THC in SO (2.5, 5.0 or 10.0 mg/2 ml/kg). Ninety min later it was given an IP injection of saline (SAL, 2 ml/kg) or one of three doses of the test drug in SAL (doses and numbers of rats tested are shown in the results section for each drug). Testing began 30 min later. These times of administration before testing were chosen from preliminary experiments to provide pharmacologically active levels of each drug alone by each route of administration at the beginning of testing. Each experiment was completed in several replications with all groups being represented in each replication.

Time Course of Radioactivity in Plasma and Brain

Treatment. Rats of the same age, strain, sex and weight as used in the other experiments were used in these experiments. They were treated daily for six days with SO (2 ml/kg, IG), Δ^9-THC (10 mg/kg, IG) or the test drug (IP). On the seventh day separate groups were treated with ^{14}C-Δ^9-THC (10 mg/kg, 40 μCi/kg, IG) and 90 min later they were injected IP with 2 ml/kg of SAL or the test drug. Blood was sampled serially under light CO_2 anesthesia by periocular puncture 1, 2, 4, 8, and 24 hr after administration of ^{14}C-Δ^9-THC. For determination of radioactivity in brain other rats were similarly treated and sacrificed at these same time points. In separate experiments rats were treated the same as described above for six days and on the seventh day they were given SO (2 ml/kg, IG) or Δ^9-THC (10 mg/kg in SO, IG) followed 90 min later by IP injections of the radiolabelled test drug. Blood was sampled serially in these rats by periocular puncture or they were sacrificed for brain analyses at selected intervals after administration of the radiolabelled test drug. All rats were treated on the seventh day between 0800 and 1000 hr.

Determination of radioactivity. The 70 μl heparinized pipettes in which the periocular whole blood was collected were centrifuged. A constant, 30-mm section of the pipet containing 30 μl of the plasma was transferred to a counting vial containing 10 ml of Oxifluor-H_2O™ (New England Nuclear). Each whole brain was homogenized in 3 volumes of distilled water. Radioactivity was determined in a 0.1-ml aliquot of the homogenate.

Radioactivity in the samples was measured with a Beckman Model LS-250 liquid scintillation system. The cpm were converted to μg/ml of plasma or μg/g of brain of Δ^9-THC or the test drug equivalents. Thus, this measure represents both the parent compounds and any of their radiolabelled metabolites. The radioactivity from known amounts of ^{14}C-Δ^9-THC or the radiolabelled test drugs was also determined and used as standards for these conversions.

Data Analysis

The data for each measure were first analyzed by analysis of variance (ANOVA) to establish the significance of any main effects or their interactions [19]. Significant F-ratios were further evaluated by *t*-tests between preselected pairs of means using the pooled degrees of freedom and residual variance from the analysis of variance. In reporting the results, whenever significant comparisons between means are given the appropriate term of the ANOVA was also significant.

Half-life estimates of total radioactivity in plasma and brain were made by linear regression analysis of $\log_{10}$ levels on time using the method of least squares. Where appropriate, separation of the disappearance curves into more than one phase was based on the goodness of fit obtained by taking subsets of time points that resulted in the largest correlation coefficient.

Drugs

Δ^9-THC as a 1% (w/v) stock solution in sesame oil was prepared by the Research Triangle Institute under contract with the NIDA. Its purity was greater than 96%. ^{14}C-Δ^9-THC was supplied by the NIDA with a specific activity of 121 μCi/mg. ^{14}C-Δ^9-THC was diluted with unlabelled Δ^9-THC in sesame oil so as to provide a concentration of 40 μCi/kg. Both unlabelled Δ^9-THC and radiolabelled Δ^9-THC were diluted with sesame oil so as to deliver the desired dose in a volume of 2 ml/kg. d-Amphetamine sulfate (Smith, Kline, and French), cocaine hydrochloride (Merck), and nicotine (Eastman Kodak) were dissolved in 0.9% saline so as to deliver the desired dose as the salt or base in a volume of 2 ml/kg. ^{3}H-d-Amphetamine sulfate (Amersham/Searle, specific activity, 7.9 Ci/mmole) was diluted with d-amphetamine in saline to deliver 2 mg/kg containing 25 μCi/kg. ^{14}C-cocaine (Cal Bionuclear, specific activity, 4.2 mCi/mmole) was diluted with cocaine hydrochloride to deliver 20 mg/kg containing 15 μCi/kg. ^{14}C-Nicotine dihydrochloride (ICM Pharmaceuticals, Inc., specific activity, 10.1 mCi/mmole) was diluted with nicotine to deliver 1.0 mg/kg containing 10 μCi/kg.

RESULTS

Interactions Between Δ^9-THC and dA

Acute interactions. Figure 1 shows the acute dose-effect relationships for Δ^9-THC and dA alone and in all combinations for the five tests in this battery. A separate 4 x 4 factorial ANOVA was computed for each measure. Two measures are shown for avoidance performance – the percentage of conditioned avoidance responses (CAR) irrespective of the CS and the number of intertrial responses (ITR) per minute. Because there were no appreciable differential effects of the treatments on the three CS, the results were combined as total CAR.

Δ^9-THC alone caused a significant dose-related reduction in the percentage CAR as determined by the ANOVA, $F(3,199) = 10.0$, $p<0.001$. Control performance (i.e., the zero dose of Δ^9-THC and the zero dose of dA) was reduced from 83% CAR to 66% CAR by 10 mg/kg Δ^9-THC, $t(199) = 2.2$, $p<0.05$, without any significant loss of the escape response. Higher doses of Δ^9-THC cause a further reduction in the percentage CAR accompanied by a dose-related loss of the escape response (Pryor, unpublished observation).

Acute administration of 1 to 4 mg/kg dA did not

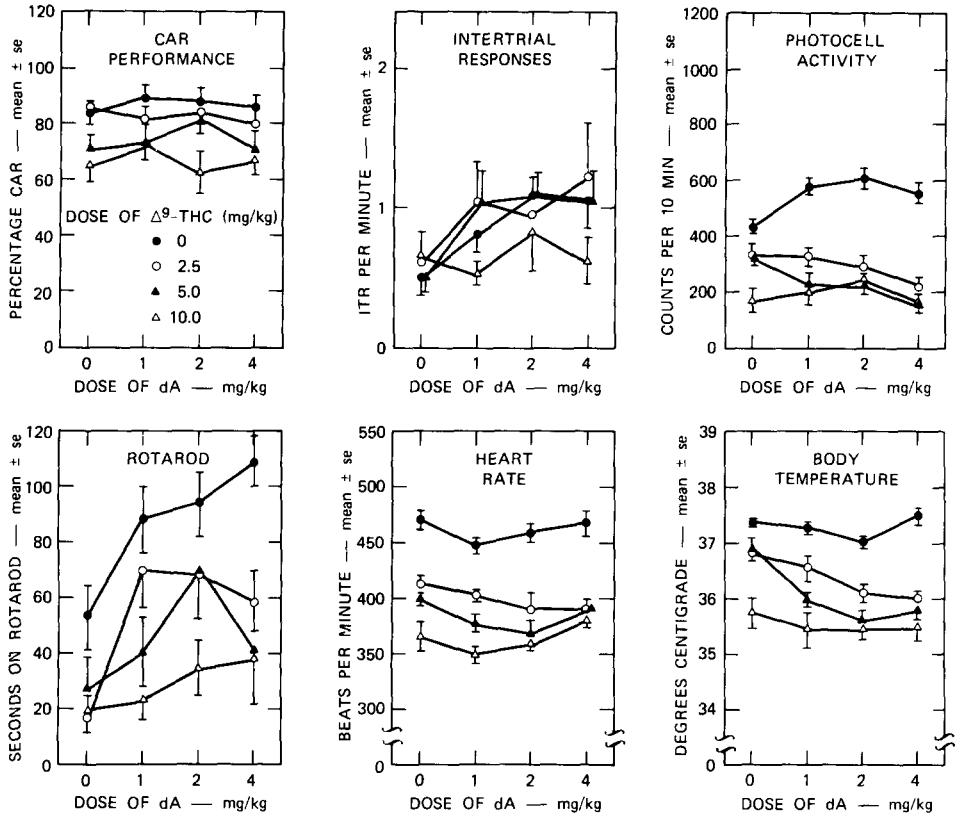

FIG. 1. Acute reciprocal dose response interactions between Δ9-THC and dA. There were 11 to 14 rats in each group for each measure.

significantly affect CAR performance or influence the impairing effect of Δ9-THC. However, dA caused a dose-related increase in intertrial responses from 0.48 ± 0.11 (SEM) to 1.10 ± 0.13 ITR/min, reflecting its known stimulant properties, $F(3,199) = 4.6$, $p<0.005$. Δ9-THC alone did not significantly influence intertrial responses, but the highest dose (10 mg/kg) tended to antagonize the increase caused by each dose of dA even though none of the differences was significant.

The stimulant effect of dA alone was more clearly demonstrated by a significant increase in photocell activity, $F(3,189) = 2.9$, $p<0.05$. The maximum increase from control values (433 ± 22 counts/10 min) was caused by 2 mg/kg dA (600 ± 34 counts/10 min), $t(174) = 3.6$, $p<0.01$. Δ9-THC alone caused a decrease in photocell activity, $F(3,189) = 88.7$, $p<0.001$. The highest dose of Δ9-THC was mainly responsible for this effect causing a 60% reduction in photocell activity to 176 ± 33 counts/10 min compared with controls, $t(174) = 6.0$, $p<0.01$. All doses of Δ9-THC antagonized the stimulant effect of all doses of dA (all $ts(174)\geq 5.3$, all $ps<0.01$ comparing comparable doses of dA with and without Δ9-THC).

dA alone significantly improved rotarod performance compared with controls, $F(3,189) = 8.2$, $p<0.001$, whereas Δ9-THC impaired performance, $F(3,189) = 17.0$, $p<0.001$. The combinations of Δ9-THC and dA appeared to be mutually antagonistic although systematic dose relationships were not evident.

Δ9-THC caused a dose-related decrease in heart rate from an average control value of 470 ± 8.1 bpm to 367 ± 13.4 bpm at 10 mg/kg, $F(3,189) = 80.7$, $p<0.001$. dA did not significantly affect heart rate either alone or in combination with Δ9-THC.

Δ9-THC alone also caused significant hypothermia, $F(3,189) = 59.1$, $p<0.001$. A 1.6°C decrease in body temperature from controls (37.4 ± 0.06°C) was caused by 10 mg/kg Δ9-THC (35.8 ± 0.33°C). dA, which is hyperthermic at high doses, did not significantly alter body temperature over this dose range in this experiment. Surprisingly, dA appeared to augment the hypothermia caused by the lower doses of Δ9-THC. When combined with 2.5 mg/kg Δ9-THC (36.8 ± 0.14°C) body temperature was further reduced by 2(36.1 ± 0.15°C) and 4(36.0 ± 0.15°C) mg/kg dA ($ts\geq 2.8$, $ps<0.01$). When combined with 5.0 mg/kg Δ9-THC (36.9 ± 0.20°C) this augmenting effect was significant for 1 (35.9 ± 0.15°C), 2 (35.6 ± 0.22°C), and 4 (35.8 ± 0.19°C) mg/kg dA (all $ts(189)\geq 3.8$, all $ps<0.01$).

