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MONOGRAPH

Interactions Between Δ^9-Tetrahydrocannabinol and Phencyclidine Hydrochloride in Rats[1,2,3]

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PRYOR, G. T., S. HUSAIN, F. LARSEN, C. E. MCKENZIE, J. D. CARR AND M. C. BRAUDE. *Interactions between Δ^9- tetrahydrocannabinol and phencyclidine hydrochloride in rats.* PHARMAC. BIOCHEM. BEHAV. 6(1) 123–136, 1977. – Δ^9-Tetrahydrocannabinol (THC; 2.5, 5.0, 10.0 mg/kg, PO) impaired avoidance and rotarod performance, and caused bradycardia and hypothermia. Phencyclidine (PCP; 1.25, 2.5, 5.0 mg/kg, IP) impaired avoidance and rotarod performance and caused a marked increase in photocell activity. When combined, the depressant properties of each drug were enhanced and the stimulation of photocell activity caused by PCP was antagonized by THC. Tolerance to many of the effects of 10.0 mg/kg THC and its interactions with PCP followed subacute treatment for six days, whereas many of the effects of PCP were enhanced after subacute treatment with a dose of 2.5 mg/kg. Open-field behavior was affected by each drug alone and in combination in a similar way as photocell activity, but the depression caused by their interaction was greater; both drugs caused an increase in urination. Response rates on an FR-10 schedule of food reinforcement were decreased by 2.5 mg/kg PCP, but not by 5.0 mg/kg THC; the combination caused greater response suppression than either drug alone. The functional interactions between THC and PCP were not related to changes in the concentrations of ^{14}C or ^{3}H in plasma or brain derived from ^{14}C-Δ^9-THC and ^{3}H-PCP, respectively.

Oral Δ^9-THC Phencyclidine Drug interactions Acute and subacute treatment CAR
Photocell activity Heart rate Body temperature Rotarod Open field FR-10
Pharmacokinetics in plasma and brain

THE isolation and synthesis of Δ^9-tetrahydrocannabinol (Δ^9-THC) [64] has made possible an extensive – although as yet incomplete – examination of the pharmacological properties of this major active ingredient of *cannabis sativa* [8]. Availability of ^{14}C-Δ^9-THC and ^{3}H-Δ^9-THC also has resulted in considerable information about the metabolic fate and distribution of Δ^9-THC when administered by various routes in several species including man [2, 3, 33, 35, 40, 47, 48, 51, 52, 53, 54, 72, 76, 77, 78]. However, comparatively little is known about the interactions between Δ^9-THC and other drugs. This relatively unexplored area is of considerable interest and importance because the widespread increase in the use of marihuana in recent years has been accompanied by a corresponding increase in multiple drug use, both licit and illicit [see 71]. The potential significance of such interactions with marihuana are generally unknown in either animals or humans. However, experience with other drug interactions (e.g., alcohol and tranquilizers) suggests that serious and/or unexpected complications might be expected to occur, and emphasizes the need for careful and systematic evaluation of the possible interactions of other drugs with marihuana. The studies that have been reported suggest significant interactions between Δ^9-THC and a number of drugs, including alcohol [57], barbiturates [18, 34, 49, 70, 79, 81, 82], phenytoin [15], amphetamines [17, 27, 34, 36, 42, 49, 50], ether [57], ketamine [83], and phencyclidine [67]. However, these studies generally have been too limited in scope to adequately characterize the interactions in terms of several important parameters, including (1) doses and blood levels of the respective drugs, (2) history of exposure to either or both drugs, and (3) the kinds of

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[3] Partial results were presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Davis, California, August 18, 1975.

measures that are used to identify the interactions.

Although the importance of this area and the need for more intensive research in it are generally recognized, investigators have been reluctant to pursue it because of the complexities and associated effort involved. We have been engaged in a systematic program to study the interactions between Δ^9-THC and a number of drugs from various pharmacological classes in the rat using a variety of measures designed to sample multiple behavioral and pharmacological parameters. The interactions have been studied after acute administration and after subacute pretreatment with either Δ^9-THC and/or the test drug [72,73]. In this and subsequent reports we will describe the results of these extensive studies in some detail. The choice of phencyclidine for this first report is based on its current resurgence as an abused street drug.