The acute effects of Δ9-THC and dA alone and in combination as determined by this battery of tests can be summarized as follows: (1) Δ9-THC alone caused impairment of CAR and rotarod performance, depressed photocell activity, and decreased heart rate and body temperature; (2) dA alone did not affect heart rate, body temperature, or CAR performance, but increased intertrial responding, stimulated photocell activity, and improved rotarod performance; and (3) when combined, the effect

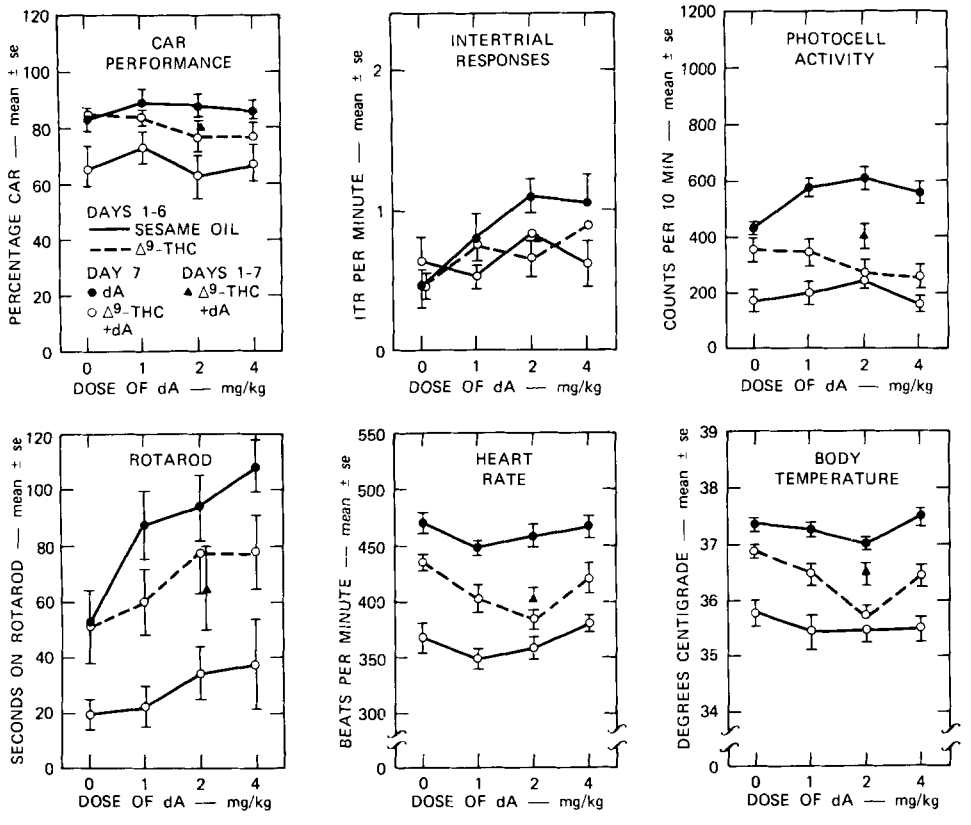

FIG. 2. Interactions between Δ⁹-THC and dA after subacute treatment with 10 mg/kg/day Δ⁹-THC for six days. There were 11 to 14 rats in each group for each measure.

was primarily an antagonism of the stimulant action of dA by Δ⁹-THC, the result resembling the depressant properties of Δ⁹-THC more than the stimulant properties of dA.

Subacute treatment with Δ⁹-THC. Figure 2 shows the acute dose-effect relationships for dA alone and in combination with 10 mg/kg Δ⁹-THC compared with the effects seen after subacute pretreatment with 10 mg/kg/day Δ⁹-THC for six days. The effects of subacute treatment with both 10 mg/kg/day Δ⁹-THC and 2 mg/kg/day dA for all seven days are also shown. Each measure was analyzed by a 5 × 5 factorial ANOVA that included the acute, reciprocal dose-response experiment just described and the groups pretreated subacutely with dA (see next section) as subsets.

Subacute treatment with Δ⁹-THC for six days caused tolerance to its impairing effects on CAR performance. Whereas 10 mg/kg Δ⁹-THC significantly reduced the percentage of CAR when it was given for the first time to rats pretreated with SO, this same dose was ineffective after subacute pretreatment with Δ⁹-THC. The percentage of CAR for these Δ⁹-THC-tolerant rats was 84 ± 3.6% compared with 83 ± 3.6% for control rats pretreated subacutely with SO and given SO and SAL on the test day. As shown in the previous section, the acute administration of 10 mg/kg Δ⁹-THC caused a decrease in percentage CAR to 66 ± 7.4%.

dA did not significantly influence CAR performance in such Δ⁹-THC-tolerant rats compared with rats treated subacutely with Δ⁹-THC alone. The performance of all groups given dA and Δ⁹-THC together after subacute treatment with Δ⁹-THC was better than the performance of all groups given the combination of Δ⁹-THC and dA acutely, reflecting the tolerance to Δ⁹-THC.

There was no significant difference in CAR performance between the group pretreated subacutely with Δ⁹-THC alone and then given 10 mg/kg Δ⁹-THC combined with 2 mg/kg dA (76 ± 6.2%) and the group pretreated subacutely with both 10 mg/kg Δ⁹-THC and 2 mg/kg dA (79 ± 4.9%). Thus, the tolerance to the effect of Δ⁹-THC on CAR performance was neither enhanced nor impeded by simultaneous treatment with dA.

Subacute treatment with Δ⁹-THC alone or in combination with dA did not significantly influence the effect of Δ⁹-THC alone or in combination with dA on intertrial reponses compared with their acute administration. The effect of Δ⁹-THC was to antagonize the increase in intertrial responses caused by dA in all groups.

Tolerance to the depressant effect of 10 mg/kg Δ⁹-THC on photocell activity was also seen. The average counts/10 min by rats pretreated subacutely with Δ⁹-THC was 356(±47) compared with 433(±22) in vehicle controls ($p>0.1$) and 176 (±33) in rats given 10 mg/kg Δ⁹-THC for the first time, $t(272) = 3.2$, $p<0.01$.

Although apparently tolerant to the depressant effects of Δ⁹-THC, dA was still unable to stimulate photocell activity as it had when given acutely to SO-treated rats. In fact, there was a trend for the combination of Δ⁹-THC

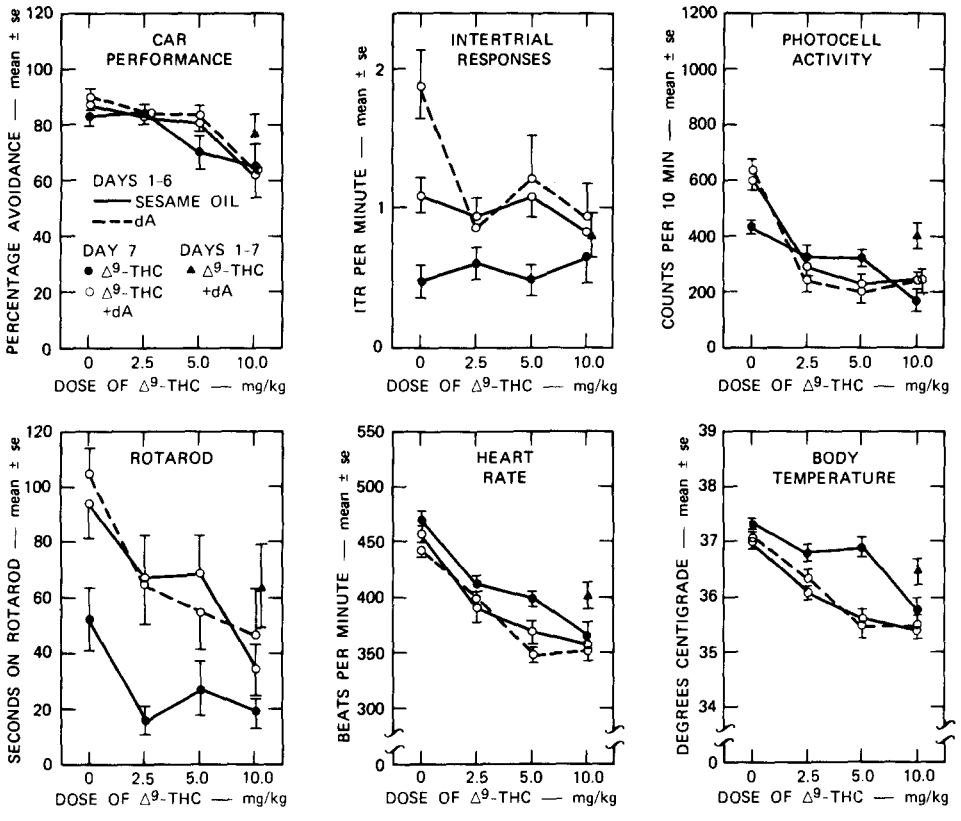

FIG. 3. Interactions between Δ9-THC and dA after subacute treatment with 2 mg/kg/day dA for six days. There were 11 to 14 rats in each group for each measure.

and dA to cause reduced photocell activity in such Δ9-THC-tolerant rats. Although not statistically significant, this trend should not be dismissed summarily because it was almost identical to that seen when 2.5 mg/kg Δ9-THC was combined acutely with dA (see Fig. 1), suggesting, perhaps, that the residual effective potency of 10 mg/kg Δ9-THC after subacute treatment was about equivalent to 2.5 mg/kg Δ9-THC given acutely.

The difference in photocell activity of the rats treated subacutely with 10 mg/kg Δ9-THC and then given the combination of 10 mg/kg Δ9-THC and 2 mg/kg dA (276 ± 45 counts/10 min) compared with those treated subacutely with the combination of 10 mg/kg Δ9-THC and 2 mg/kg dA (402 ± 43 counts/10 min) was significant, $t(272) = 2.3$, $p<0.05$. This result suggests that in addition to the tolerance to Δ9-THC a sensitization to dA may also have occurred on this measure.

Rotarod performance was improved after subacute treatment with 10 mg/kg Δ9-THC compared with acute treatment indicating tolerance on this measure as well, although the difference failed to reach an acceptable level of significance, $t(272) = 1.7$, $p<0.10$. Performance improved further as a function of dose of dA, but because of the variability on this measure the differences were not significant. However, the differences between comparably dosed rats given both drugs acutely and those given both drugs after subacute treatment with Δ9-THC were all significant (all $ts(272)\geq 2.1$, all $ps<0.05$). Subacute pretreatment with both Δ9-THC and dA did not significantly alter rotarod performance compared with subacute treatment with Δ9-THC alone.

Heart rate and body temperature showed similar responses to Δ9-THC and its combination with dA after subacute pretreatment with Δ9-THC. There was significant tolerance to the effects of Δ9-THC compared with acute treatment on both measures ($ts(271) = 4.9$ and 4.0 for heart rate and body temperature, respectively, $ps<0.01$). dA caused a decrease in both heart rate and body temperature in such Δ9-THC-tolerant rats that appeared to be a nonlinear function of dose of dA. The differences for 1 and 2 mg/kg dA were significant for heart rate, $ts(271)\geq 2.4$, $ps<0.01$. It will be recalled (see Fig. 1) that a similar potentiating effect of dA on the bradycardia and hypothermia caused by the lower doses of Δ9-THC was found for their acute combination. This result again suggests a residual potency of about 2.5 to 5.0 mg/kg Δ9-THC after subacute treatment for six days.

The subacute combination of 10 mg/kg Δ9-THC and 2 mg/kg dA resulted in less of a decreasing effect on heart rate and body temperature than the acute combination or after subacute treatment with Δ9-THC alone. This result again may represent a sensitization to the stimulant effect of dA after its subacute treatment.

The effects of Δ9-THC and dA given alone and in combination after subacute treatment with 10 mg/kg Δ9-THC alone or in combination with 2 mg/kg dA may be summarized as follows: (1) tolerance developed to the effects of 10 mg/kg Δ9-THC on all measures, the residual

potency being equivalent to about 2.5 to 5.0 mg/kg Δ^9-THC given acutely; (2) this tolerance to 10 mg/kg Δ^9-THC extended to its interactions with dA, the result resembling the acute interactions seen with the lower doses of Δ^9-THC; and (3) there was an apparent sensitization to dA along with tolerance to the depressing effects of Δ^9-THC on photocell activity, heart rate, and body temperature.

Subacute treatment with dA. Figure 3 shows the results after subacute treatment with 2 mg/kg/day dA. The results after subacute treatment with both Δ^9-THC and dA are repeated for comparison.

There were no significant differences among groups for any of the measures except intertrial responses caused by subacute treatment with dA compared with acute treatment. The increase in intertrial responses caused by acute administration of 2 mg/kg dA was significantly enhanced by subacute treatment with dA, $t(293) = 3.0$, $p<0.01$.

Effects of dA on ^{14}C-Δ^9-THC. Table 1 shows the plasma and brain levels of radioactivity as a function of time after oral administration of ^{14}C-Δ^9-THC (10 mg/kg, 40 μCi/kg). Levels of radioactivity in both plasma and brain of control rats treated subacutely with SO increased to reach a maximum after 2 to 4 hr and declined thereafter. Higher levels of radioactivity were attained after 2 hr in rats treated subacutely with 10 mg/kg Δ^9-THC than in rats treated subacutely with SO. The differences were significant in both plasma (0.82 ± 0.09 μg/ml compared with 0.61 ± 0.05 μg/ml) and brain (0.75 ± 0.08 μg/g compared with 0.48 ± 0.05 μg/g) as determined by *t*-tests following the ANOVA ($t(390) = 3.7$, $p<0.01$ and $t(226) = 3.4$, $p<0.01$, respectively).

A dose of 2 mg/kg dA was administered 1.5 hr after ^{14}C-Δ^9-THC. This treatment caused a significant decrease in plasma radioactivity at 4 hr in rats treated subacutely with SO (0.46 ± 0.03 μg/ml compared with 0.66 ± 0.06 μg/ml; $t(390) = 3.5$, $p<0.01$). Subacute treatment with 2 mg/kg/day dA did not significantly influence this acute effect of dA (0.39 ± 0.03 μg/ml). dA also caused a decrease in plasma radioactivity in rats treated subacutely with Δ^9-THC, but the effect appeared earlier at 2 hr (0.66 ± 0.04 μg/ml compared with 0.82 ± 0.09 μg/ml; $t(390) = 3.7$, $p<0.01$).

In brain, acute administration of dA caused a significant increase in radioactivity in rats treated subacutely with SO (0.67 ± 0.08 μg/g compared with 0.48 ± 0.05 μg/g; $t(226) = 2.4$, $p<0.05$). A similar trend was seen after subacute treatment with dA (0.60 ± 0.06 μg/g) but the effect was not significant. In rats treated subacutely with Δ^9-THC, dA caused a significant increase in brain at 4 hr (1.04 ± 0.07 μg/g compared with 0.78 ± 0.07 μg/g; $t(226) = 3.3$, $p<0.01$).

These results can be summarized as follows: (1) higher levels of radioactivity were reached in both plasma and brain after subacute treatment with Δ^9-THC than after subacute treatment with SO; (2) dA caused the plasma levels of radioactivity initially to decline faster in rats treated subacutely with SO than controls and prevented the increase in plasma radioactivity caused by subacute treatment with Δ^9-THC; and (3) dA caused higher levels of radioactivity to be reached in brain at 2 hr after subacute treatment with SO and at 4 hr after subacute treatment with Δ^9-THC.

Effect of Δ^9-THC on ^{3}H-dA. Table 2 shows the plasma and brain levels of radioactivity derived from ^{3}H-dA as a function of time after its IP administration. Control levels of radioactivity in both plasma and brain were maximum within 0.25 hr after administration. Maximum control levels in brain (1.71 ± 0.11 μg/g) were almost threefold higher than in plasma (0.67 ± 0.05 μg/ml). Radioactivity disappeared in two phases from both tissues with the initial rate of disappearance being faster from brain than from plasma. The estimated $t_{1/2}$ for the first phase was 1.8 hr in plasma and 0.9 hr in brain. The $t_{1/2}$ for the second phase was 7.7 hr in plasma and 7.4 hr in brain.

Acute or subacute treatment with Δ^9-THC or subacute treatment with dA did not significantly influence the levels or the disappearance characteristics of radioactivity derived from ^{3}H-dA in either plasma or brain.

Interactions Between Δ^9-THC and COC

Acute interactions. Figure 4 shows the acute dose-effect relationships for Δ^9-THC and COC alone and in all combinations for the five tests in this battery. The results for Δ^9-THC alone were similar in all respects to those in the previous experiment. Δ^9-THC significantly impaired CAR ($F(3,298) = 40.1$, $p<0.001$) and rotarod ($F(3,203) = 9.3$, $p<0.001$) performance, depressed photocell activity at the highest dose, $F(3,215) = 10.3$, $p<0.001$, and caused dose-related bradycardia, $F(3,213) = 58.4$, $p<0.001$, and hypothermia, $F(3,216) = 55.4$, $p<0.001$.

COC alone did not significantly influence CAR performance although there was a trend toward enhanced performance accompanied by a significant, dose-related increase in intertrial responses, $F(3,298) = 5.9$, $p<0.001$. The highest dose of COC (30 mg/kg, 1.25 ± 0.19) caused a twofold increase in intertrial responses per minute compared with controls treated subacutely with SO and given SO before COC on the test day (0.68 ± 0.10; $t(298) = 3.0$, $p<0.01$). COC did not significantly influence the impairment of CAR performance caused by Δ^9-THC. However, the highest dose of Δ^9-THC antagonized the increase in intertrial responses caused by all doses of COC (all $ts(298) \geq 3.0$, all $ps<0.01$ comparing comparable doses of COC with and without 10 mg/kg Δ^9-THC.

Photocell activity was increased as a function of increasing doses of COC, $F(3,215) = 19.4$, $p<0.001$. Compared with controls (341 ± 19 counts/10 min), this effect was significant for 20 (562 ± 35 counts/10 min) and 30 (649 ± 68 counts/10 min) mg/kg COC ($ts(215) = 3.6$ and 4.9, $ps<0.01$). There was mutual antagonism between the effects of Δ^9-THC and COC on photocell activity depending on the dose of each. Δ^9-THC caused a significant dose-related reduction in COC-stimulated photocell activity at the two highest doses of COC. On the other hand the decrease in photocell activity caused by 10 mg/kg Δ^9-THC (282 ± 47 counts/10 min) was restored to control levels by the highest dose of COC (398 ± 63 counts/10 min).

COC alone did not significantly affect rotarod performance. Nor was there any interaction between COC and doses of 2.5 and 10.0 mg/kg Δ^9-THC. The impairment caused by 5.0 mg/kg Δ^9-THC appeared to be antagonized by 20 and 30 mg/kg COC ($ts(213) = 3.6$ and 2.6, $ps<0.01$). However, in view of the variability within and between experiments on this measure and the nonsystematic nature of this effect, we are hesitant to attribute any importance to this result.

Doses of 20 and 30 mg/kg COC caused a slight but significant decrease in heart rate from 512 ± 8.7 bpm to

TABLE 1

EFFECTS OF ACUTE AND SUBACUTE TREATMENT WITH dA ON THE TIME COURSE OF RADIOACTIVITY DERIVED FROM ^{14}C-Δ^9-THC IN PLASMA AND BRAIN*

Treatment†				Time Sampled After Administration of ^{14}C-THC (Hr)									
Days 1–6	Day 7‡			1		2		4		8		24	
	T_0	$T_{1.5}$	Tissue	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SO	^{14}C-THC	SAL	Plasma	0.47	0.033	0.61	0.053	0.66	0.061	0.44	0.037	0.16	0.011
SO	^{14}C-THC	dA		0.40	0.023	0.62	0.050	0.46§	0.031	0.41	0.044	0.19	0.012
dA	^{14}C-THC	dA		0.38	0.036	0.59	0.044	0.39§	0.027	0.35	0.026	0.16	0.011
THC	^{14}C-THC	SAL		0.46	0.039	0.82§	0.091	0.64	0.052	0.41	0.026	0.17	0.013
THC	^{14}C-THC	dA		0.48	0.047	0.66¶	0.044	0.55	0.023	0.44	0.024	0.20	0.012
SO	^{14}C-THC	SAL	Brain	0.27	0.019	0.48	0.047	0.63	0.102	0.45	0.053	0.23	0.017
SO	^{14}C-THC	dA		0.28	0.018	0.67§	0.085	0.75	0.086	0.43	0.057	0.30	0.052
dA	^{14}C-THC	dA		0.28	0.021	0.60	0.065	0.64	0.098	0.46	0.032	0.26	0.035
THC	^{14}C-THC	SAL		0.28	0.028	0.75§	0.079	0.78	0.071	0.53	0.031	0.24	0.034
THC	^{14}C-THC	dA		0.26	0.018	0.66§	0.060	1.04§¶	0.070	0.53	0.039	0.24	0.018

*Values are expressed as μg equivalents of Δ^9-THC per milliliter of plasma or per gram of brain. There were 16 or 17 rats per group sampled serially for plasma and 10 rats per group sacrificed at each time point for brain.

†SO represents sesame oil and SAL represents saline.

‡^{14}C-THC was given orally in sesame oil at time zero (T_0) followed 1.5 hr later ($T_{1.5}$) by an IP injection of saline or dA in saline.

§$p<0.05$ compared with SO pretreatment

¶$p<0.05$ compared with THC-pretreatment

TABLE 2

EFFECTS OF ACUTE AND SUBACUTE TREATMENT WITH Δ^9-THC ON THE TIME COURSE OF RADIOACTIVITY DERIVED FROM ^{3}H-dA IN PLASMA AND BRAIN*

Treatment†				Time Sampled After Administration of ^{3}H-dA (Hr)									
Days 1–6	Day 7‡			0.25		0.5		1.5		2.5		6.5	
	T_0	$T_{1.5}$	Tissue	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SO	SO	^{3}H-dA	Plasma	0.67	0.053	0.57	0.019	0.41	0.026	0.37	0.015	0.26	0.008
SO	THC	^{3}H-dA		0.61	0.039	0.57	0.039	0.42	0.014	0.36	0.020	0.26	0.012
THC	THC	^{3}H-dA		0.61	0.042	0.63	0.019	0.46	0.027	0.41	0.021	0.28	0.015
dA	SO	^{3}H-dA		0.52	0.066	0.52	0.035	0.37	0.020	0.36	0.024	0.28	0.017
dA	THC	^{3}H-dA		0.48	0.054	0.52	0.043	0.38	0.008	0.34	0.013	0.28	0.024
SO	SO	^{3}H-dA	Brain	1.71	0.109	1.58	0.104	0.58	0.025	0.32	0.015	0.22	0.022
SO	THC	^{3}H-dA		2.06	0.029	1.72	0.009	0.54	0.037	0.34	0.026	0.25	0.029
THC	THC	^{3}H-dA		2.02	0.141	1.79	0.068	0.68§	0.041	0.31	0.020	0.18	0.017
dA	SO	^{3}H-dA		1.81	0.214	1.52	0.110	0.54	0.016	0.32	0.027	0.19	0.017
dA	THC	^{3}H-dA		2.08	0.156	1.54	0.021	0.65¶	0.034	0.34	0.016	0.21	0.016

*Values are expressed as μg equivalents of dA per milliliter of plasma or per gram of brain. There were 5 or 6 rats per group sampled serially for plasma and 5 rats per group sacrificed at each time point for brain.

†SO represents sesame oil.

‡Sesame oil or THC in sesame oil was given orally at time zero (T_0) followed 1.5 hr later ($T_{1.5}$) by an IP injection of ^{3}H-dA in saline.

§$p<0.05$ compared with SO pretreatment

¶$p<0.05$ compared with dA pretreatment

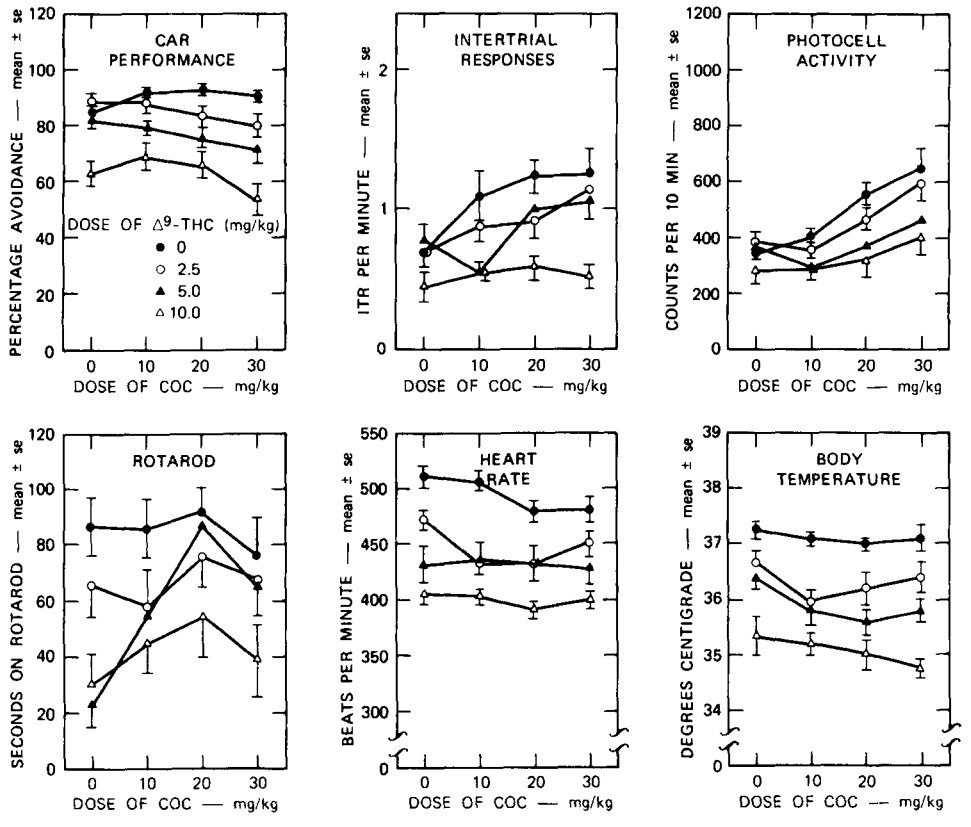

FIG. 4. Acute reciprocal dose response interactions between Δ9-THC and COC. There were 18 to 20 rats in each group tested for CAR performance and 13 to 17 for the other measures.