Phencyclidine is 1-(1-phenylcyclohexyl) piperidine-HCl (PCP). Its preclinical pharmacology has been studied in several species [11, 12, 23]. In mice and rats PCP causes increased locomotor activity up to doses that are cataleptic, whereas in other species the effect is dose-related depression that proceeds to anesthesia, catalepsy, and convulsions. Patented in 1963 as an anesthetic in man (Sernyl), it was removed from the market because of adverse side effects that occurred during emergence, including extreme agitation, disorientation, delirium, and hallucinations [23]. In 1967 it was reintroduced as Sernylan for veterinary use only; it is used widely as an anesthetic in primates.

Because of its psychotomimetic effects and ease of clandestine manufacture, PCP has gained widespread use as an ubiquitous street drug [39, 66, 74]. In addition to being sold as PCP, it is frequently misrepresented as LSD, cocaine, mescaline, psilocybin, and Δ^9-THC among others. As a powder, PCP is frequently mixed with dried parsley or marihuana and smoked. This is the preferred and safest street route of ingestion. In the form of a marihuana joint, the possibility of any interactions between Δ^9-THC and PCP is, of course, maximized.

A small amount of laboratory research has been reported that suggests significant interactions between Δ^9-THC and PCP. Pretreatment with 20 and 40 mg/kg of Δ^9-THC was reported to produce a significant increase in the duration and number of mice exhibiting a loss of righting reflex caused by ketamine, a congener of PCP [83]. A similar result was more recently reported for PCP and the effect was related to the dose of Δ^9-THC (2.5–10 mg/kg [67]). The response suppression in a VI-60 operant schedule in rats caused by 2 mg/kg of PCP also was increased by 0.5 mg/kg of Δ^9-THC, but doses of 20 and 40 mg/kg of Δ^9-THC did not affect the LD_{50} of PCP (68.5 mg/kg) in mice [67].

The experiments reported here were designed to systematically investigate the interactions between Δ^9-THC and PCP in terms of the parameters stated above and to provide pharmacokinetic data that might be related to the behavioral and pharmacological responses.

EXPERIMENT 1

The purpose of this experiment was to establish the dose-effect relationships of Δ^9-THC and PCP alone and in all combinations using a battery of behavioral and pharmacological tests. The acute effects of the two drugs were examined as well as their effects alone and in combination after subacute pretreatment with either Δ^9-THC, PCP, or both given daily for six days. Only one dose of Δ^9-THC (10mg/kg) and one dose of PCP (2.5 mg/kg) were administered subacutely because a full dose-effect experiment during this phase would have been prohibitive. The doses used for subacute treatment were chosen from the results of preliminary experiments during which tolerance developed to 10 mg/kg of Δ^9-THC and cumulative effects of 2.5 mg/kg of PCP were suggested.

Method

Animals. Male rats of the inbred Fischer strain were used in this and all subsequent experiments. They were 55–60 days old (140–160 g) when received from Simonsen's Laboratories, Gilroy, California. They were housed singly in wire mesh hanging cages with food and water available at all times (except when food-deprived for operant tests). The ambient temperature was 22° C and the lights in the room were turned on at 0700 and off at 1900 daily.

Apparatus. Each avoidance chamber consisted of a 30 × 36 × 40 cm wooden box housed inside a larger, sound-attenuated cabinet. Scrambled, constant current 1.0 mA shock applied to 0.32-cm-diameter brass rods spaced 1.27 cm apart served as the unconditioned stimulus (UCS). Downward displacement (0.16 cm) of a 1.27-cm-diameter aluminum pole suspended from the center of the ceiling served as the conditioned response. A 7.5-W light and a 11.4-cm-diameter loudspeaker provided ambient light (0.44-foot-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). An increase in intensity above ambient, which occurred at the rate of 2.5 times per sec of either the light (to 0.88 foot-candles) or the tone (to 63 dB), or a low-intensity, nonaversive current (120 μA) on the floor served as conditioned stimuli (CS). Each chamber had its own air-circulation system. Twelve such chambers were interfaced with a Digital Equipment Corporation PDP 8/F computer (located in an adjoining room) that provided automatic control and data collection.

For measuring spontaneous motor activity, a single rat was placed in a black, cylindrical chamber 30 cm in diameter 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 larger, sound-attenuated box equipped with a 7.5-W light located above the center of the chamber and had its own ventilation system.