477 ± 7.4 and 480 ± 11.4 bpm, respectively, $ts(213) = 2.4$ and 2.3, $ps<0.05$. The decrease in heart rate caused by 2.5 mg/kg Δ9-THC (471 ± 7.8 bpm) was significantly augmented by 10 (432 ± 10.1 bpm) and 20 (433 ± 13.7 bpm) mg/kg COC, $ts(213) = 2.6$, $ps<0.01$. However, there were no differences in the bradycardia caused by 5 and 10 mg/kg Δ9-THC attributable to any dose of COC.

Body temperature was not significantly affected by COC alone. A significant potentiation of the hypothermia caused by Δ9-THC was seen for certain combinations of both drugs (2.5 mg/kg Δ9-THC plus 10 mg/kg COC, $t(203) = 2.1$, $p<0.05$, 5 mg/kg Δ9-THC plus 20 mg/kg COC, $t(203) = 2.4$, $p<0.05$, 10 mg/kg Δ9-THC plus 30 mg/kg COC, $t(203) = 2.0$, $p<0.05$. However, these differences, although statistically significant, were all less than 1°C.

The acute effects of Δ9-THC and COC alone and in combination as determined by this battery of tests can be summarized as follows: (1) Δ9-THC alone caused impairment of CAR and rotarod performance, depressed photocell activity, and decreased heart rate and body temperature; (2) COC alone increased intertrial responding that resulted in a trend (not significant) toward an increase in CAR. This general stimulant action of COC was clearly reflected in a dose-related increase in photocell activity; (3) COC was unable to systematically antagonize the impairment of CAR and rotarod performance caused by Δ9-THC, but the two drugs were mutually antagonistic of their opposing effects on photocell activity, the net result depending on the respective doses of each; and (4) COC alone caused a slight decrease in heart rate but it had no effect on body temperature. COC appeared to augment the bradycardia and hypothermia caused by Δ9-THC at some dose combinations. However, because of the lack of any systematic dose-effect relationships in this regard, the importance of these interactive effects is questionable.

Subacute treatment with Δ9-THC. Figure 5 shows the acute dose-effect relationships for COC alone and in combination with 10 mg/kg Δ9-THC compared with the effects seen after subacute pretreatment with 10 mg/kg/day Δ9-THC for six days. The effects of subacute treatment with both 10 mg/kg/day Δ9-THC and 20 mg/kg/day COC for all seven days are also shown.

As in the previous experiment there was clear tolerance to the impairing effect of Δ9-THC on CAR performance. Acute administration of 10 mg/kg Δ9-THC reduced performance to 60 ± 4.4% compared with 84 ± 3.6% CAR in controls. Subacute treatment with Δ9-THC restored performance to 81 ± 3.7%, which was not significantly different from controls ($p> 0.1$).

The dose response curves for COC combined with 10 mg/kg Δ9-THC in SO-pretreated and Δ9-THC-pretreated rats were essentially parallel. In neither case did any dose of COC cause any significant differences in CAR performance compared with the zero dose of COC combined with Δ9-THC (all $ps>0.1$). CAR performance was significantly better in the rats treated subacutely with Δ9-THC and given the combination of Δ9-THC and COC

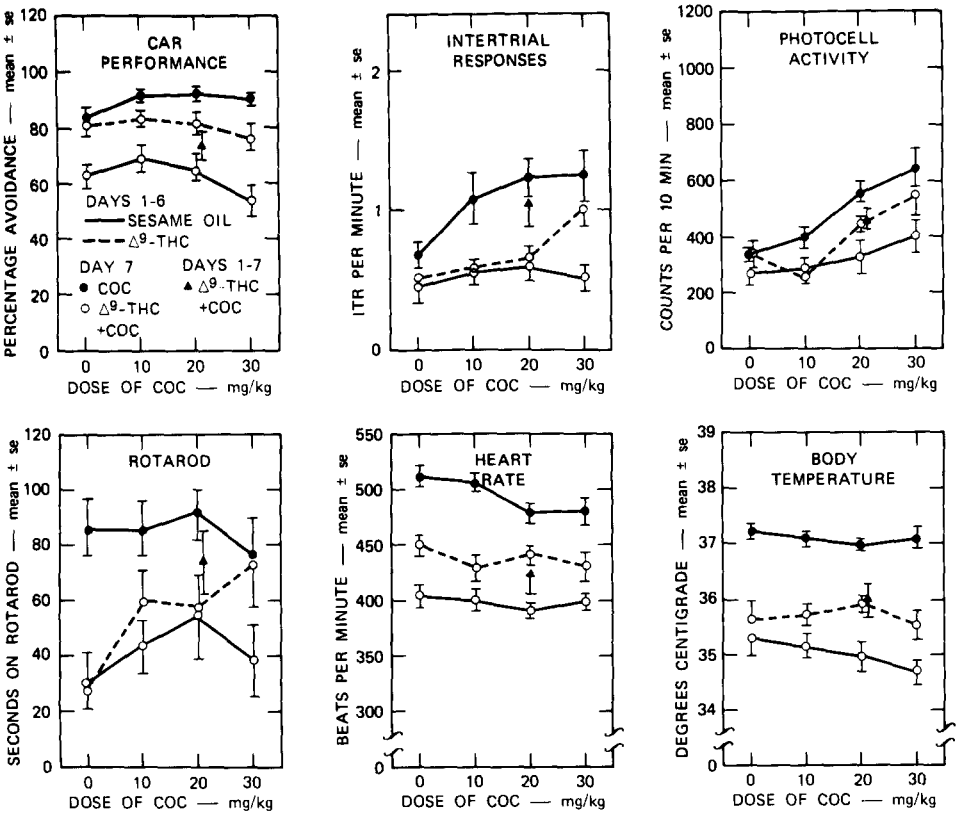

FIG. 5. Interactions between Δ⁹-THC and COC after subacute treatment with 10 mg/kg/day Δ⁹-THC for six days. There were 18 to 20 rats in each group tested for CAR performance and 13 to 17 for the other measures.

than in the rats treated subacutely with SO and given the combination of Δ⁹-THC and COC for all doses of COC (all $ts(462) \geq 2.5$, all $ps < 0.05$), reflecting the tolerance to Δ⁹-THC. Subacute treatment with both 10 mg/kg Δ⁹-THC and 20 mg/kg COC resulted in CAR performance that was intermediate between the performance caused by this combination given to SO-pretreated and Δ⁹-THC-pretreated rats.

Although CAR performance was restored to control levels after subacute treatment with Δ⁹-THC, the tolerance was not generally complete. Sufficient residual activity of Δ⁹-THC remained to significantly antagonize the increase in intertrial responses caused by COC. Intertrial responses were not significantly different in rats treated subacutely with SO or 10 mg/kg Δ⁹-THC and given the combinations of Δ⁹-THC and 10 or 20 mg/kg COC ($ps > 0.1$), but they were significantly lower than when these doses of COC were given alone to rats treated subacutely with SO, $ts(462) \geq 2.5$, $ps < 0.05$. This residual antagonism of intertrial responses by Δ⁹-THC in Δ⁹-THC-tolerant rats also appeared to reduce the percentage CAR, but the differences were not significant. Nevertheless, this result again suggests that the slight, although nonsignificant, increase in CAR after acute treatment with COC alone was caused by the increase in intertrial responses that resulted in fortuitous responses, rather than enhanced performance. Only the highest dose of COC (30 mg/kg) caused any apparent increase in intertrial responses in rats treated subacutely with Δ⁹-THC compared with controls but it was not significant, $t(462) = 1.8$, $p < 0.1$. However, compared with the acute combination the increase was significant, $t(462) = 2.5$, $p < 0.05$. Although there was no difference in intertrial responses caused by the combination of 10 mg/kg Δ⁹-THC and 20 mg/kg COC in rats treated subacutely with SO (0.58 ± 0.08 ITR/min) or Δ⁹-THC (0.66 ± 0.07 ITR/min), there was a significant increase in intertrial responses by the rats treated subacutely with both drugs at these doses (1.05 ± 0.18 ITR/min, $t(462) = 2.3$, $p < 0.05$.

Tolerance to the depressant effect of 10 mg/kg Δ⁹-THC on photocell activity was again apparent in this experiment. Photocell activity in rats treated subacutely with Δ⁹-THC was 342 ± 50 counts/10 min compared with 341 ± 19 counts/10 min in controls and 282 ± 47 counts/10 min in rats treated acutely with Δ⁹-THC. Photocell activity of rats treated subacutely with Δ⁹-THC and given the combination of Δ⁹-THC and 20 or 30 mg/kg COC was intermediate between that of rats treated with COC alone and the combination after subacute treatment with SO. This result again suggests a residual antagonistic activity of Δ⁹-THC remaining after subacute treatment with Δ⁹-THC for six days. The combination of Δ⁹-THC with 10 mg/kg COC did not conform to the above results for reasons that are not apparent. Nor was there any indication of any sensitization to the effect of COC on photocell activity in the group treated subacutely with both Δ⁹-THC

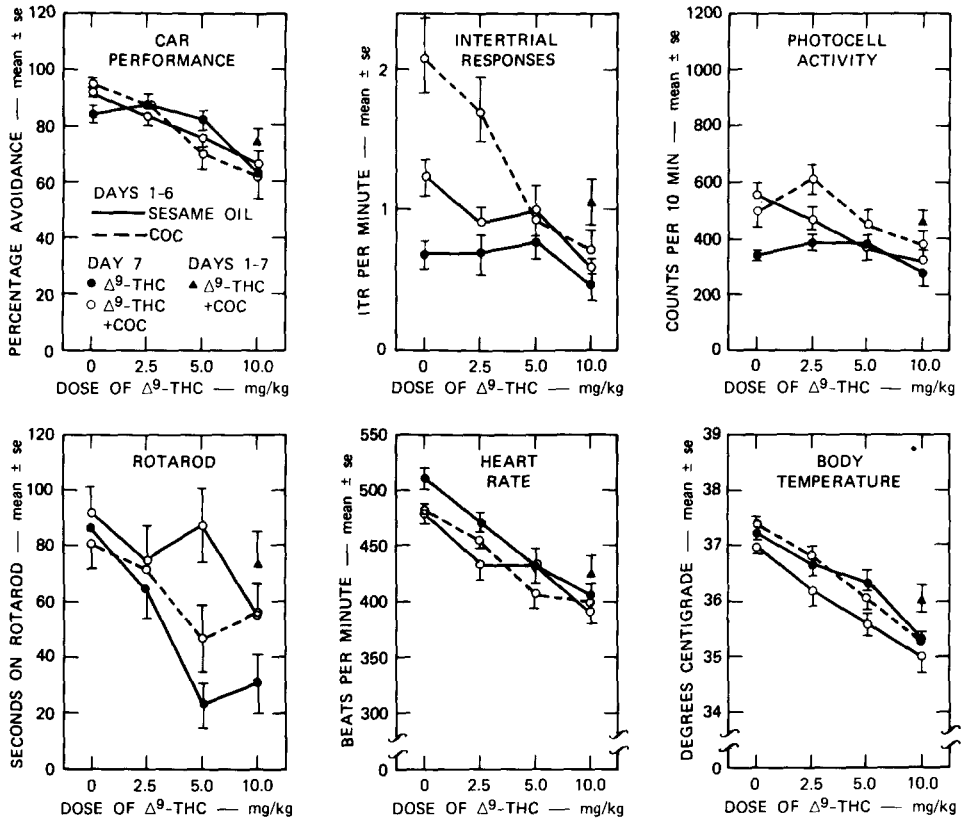

FIG. 6. Interactions between Δ9-THC and COC after subacute treatment with 20 mg/kg/day COC for six days. There were 18 to 20 rats in each group tested for CAR performance and 13 to 17 for the other measures.

and COC (461 ± 38 counts/10 min) compared with the same combination after subacute treatment with Δ9-THC alone (436 ± 27 counts/10 min).

Contrary to the results of the previous (and the subsequent) experiment, there was no evidence for tolerance to the impairing effect of Δ9-THC on rotarod performance in this experiment. Inspection of the raw data did not reveal any insights into this discrepancy. As noted before, the variability, both within and between experiments, on this measure has been troublesome and precludes firm conclusions based on single experiments. Because of this discrepancy further discussion of the results for this measure are considered unwarranted for this part of the experiment.

Partial tolerance to the bradycardic effects of Δ9-THC was again seen in this experiment ($t(337) = 2.6$, $p<0.01$, comparing acute and subacute Δ9-THC alone). The combination of Δ9-THC and any dose of COC was not significantly different for Δ9-THC alone in such groups treated subacutely with Δ9-THC. Heart rate was higher in all groups treated subacutely with Δ9-THC than in the groups treated subacutely with SO and then given the combination, reflecting the tolerance to Δ9-THC. Subacute treatment with both Δ9-THC and COC did not cause any significant difference in heart rate compared with subacute treatment with Δ9-THC alone.

Tolerance to the hypothermia caused by Δ9-THC was not significant in this experiment. However, the hypothermia was less in rats treated subacutely with Δ9-THC and given Δ9-THC and 20 or 30 mg/kg COC than in comparable groups treated subacutely with SO, $ts(342) = 2.2$ and 2.5, $ps<0.05$ and 0.01, respectively. The subacute combination of Δ9-THC and COC did not cause any changes in body temperature from those caused by subacute treatment with Δ9-THC alone.

The effects of Δ9-THC and COC given alone and in combination after subacute treatment with 10 mg/kg Δ9-THC alone or in combination with 20 mg/kg COC can be summarized as follows: (1) tolerance developed to most of the effects of 10 mg/kg Δ9-THC, but sufficient residual activity remained to partially antagonize the stimulant effects of COC reflected as increases in intertrial responses and photocell activity; (2) CAR performance was better and the bradycardia and hypothermia were less after subacute than acute treatment with 10 mg/kg Δ9-THC and these responses were not influenced by COC; and (3) there was some evidence for sensitization to the stimulant effects of COC in rats treated subacutely with both Δ9-THC and COC as reflected by a greater number of intertrial responses compared with Δ9-THC-tolerant rats treated acutely with COC.

Subacute treatment with COC. Figure 6 shows the results after subacute treatment with 20 mg/kg/day COC. The results after subacute treatment with both Δ9-THC and COC are repeated for comparison.

There were no significant effects on CAR performance

caused by subacute treatment with COC, the results resembling those seen after acute treatment with COC alone or in combination with Δ⁹-THC. However, subacute treatment with COC caused a significantly greater increase in intertrial responses (308% of controls) than the increase caused by acute treatment (181% of controls), $t(462) = 4.3$, $p<0.01$. Δ⁹-THC caused a dose-related antagonism of this sensitizing effect of subacute treatment with COC. The sensitizing effect of subacute treatment with COC was also unmasked in the rats treated subacutely with Δ⁹-THC and COC and tolerant to the antagonistic effects of Δ⁹-THC. These rats made 46% more ITR/min than the comparably dosed rats treated subacutely with COC alone.

The sensitizing effect of subacute treatment with COC was not reflected in a greater increase in photocell activity than that caused by acute treatment. However, photocell activity was significantly higher in the COC-pretreated rats given 2.5 mg/kg Δ⁹-THC in combination with COC than in the comparably dosed rats not subacutely pretreated with COC, $t(341) = 2.2$, $p< 0.05$. This effect was also apparent for the higher doses of Δ⁹-THC, but the differences were not significant. However, in the rats made tolerant to the depressant effects of Δ⁹-THC along with the cumulative effects of COC (i.e., given both drugs subacutely) the increase (46%) was significant, $t(341) = 2.2$, $p<0.05$.

Although the results for rotarod performance were considered unsatisfactory, there was some evidence that acute treatment with COC offset the impairing effect of Δ⁹-THC on this measure. A similar result was seen after subacute treatment with COC, but in no comparison was the difference significant. If anything, subacute treatment with COC was less effective in this regard than acute treatment.

There were no significant effects of subacute treatment with COC on heart rate compared with acute treatment either alone or in combination with Δ⁹-THC. However, the enhanced hypothermia caused by the acute combination of Δ⁹-THC and COC appeared to be offset by subacute treatment with COC; this effect was significant for the 2.5 mg/kg dose of Δ⁹-THC, $t(243) = 2.0$, $p<0.05$.

The main result of these experiments was a further suggestion, implied from the previous section in which both Δ⁹-THC and COC were administered subacutely, that some of the stimulant properties of COC were enhanced by subacute treatment. The evidence for this conclusion was the greater increase in intertrial responding after subacute than acute treatment with COC and a resistance to the antagonism by Δ⁹-THC of COC-stimulated photocell activity.

Effects of COC on ¹⁴C–Δ⁹-THC. Only the radioactivity in plasma was sampled in this experiment. Table 3 shows the levels of radioactivity in plasma as a function of time after administration of ¹⁴C–Δ⁹-THC. Although the absolute values were higher in this experiment than they were in the previous experiment, the shape of the curve for controls (i.e., given ¹⁴C–Δ⁹-THC and vehicles only) was almost identical. Subacute treatment with Δ⁹-THC again caused an increase in the average peak levels of radioactivity at 2 (19%) and 4 (27%) hr in this experiment, but the differences were not significant. Acute treatment with COC did not significantly affect the levels of radioactivity at any time point compared with the levels in controls given only ¹⁴C–Δ⁹-THC. Nor did COC significantly affect the increased levels of radioactivity at 2 and 4 hr caused by subacute treatment with Δ⁹-THC. Subacute treatment with COC tended to lower the levels of radioactivity at all time points compared with controls given ¹⁴C–Δ⁹-THC only, but none of the difference were significant.

In summary, acute or subacute treatment with COC did not significantly alter the time course of total radioactivity derived from ¹⁴C–Δ⁹-THC in plasma under any of the treatment conditions of this experiment.

Effects of Δ⁹-THC on ¹⁴C–COC. Table 4 shows the results of the reciprocal experiment done to examine any possible effects of Δ⁹-THC on the time course of ¹⁴C–COC in plasma.

Radioactivity in the control rats treated with ¹⁴C–COC and vehicle only reached a maximum within the first 30 min (the earliest time point sampled) after IP injection and declined in two phases as judged by the shape of the semilog plot and the best fit lines. The estimated $t_{1/2}$ for the first phase was 1.2 hr and for the second phase it was 16.3 hr. There were no significant differences in plasma radioactivity caused by the treatment conditions of this experiment at any time point sampled.

Interaction Between Δ⁹-THC and NIC

Acute interactions. Figure 7 shows the acute dose-effect relationships for Δ⁹-THC and NIC alone and in all combinations for the five tests in this battery. The results for Δ⁹-THC alone were essentially the same as they were in the previous two experiments. Δ⁹-THC significantly impaired CAR ($F(3,228) = 23.4$, $p<0.001$) and rotarod ($F(3,148) = 26.6$, $p<0.001$) performance, depressed photocell activity ($F(3,148) = 48.9$, $p<0.001$), and caused dose-related bradycardia ($F(3,146) = 107.4$, $p<0.001$) and hypothermia ($F(3,148) = 63.8$, $p<0.001$).

Acute administration of 0.25 to 1.0 mg/kg NIC alone did not significantly influence CAR performance nor, unlike dA and COC, did it show any stimulating effect on intertrial responding. There were no significant interactions between Δ⁹-THC and NIC on these two measures.

NIC, in contrast to dA and COC, also did not significantly influence photocell activity when it was administered acutely alone at these doses. However, NIC interacted with Δ⁹-THC to almost completely abolish photocell activity, $F(9,148) = 3.4$, $p<0.01$. Whereas there was antagonism between the depressant effects of Δ⁹-THC and the stimulant effects of dA and COC, NIC clearly and markedly potentiated the depression caused by Δ⁹-THC. For all combinations of Δ⁹-THC and NIC the decrease in photocell activity was significantly greater than that caused by Δ⁹-THC alone (all ts(146)>4.0, all ps<0.01 compared with any dose of Δ⁹-THC alone).

NIC also augmented the depressant properties of Δ⁹-THC on the other measures in the test battery. Rotarod performance was severely impaired by the combination of all doses of NIC with all doses of Δ⁹-THC even though NIC alone did not significantly influence rotarod performance. Only the highest dose of NIC alone slightly but significantly reduced heart rate, $t(146) = 2.0$, $p<0.05$, and body temperature, $t(148) = 2.2$, $p<0.05$. However, in combination with Δ⁹-THC all doses of NIC caused greater bradycardia (all ts(146)>2.5, all ps<0.05 except 5 mg/kg Δ⁹-THC plus 0.5 mg/kg NIC and 10 mg/kg Δ⁹-THC plus 0.25 mg/kg NIC that were not significant) and hypothermia (all ts(148)>3.8, all ps<0.01) than caused by Δ⁹-THC alone.

The acute effects of Δ⁹-THC and NIC alone and in

TABLE 3

EFFECTS OF ACUTE AND SUBACUTE TREATMENT WITH COC ON THE TIME COURSE OF RADIOACTIVITY DERIVED FROM ^{14}C-THC IN PLASMA*

Treatment†			Time Sampled After Administration of ^{14}C-THC (Hr)									
Days 1–6	Day 7‡		1		2		4		8		24	
	T_0	$T_{1.5}$	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SO	^{14}C-THC	SAL	0.48	0.043	1.17	0.092	1.29	0.189	0.87	0.104	0.49	0.073
SO	^{14}C-THC	COC	0.42	0.037	1.14	0.118	1.15	0.151	0.91	0.106	0.40	0.071
COC	^{14}C-THC	COC	0.39	0.047	1.10	0.085	0.96	0.084	0.77	0 097	0.44	0.060
THC	^{14}C-THC	SAL	0.46	0.059	1.39	0.079	1.64	0.195	0.96	0.077	0.42	0.066
THC	^{14}C-THC	COC	0.48	0.074	1.34	0.193	1.59	0.189	1.11	0.087	0.47	0.097

*Values are expressed as μg equivalents of Δ^9-THC per milliliter of plasma. There were 5 or 6 rats per group sampled serially.
†SO represents sesame oil and SAL represents saline.
‡^{14}C-THC was given orally in sesame oil at time zero (T_0) followed 1.5 hr later ($T_{1.5}$) by an IP injection of saline or COC in saline.

TABLE 4

EFFECTS OF ACUTE AND SUBACUTE TREATMENT WITH Δ^9-THC ON THE TIME COURSE OF RADIOACTIVITY DERIVED FROM ^{14}C-COC IN PLASMA*

Treatment†			Time Sampled After Administration of ^{14}C-COC (Hr)									
Days 1–6	Day 7‡		0.5		1.0		2.5		6.5		22.5	
	T_0	$T_{1.5}$	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SO	SO	^{14}C-COC	7.3	0.31	5.4	0.21	4.6	0.23	4.1	0.24	2.0	0.11
SO	THC	^{14}C-COC	7.6	0.15	5.5	0.13	4.5	0.16	4.6	0.21	2.5	0.10
THC	THC	^{14}C-COC	7.9	0.24	5.9	0.18	4.7	0.30	4.9	0.14	2.5	0.20
COC	SO	^{14}C-COC	7.1	0.27	5.6	0.20	4.6	0.22	4.3	0.23	2.3	0.15
COC	THC	^{14}C-COC	7.1	0.38	5.0	0.46	4.3	0.27	4.6	0.26	2.2	0.10

*Values are expressed as μg equivalents of COC per milliliter of plasma. There were 9 to 11 rats per group sampled serially.
†SO represents sesame oil.
‡Sesame oil or THC was given orally at time zero (T_0) followed 1.5 hr later ($T_{1.5}$) by an IP injection of ^{14}C-COC in saline.

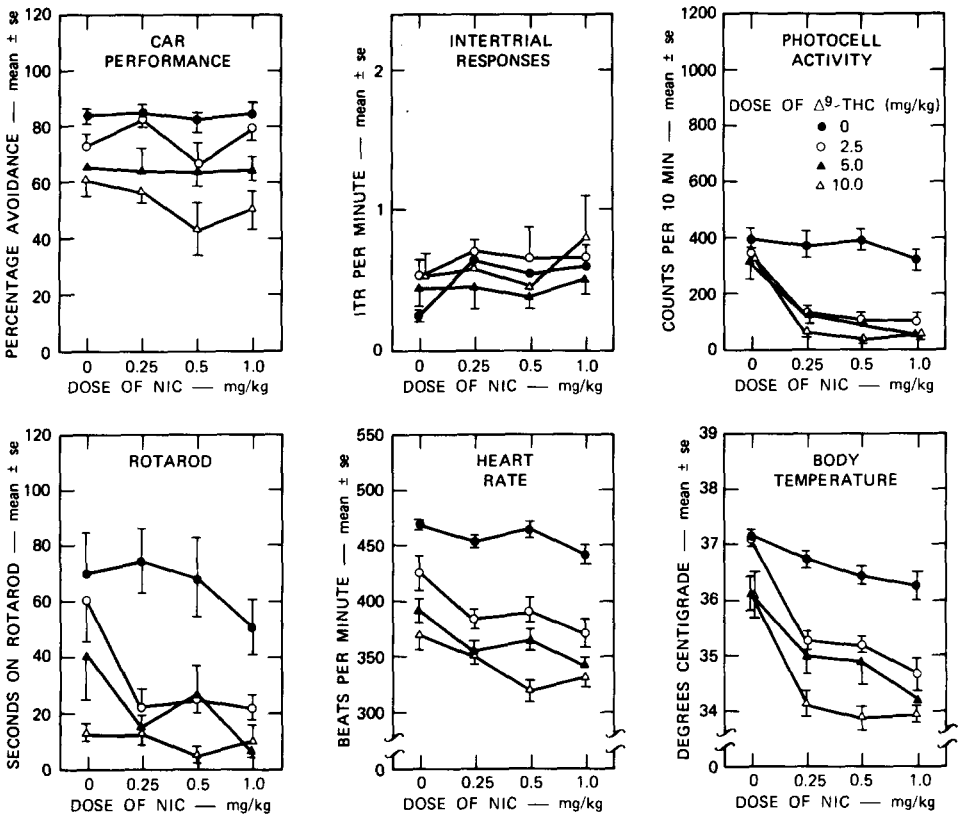

FIG. 7. Acute reciprocal dose response interactions between Δ9-THC and NIC. There were 14 to 17 rats in each group tested for CAR performance and 9 to 11 for the other measures.

combination can be summarized as follows: (1) confirmatory of the results of the two previous experiments, Δ9-THC alone caused impairment of CAR and rotarod performance, depressed photocell activity, and decreased heart rate and body temperature; (2) acute treatment with NIC alone did not appreciably influence any of the behavioral or physiological measures in this test battery; (3) however, NIC interacted with Δ9-THC to potentiate markedly the latter's depressant effects on rotarod performance, photocell activity, heart rate, and body temperature; and (4) this interactive effect was not the result of a complete motor collapse because CAR performance was spared.

Subacute treatment with Δ9-THC. Figure 8 shows the acute dose-effect relationships for NIC alone and in combination with 10 mg/kg Δ9-THC compared with the effects seen after subacute treatment with 10 mg/kg/day Δ9-THC for six days. The effects of subacute treatment with both 10 mg/kg/day Δ9-THC and 0.5 mg/kg/day NIC for all seven days are also shown.

Clear tolerance to the effects of Δ9-THC on CAR performance was again seen in this experiment. Whereas acute administration of 10 mg/kg Δ9-THC caused a 27% reduction in CAR compared with SO-treated controls, the difference was only 5% after subacute treatment (NS). Similar to the results after acute administration, there were no significant interactive effects between Δ9-THC and NIC in such Δ9-THC-tolerant rats on CAR performance or intertrial responding. The response to subacute treatment with both Δ9-THC and NIC was not significantly different from that in rats treated subacutely with Δ9-THC alone on these two measures at comparable doses.

There was no significant difference in photocell activity between the depressant effects caused by acute and subacute treatment with 10 mg/kg Δ9-THC in this experiment. However, tolerance to this effect of Δ9-THC was again indicated by the attenuated response to the combination of Δ9-THC and NIC after subacute treatment with Δ9-THC. Whereas the acute combination of 10 mg/kg Δ9-THC and as little as 0.25 mg/kg NIC almost completely eliminated photocell activity, this interactive effect was significantly less after subacute treatment with Δ9-THC, $t(230) = 3.3$, $p<0.01$. However, the highest dose of NIC (1.0 mg/kg) was still as effective in completely suppressing photocell activity when given in combination with 10 mg/kg Δ9-THC after subacute treatment with Δ9-THC as after their acute combination.

A similar result was found for rotarod performance. Complete tolerance to the impairing effect of Δ9-THC was seen in this experiment. NIC caused a dose-related impairment of rotarod performance in such Δ9-THC-tolerant animals, $ts(230) = 1.6$, 2.4 and 4.2; $ps<0.1$, 0.05, and 0.01 for 0.25, 0.5 and 1.0 mg/kg NIC, respectively.

There was significant tolerance to the bradycardia, $t(228) = 3.6$, $p<0.01$, and hypothermia, $t(230) = 2.1$, $p<0.05$, caused by Δ9-THC. The enhanced dose-response interaction to the combination of Δ9-THC and NIC in such Δ9-THC-tolerant animals was similar in shape to that

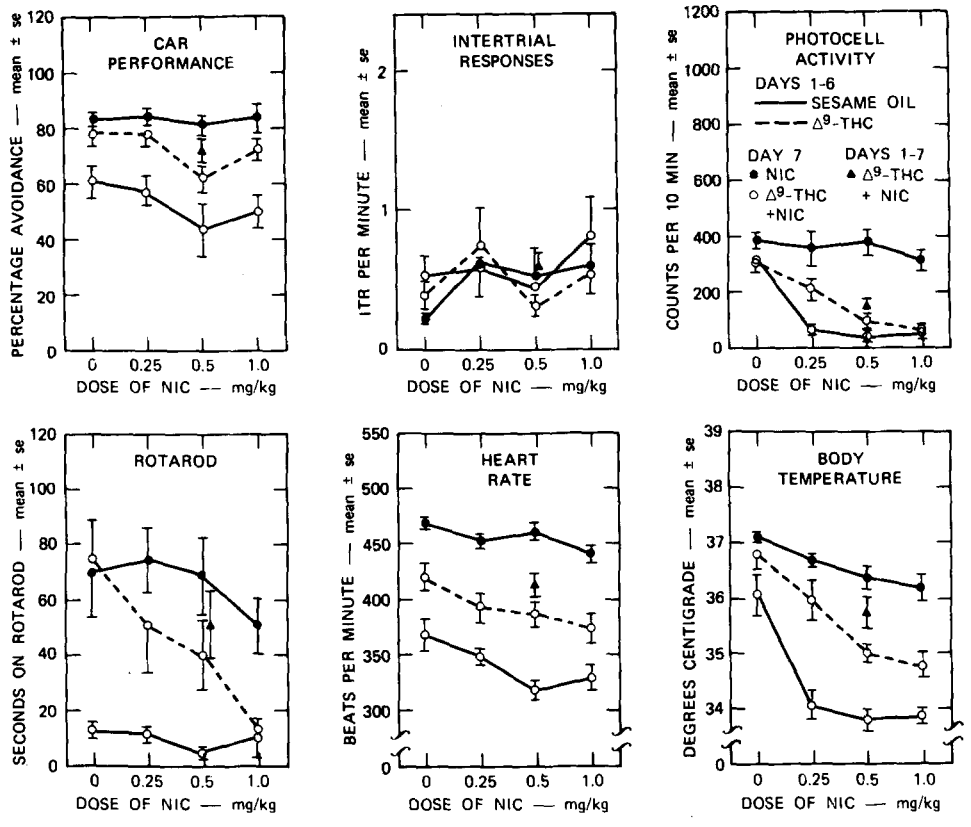

FIG. 8. Interactions between Δ^9-THC and NIC after subacute treatment with 10 mg/kg/day Δ^9-THC for six days. There were 14 to 17 rats in each group tested for CAR performance and 9 to 11 for the other measures.

seen after their acute combination but the effects were less, reflecting the tolerance to Δ^9-THC.

For all measures the effects of subacute treatment with both Δ^9-THC and NIC were not significantly different from the same combination of doses after subacute treatment with Δ^9-THC alone.

The results of this experiment again demonstrated the development of tolerance to the effects of Δ^9-THC on the measures in this test battery. However, again the tolerance was not complete for all measures as revealed especially by the interaction with NIC. Sufficient residual Δ^9-THC activity remained after subacute treatment for six days to cause almost complete suppression of photocell activity and rotarod performance when combined with the highest doses of NIC, even though these doses of NIC did not cause any significant effects on these parameters when given acutely alone.

Subacute treatment with NIC. Figure 9 shows the results after subacute treatment with 0.5 mg/kg/day NIC. The results after subacute treatment with both Δ^9-THC and NIC are repeated for comparison.

With one exception there were no significant differences between acute and subacute treatment with NIC or its interaction with Δ^9-THC on any of the measures in this test battery. The exception was that intertrial responses were significantly increased after subacute treatment with NIC compared with controls, $t(351) = 2.8$, $p<0.01$. This effect was completely antagonized by the lowest dose of Δ^9-THC (2.5 mg/kg).

Effects of NIC on ^{14}C-Δ^9-THC. Table 5 shows the plasma and brain levels of radioactivity derived from ^{14}C-Δ^9-THC as a function of time after its oral administration. The results after acute administration of ^{14}C-Δ^9-THC alone were essentially the same in this as in the two previous experiments – levels rose to reach a maximum 2 to 4 hr after oral administration in both plasma and brain and declined thereafter. Also, the increases in plasma levels of radioactivity caused by subacute treatment with Δ^9-THC were significant at 1, 2 and 4 hr (all $ts(93)>2.9$, all $ps<0.01$).

Both acute and subacute treatment with NIC appeared to cause the maximum levels of radio activity in plasma to be less than in comparable controls. This effect was significant after acute treatment with NIC at 4 hr in the group treated subacutely with sesame oil, $t(93) = 2.0$, $p<0.05$, and in the group treated subacutely with NIC, $t(93) = 3.9$, $p<0.01$. In the groups treated subacutely with Δ^9-THC, and in which levels of radioactivity were elevated, this effect of NIC was similar to that just described at 2 and 4 hr but the differences did not reach statistical significance, $ts(93) = 1.7$, $p<0.10$. There were no significant interactive effects of NIC on brain levels of radioactivity.

Effects of Δ^9-THC on ^{14}C-NIC. Table 6 shows the reciprocal effects of Δ^9-THC on ^{14}C-NIC as a function of

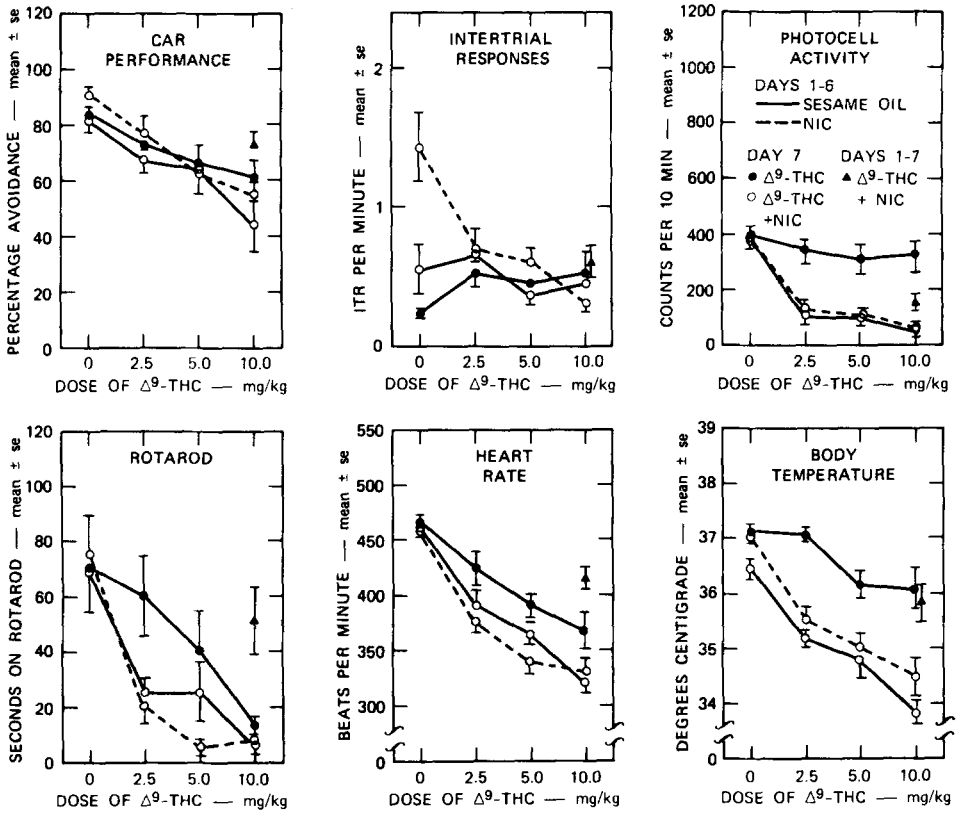

FIG. 9. Interactions between Δ^9-THC and NIC after subacute treatment with 0.5 mg/kg/day NIC for six days. There were 14 to 17 rats in each group tested for CAR performance and 9 to 11 for the other measures.

time after the IP administration of ^{14}C–NIC. There were no significant differences in the levels of radioactivity in either plasma or brain caused by subacute treatment with NIC compared with subacute treatment with sesame oil. Maximum levels were reached within 15 min after IP injection and disappeared in two phases. Disappearance of radioactivity was faster from brain than from plasma. The estimated $t_{1/2}$ for the first phase was 0.6 hr in brain compared with 1.2 hr in plasma. The $t_{1/2}$ for the second phase was 2.7 hr in brain compared with 4.6 hr in plasma.

Significantly higher levels of radioactivity were found at 0.25 hr in the plasma of rats treated subacutely with Δ^9-THC compared with controls treated only with SO and ^{14}C–NIC. A similar elevation in plasma radioactivity at 0.25 hr was also found in the rats treated subacutely with NIC and given Δ^9-THC acutely before ^{14}C–NIC. However, there were no other significant effects of acute or subacute treatment with Δ^9-THC at any other time sampled in plasma or at any time in brain. Therefore, we hesitate to attribute any major importance to these initial perturbations until their reliability has been confirmed.

DISCUSSION

Our purpose in conducting these and other similar experiments [25, 26, 27, 28] was to investigate the possible interactions between commonly used and/or abused drugs and a constituent in marihuana – Δ^9-THC. The three stimulants discussed here represent diverse chemical structures that may produce their pharmacological effects by somewhat different mechanisms [12]. dA and COC are thought to interact primarily with the catecholamine and/or serotonin systems [3], whereas NIC is generally regarded as a cholinergic drug [7,30]. Because some of the effects of cannabis may also involve these neurohumoral systems (see [14] for recent review), interactions at this level with all three stimulants might be expected. Significant interactions could also occur at the drug dispositional level (see [24]). Thus, the magnitude or duration of action of the respective drugs could be influenced by changes in their absorption, distribution, metabolism, or elimination caused by the interacting drug. This mode of interaction would be more likely if the drugs were given repeatedly because of the possible accumulation in tissues and/or enzyme induction. On the other hand, acute interactions could also occur at this level by competition for plasma binding sites, metabolic enzymes, or clearance routes. Regardless of the mechanisms involved, an empirical investigation of the kind reported here was considered necessary as a first step to identify any potential hazards or unusual reactions or interactions.

Acute Interactions

Although dA, COC, and NIC are all considered to be CNS stimulants, their effects alone and in combination with

TABLE 5

EFFECTS OF ACUTE AND SUBACUTE TREATMENT WITH NIC ON THE TIME COURSE OF RADIOACTIVITY DERIVED FROM ^{14}C-THC IN PLASMA AND BRAIN*

Treatment†				Time Sampled After Administration of ^{14}C-THC (Hr)									
Days 1–6	Day 7‡			1		2		4		8		24	
	T_0	$T_{1.5}$	Tissue	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SO	^{14}C-THC	SAL	Plasma	0.36	0.024	0.51	0.033	0.68	0.045	0.42	0.032	0.17	0.010
SO	^{14}C-THC	NIC		0.38	0.026	0.52	0.042	0.55§	0.038	0.50	0.034	0.20	0.014
NIC	^{14}C-THC	NIC		0.37	0.024	0.48	0.028	0.44§	0.026	0.49	0.034	0.20	0.012
THC	^{14}C-THC	SAL		0.49§	0.038	0.94§	0.081	0.89§	0.049	0.45	0.018	0.17	0.009
THC	^{14}C-THC	NIC		0.53§	0.038	0.81§	0.072	0.79	0.050	0.50	0.036	0.18	0.010
SO	^{14}C-THC	SAL	Brain	0.22	0.038	0.52	0.044	0.57	0.104	0.33	0.060	0.15	0.009
SO	^{14}C-THC	NIC		0.21	0.038	0.46	0.106	0.55	0.073	0.46	0.049	0.18	0.021
NIC	^{14}C-THC	NIC		0.20	0.034	0.48	0.044	0.49	0.064	0.53	0.031	0.21	0.021
THC	^{14}C-THC	SAL		0.24	0.022	0.62	0.059	0.72	0.095	0.49	0.109	0.16	0.021
THC	^{14}C-THC	NIC		0.27	0.031	0.70	0.077	0.57	0.071	0.48	0.102	0.16	0.017

*Values are expressed as μg equivalents of Δ^9-THC per milliliter of plasma or per gram of brain. There were 16 to 23 rats per group samples serially for plasma and 5 rats per group sacrificed at each time point for brain.

†SO represents sesame oil and SAL represents saline.

‡^{14}C-THC was given orally in sesame oil at time zero (T_0) followed 1.5 hr later ($T_{1.5}$) by an IP injection of saline or NIC in saline.

§$p<0.05$ compared with SO pretreatment

TABLE 6

EFFECTS OF ACUTE AND SUBACUTE TREATMENT WITH Δ^9-THC ON THE TIME COURSE OF RADIOACTIVITY DERIVED FROM ^{14}C-NIC IN PLASMA AND BRAIN*

Treatment†				Time Sampled After Administration of ^{14}C-NIC (Hr)									
Days 1–6	Day 7‡			0.25		0.5		1.5		2.5		6.5	
	T_0	$T_{1.5}$	Tissue	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SO	SO	^{14}C-NIC	Plasma	0.75	0.021	0.65	0.014	0.54	0.017	0 45	0.017	0.26	0.012
SO	THC	^{14}C-NIC		0.74	0.022	0.65	0.015	0.54	0.024	0.47	0.019	0.28	0.014
THC	THC	^{14}C-NIC		0.82§	0.024	0.70	0.031	0.54	0.024	0.46	0.016	0.25	0.008
NIC	SO	^{14}C-NIC		0.75	0.018	0.67	0.009	0.55	0.021	0.47	0.020	0.25	0.011
NIC	THC	^{14}C-NIC		0.84§¶	0.019	0.69	0.022	0.55	0.033	0.46	0.032	0.28	0.016
SO	SO	^{14}C-NIC	Brain	0.81	0.040	0.59	0.047	0.41	0.027	0.30	0.027	0.12	0.016
SO	THC	^{14}C-NIC		0.71	0.082	0.55	0.057	0.41	0.042	0.26	0.031	0.12	0.021
THC	THC	^{14}C-NIC		0.75	0.107	0.52	0.075	0.32	0.015	0.26	0.018	0.10	0.020
NIC	SO	^{14}C-NIC		0.86	0.050	0.64	0.035	0.39	0.041	0.33	0.024	0.12	0.015
NIC	THC	^{14}C-NIC		0.77	0.098	0.59	0.033	0.35	0.041	0.31	0.026	0.14	0.022

*Values are expressed as μg equivalents of nicotine per milliliter of plasma or per gram of brain. There were 9 to 12 rats per group sampled serially for plasma and 5 rats per group sacrificed at each time point for brain.

†SO represents sesame oil.

‡Sesame oil or THC was given orally at time zero (T_0) followed 1.5 hr later ($T_{1.5}$) by an IP injection of ^{14}C-NIC in saline.

§$p<0.05$ compared with SO pretreatment

¶$p<0.05$ compared with NIC pretreatment

Δ^9-THC were different depending on the tests used in these experiments. None of the three drugs when given alone at the doses used had any appreciable effect on performance of a conditioned avoidance task. Nor did they influence the dose-related impairment caused by Δ^9-THC on this task. However, both dA and COC caused an increase in intertrial responding that led to a slightly higher percentage of avoidance than controls. The highest dose of Δ^9-THC antagonized this increase in intertrial responding with a consequent reduction in CAR. Acute treatment with NIC, on the other hand, did not affect intertrial responding at any dose.

Δ^9-THC completely antagonized the increase in photocell activity caused by all doses of dA and the net result resembled that caused by Δ^9-THC alone on this measure. The interaction between cannabis and/or Δ^9-THC and the amphetamines for their effects on activity measures has received some experimental attention but the results have been equivocal. An early study by Garriott *et al.* [11] indicated that cannabis extract or Δ^9-THC potentiated and prolonged the stimulation of photocell activity caused by *dl*-amphetamine in aggregated mice, and this effect has been confirmed by others [6,33]. Recently, however, although a similar potentiation of methamphetamine-induced stimulation by Δ^9-THC was found in aggregated mice, a dose-related depression was found in mice tested singly [9]. Similar differences in results caused by housing density were reported for the effects of cannabis on amphetamine-induced lethality [15].

In rats the picture is more consistent in that no one to our knowledge has reported potentiation of amphetamine-induced motor stimulation by Δ^9-THC. However, the question of antagonism is unclear. Kubena and Barry [16] reported that a dose of 4 mg/kg Δ^9-THC antagonized the stimulant effect of 2 mg/kg methamphetamine, whereas a dose of 16 mg/kg Δ^9-THC failed to diminish the stimulant effect of 0.5 and 2.0 mg/kg methamphetamine (both drugs given IP). Because the higher dose of Δ^9-THC was itself depressant they suggested that methamphetamine antagonized this effect of Δ^9-THC. On the other hand, Pirch *et al.* [22] administered marihuana extract and reported a dose-related antagonism of the stimulation caused by IP-administered dA. Interestingly, the studies reported for rats have all been done with single animals and the question of potentiation in aggregated rats remains unanswered.

In rabbits 0.1 mg/kg methamphetamine reversed the EEG alterations caused by 0.5 mg/kg Δ^9-THC (both IV) and antagonized Δ^9-THC-induced postural and activity behaviors [4,5]. However, the combination of methamphetamine and Δ^9-THC also caused increased ataxia and stereotypy typical of high doses of the amphetamines. A similar mixed antagonism-potentiation by cannabis extract of various stereotyped behaviors caused by amphetamine was also reported for rats [13].

Thus, our results are consistent with those of Pirch *et al.* [22] who tested rats singly, but at variance with those of Garriott *et al.* [11] and others [6,33] who tested mice in groups. The difference in results could be due to the different species used or to the housing density of the animals during testing. In view of the results of Evans *et al.* [9] who reported enhanced stimulation in aggregated mice but depression in mice tested singly, we suggest that housing density may be the critical variable rather than species.

In humans two studies indicated that the psychological and physiological effects of smoked marihuana in combination with orally-ingested amphetamine were generally the same as would be expected from the additive effects of each substance [10,32].

Information about the interactions between cannabis and stimulants other than the amphetamines is sparse or nonexistent. Consroe *et al.* [5] recently reported that IV COC (1 mg/kg) and caffeine (12.5 mg/kg) antagonized changes in cortical and hippocampal EEG in rabbits caused by 0.5 mg/kg Δ^9-THC (IV). Postural and activity behaviors were also reversed by caffeine and, very briefly, by COC. However, the combination of Δ^9-THC and COC resulted in stereotypy similar to that noted above for methamphetamine.

Our results indicated that the interaction between Δ^9-THC and COC on photocell activity was one of mutual antagonism that depended on the respective doses of the two drugs. Thus, low doses of Δ^9-THC were only slightly effective in antagonizing the stimulation caused by COC, whereas the highest dose of Δ^9-THC used (10 mg/kg) completely antagonized this effect. For the lower doses of COC the net result was the depression caused by 10 mg/kg Δ^9-THC. However, the highest dose of COC effectively antagonized the depression caused by Δ^9-THC and the net result was not different from controls.

Although NIC is considered to be a CNS stimulant and acts directly on acetylcholine receptors [12] others have shown that the behavioral effects of this drug depend on a number of variables including species, sex, and baseline performance (see [17]). In our experiment NIC did not significantly affect photocell activity over the dose range tested (0.25 to 1.0 mg/kg, IP) although a trend toward reduced activity was apparent for the highest dose. The combination of all doses of Δ^9-THC and NIC caused almost complete elimination of photocell activity.

Consroe *et al.* [5] found that 0.02 mg/kg NIC (IV) reversed the EEG alterations caused by 0.5 mg/kg Δ^9-THC, but that this combination caused behavioral collapse preceded by behavioral disturbance. On the other hand, Sofia and Knobloch [31] found no effect of 20 mg/kg Δ^9-THC (IP) on the ED_{50} of NIC given by the same route in mice. Our results in rats appear to be similar to those of Consroe *et al.* [5] in rabbits. However, the term behavioral collapse may not be an appropriate description of our results because it implies an inability to perform any motor task. Clearly, when our rats were sufficiently motivated as in the performance of the CAR, they were able to coordinate their acquired sensory-motor skills in such a way as to avoid footshock quite efficiently. Nevertheless, it is clear that the effects of the interaction between Δ^9-THC and NIC on those two measures was quite different and that the effects were completely unlike those found for dA and COC.

The effects of these three stimulant drugs and their combinations with Δ^9-THC on rotarod performance were clouded in these experiments by excessive variability and a lack of consistency on this measure. Nevertheless, it appeared that some doses of dA and COC were able to counteract partially the impairment of rotarod performance caused by Δ^9-THC, whereas the impairment was as great or greater when Δ^9-THC and NIC were combined than after Δ^9-THC alone.

Both heart rate and body temperature were decreased as a function of dose of Δ^9-THC. The three stimulants had only slight or no effects on these measures over the dose

ranges used. NIC significantly reduced body temperature as a function of dose but the maximum effect was less than 1°C. All three drugs appeared to potentiate the bradycardia and hypothermia caused by Δ^9-THC at some dose combinations. NIC was the most consistent in this regard and interacted with Δ^9-THC in a dose-related way on both measures. There was no evidence that any of the three stimulants antagonized these effects of Δ^9-THC.

Taken together the results of these acute experiments suggest that the interactions of dA or COC with Δ^9-THC were similar to each other in many respects and that they differed markedly from those of NIC. Whereas dA and COC exhibited clear stimulant properties on intertrial responding and photocell activity, NIC was without effect or tended to be depressant. Moreover, whereas dA and COC interacted with Δ^9-THC in an antagonistic way on photocell activity, NIC interacted to potentiate depression. These differences may be related to the ways in which these drugs interact with the various neurohumoral systems in the CNS. Both dA and COC have been shown to interact with the catecholamine systems causing release from and/or preventing uptake of norepinephrine into neurons [3]. On the other hand, NIC is primarily cholinergic and, indeed, has served as a useful tool in studying this neurohumoral system [30]. There is also some evidence that Δ^9-THC has anticholinergic properties (e.g., [2]) and that it influences the monoamine systems in brain [14]. Thus, the interactions that we have observed can be expected to be the result of a complex interplay of the various drugs with their respective receptor cites and the neurohumoral systems involved.

Subacute Δ^9-THC. Some degree of tolerance developed to all of the effects of Δ^9-THC in one or more of the experiments reported herein. The most consistent measure in this regard was CAR performance. In all three experiments the impairment caused by 10 mg/kg Δ^9-THC was abolished when preceded by six daily treatments. For the other measures the tolerance was less complete or less consistent from experiment to experiment than for CAR performance. In all three experiments partial tolerance to the effects of Δ^9-THC on heart rate and body temperature were found. Photocell activity was generally depressed by 10 mg/kg Δ^9-THC in these experiments. In two of the three experiments where depression was evident tolerance to this effect was found. In two of the three experiments there was apparent complete tolerance to the marked impairment of rotarod performance caused by Δ^9-THC.

The tolerance that developed to the effects of Δ^9-THC alone also often extended to the interactions of Δ^9-THC with the other drugs when such interactions were present. Thus, the antagonism by Δ^9-THC to the stimulation of photocell activity caused by COC was markedly attenuated after subacute treatment with Δ^9-THC. In such Δ^9-THC-tolerant rats the dose-related stimulant properties of COC again emerged. Similarly, the marked depression caused by the acute combination of Δ^9-THC and NIC was attenuated considerably after subacute treatment with Δ^9-THC. On the other hand, there appeared to be sufficient residual activity of 10 mg/kg Δ^9-THC remaining after subacute treatment to completely antagonize the stimulation of photocell activity caused by dA. Because acute doses of only 2.5 mg/kg Δ^9-THC antagonized this effect of dA to about the same extent, it appears that the residual activity remaining after subacute treatment with 10 mg/kg Δ^9-THC under this treatment schedule was about the same as an acute dose of 2.5 mg/kg Δ^9-THC. Indeed, many of the dose-response curves for all three stimulants found after subacute treatment with 10 mg/kg Δ^9-THC were very similar to those found after the acute combination of 2.5 mg/kg Δ^9-THC with each of the three stimulants.

Subacute stimulants. Subacute treatment with each of the three stimulants generally resulted in effects that were similar, if not identical, to those seen after acute treatment. There was one exception to this general finding. Intertrial responses were increased to a greater extent after subacute treatment with each of the three stimulants than after acute treatment. For dA this effect was significant and it was antagonized by the lowest dose of Δ^9-THC. For COC this effect was also significant and it was antagonized as a function of dose of Δ^9-THC. Finally, for NIC, which did not show any stimulant effects when given acutely, this effect was significant and it was also antagonized by the lowest dose of Δ^9-THC. These results suggest a sensitization to some of the effects of these stimulants after repeated administration. On the other hand, there was no evidence for tolerance to the effects of these drugs on any of the measures used and subacute treatment did not offset or appreciably influence the acute effects of their combination with Δ^9-THC.

Subacute Δ^9-THC plus stimulants. In almost all comparisons subacute treatment with both Δ^9-THC and each of the three stimulants resulted in effects that were about the same as those seen after subacute treatment with Δ^9-THC alone. This result suggests that no additional interactions had occurred from their being administered together subacutely than would be expected from their separate administration.

Plasma and tissue levels of radioactivity. Levels of radioactivity reached a peak in both plasma and brain between 2 and 4 hr after oral administration of ^{14}C-Δ^9-THC and then declined steadily thereafter. This time interval of peak tissue levels corresponds to the interval over which behavioral and pharmacological testing was done. Thus, the behavioral and pharmacological responses to Δ^9-THC were temporally related to the tissue levels of radioactivity even though the latter represent an undetermined distribution of unchanged Δ^9-THC and its metabolites.

Subacute pretreatment with unlabelled Δ^9-THC caused the tissue levels of radioactivity derived from the acute administration of ^{14}C-Δ^9-THC to reach higher levels in both plasma and brain than after subacute pretreatment with sesame oil. We have repeatedly observed this phenomenon in our laboratory and its reliability seems well established [25, 26, 27, 28]. However, others were unable to find any differences in total radioactivity, unchanged Δ^9-THC, or any of its metabolites in the plasma or tissues of behaviorally tolerant pigeons [8,21] and dogs [20]. On the other hand, Lemberger *et al.* [18] reported that Δ^9-THC was eliminated more rapidly in heavy users of marihuana than in naive subjects. They suggested that Δ^9-THC might induce its own hepatic metabolism with repeated use, but othes (e.g. [29]) have been unable to reliably verify this effect in animals. The increased levels of radioactivity we have observed may represent dilution of the isotope with the tissue pools derived from repeated treatment with unlabelled Δ^9-THC. However, the pos-

sibility that this effect is in some way related to the tolerance that develops to Δ^9-THC remains to be established.

Acute or subacute treatment with dA caused the levels of radioactivity derived from ^{14}C-Δ^9-THC to disappear sooner from plasma than in comparable groups treated with sesame oil or Δ^9-THC. This effect was accompanied by a trend toward an increase in levels of radioactivity in brain. These results suggest that dA may have influenced the distribution of Δ^9-THC and/or its metabolites with more of the radioactivity leaving the plasma and entering the tissue. NIC also reduced the levels of radioactivity derived from ^{14}C-Δ^9-THC in plasma. However, unlike dA, this effect in plasma was unaccompanied by any apparent change in brain. COC did not affect the levels of radioactivity in plasma.

Levels of radioactivity derived from ^{3}H-dA, ^{14}C-COC, and ^{14}C-NIC all reached maximum levels in both plasma and brain within 15 to 30 min after IP injection. Radioactivity from ^{3}H-dA and ^{14}C-COC disappeared in what appeared to be two distinct phases, whereas that from ^{14}C-NIC disappeared in a single phase after the initial distribution phase.

Δ^9-THC did not affect the plasma levels of radioactivity derived from ^{3}H-dA but tended to increase the initial levels in brain. There were no differences in the disappearance of radioactivity in either tissue caused by Δ^9-THC. The levels and disappearance of radioactivity derived from ^{14}C-COC and ^{14}C-NIC were not significantly influenced by Δ^9-THC.

General comments. The results of these experiments provide considerable information about the acute and subacute interactions between Δ^9-THC and three stimulants – dA, COC, and NIC. In general the behavioral interactions between Δ^9-THC and dA or COC could be characterized as antagonistic, whereas the interactions between Δ^9-THC and NIC resulted in potentiation of the depressant effects of Δ^9-THC. Also, in general, these interactive effects could not be explained in terms of any major alterations in the disposition of the respective drugs in plasma or brain. We recognize that our measure of total radioactivity was limited and that further examination of the parent compounds and specific metabolites may have been more revealing. However, had any major changes in absorption, distribution, or elimination been present they would likely have been reflected in corresponding changes in total radioactivity, especially for such profound pharmacological interactions as those seen with NIC.

If the sites of these interactions were not at the dispositional level, then it is logical to expect them to have occurred at the points where the drugs interact with their respective target receptors, namely at the neural level including any or all of the biochemical machinery involved. These processes are still poorly understood. However, information has been accumulating over the past few years about some of the neurohumoral interactions with these drugs, that may eventually lead to our understanding of the mechanisms involved.

The results of the experiments reported here provide new information about the interactions between Δ^9-THC and three widely used stimulants. The data are preclinical and apply to only one species. Although limited in this regard, they provide a framework for possible future work in this area with other relevant tests and additional species. Two outcomes of such efforts can be anticipated: (1) we will better understand the characterics and, possibly, the mechanisms of action of these drugs and how they interact and (2) the potential significance of such interactions in terms of human safety will become better established.

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