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

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

The rotarod was an 8.9-cm-diameter wooden rod mea-

suring 91-cm long and suspended 46 cm above the test surface. 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 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 (15–120 sec). Then, they were given three daily 60-trial sessions to learn to avoid footshock by pulling a 13-cm pole in the presence of each CS, which preceded the UCS by 10 sec. The CS and UCS remained on for 30 sec, unless it was terminated earlier by a pole-displacement response. The three CS (tone, light, or nonaversive footshock) were presented randomly for 20 trials each. The entire 60-trial session required 2–2.5 hr. The intertrial interval was variable, averaging 1.5 min (15 sec to 3 min). Response latencies and intertrial responses (ITR) were recorded on paper tape for processing on a CDC 6400 computer. Animals 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 that followed acute or subacute drug treatment was conducted in the same way as the training sessions. No appreciable loss of the avoidance response has been noted following intervals of up to 14 days between training and testing in control animals using this procedure.

The second group of animals was used for measuring photocell activity, heart rate, body temperature, and rotarod performance. Before receiving any drug treatment, each animal was given a 5-min pretest in the photocell activity chamber, and, based on its score, was matched and assigned to a control or drug treatment group. After the photocell activity pretest, each animal 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 animals met this criterion; rotarod data from animals that failed this criterion were not used. On the test day, photocell activity was measured for 10 min. Electrodes were attached and heart rate was then monitored in the same chamber for the next 2 min. The animal was removed and body temperature was measured after a 1 min equilibration period. The animal was then placed on the stationary rotarod and the rotation was gradually increased to 11 rpm. The amount of time that the animal was able to remain on the rod – up to 120 sec – was recorded.

Experimental design. After pretraining or pretesting each animal was assigned to 1 of 25 groups. For the next 6 days each animal was intubated with sesame oil (2 ml/kg) or 10 mg/kg of Δ^9-THC dissolved in sesame oil, or injected intraperitoneally (IP) with 2.5 mg/kg of PCP. No further training or testing occurred during this subacute treatment phase to ensure that any tolerance or cumulative effects of the drugs could be interpreted simply and would not be confounded with the test procedures. On the seventh day each animal was intubated with sesame oil (2 ml/kg) or 1 of 3 doses of Δ^9-THC in sesame oil (2.5, 5.0, or 10.0 mg/2 ml/kg). Ninety min later they were given an IP injection of saline (2 ml/kg) or 1 of 3 doses of PCP in saline (1.25, 2.5, or 5.0 mg/2 ml/kg). 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. The drug design and the number of animals tested in each group is shown in Table 1. The experiment was completed in several replications with all groups being represented in each replication.

TABLE 1

DRUG DESIGN FOR RECIPROCAL DOSE-EFFECT INTERACTIONS BETWEEN Δ^9-THC AND PCP

Days 1-6		Day 7		Number of Animals Tested	
Drug	Dose (mg/kg/day)	Dose of Δ^9-THC (mg/kg)	Dose of PCP (mg/kg)	CAR	Activity etc.
Sesame oil	2 ml/kg	0.0	0.0	17	13
		0.0	1.25	19	13
		0.0	2.5	16	13
		0.0	5.0	16	13
		2.5	0.0	17	13
		2.5	1.25	16	12
		2.5	2.5	17	13
		2.5	5.0	17	12
		5.0	0.0	18	12
		5.0	1.25	17	14
		5.0	2.5	18	13
		5.0	5.0	18	13
		10.0	0.0	17	13
		10.0	1.25	18	13
		10.0	2.5	16	13
		10.0	5.0	17	13
Δ^9-THC	10.0	10.0	0.0	16	13
		10.0	1.25	18	13
		10.0	2.5	17	13
		10.0	5.0	17	13
PCP	2.5	0.0	2.5	16	11
		2.5	2.5	17	12
		5.0	2.5	16	12
		10.0	2.5	17	13
Δ^9-THC+ PCP	10.0 2.5	10.0	2.5	18	12

Data analysis. The data for each measure were first analyzed by analysis of variance to establish the significance of any main effects or their interactions [55]. Then, *t*-tests were computed between selected pairs of means using the pooled degrees of freedom and error variance from the analysis of variance.

Results and Discussion

Acute interactions. Figure 1 shows the acute dose-effect relationships of Δ^9-THC and PCP alone and in all combinations for the five tests in this battery. Since there were no appreciable differential effects of the treatments on the three CS, the results were combined as total conditioned avoidance responses (CAR). Analysis of variance showed the main effects of Δ^9-THC ($F(3,258) = 50.8$, $p < 0.01$) and PCP ($F(3,258) = 65.4$, $p < 0.01$) on CAR performance to be statistically significant. The interaction was also significant ($F(9,258) = 2.1$, $p < 0.05$). Δ^9-THC caused a dose-related impairment of CAR performance, but the effect did not reach statistical significance until a dose of 10 mg/kg was used ($t(258) = 3.8$, $p < 0.01$) in this

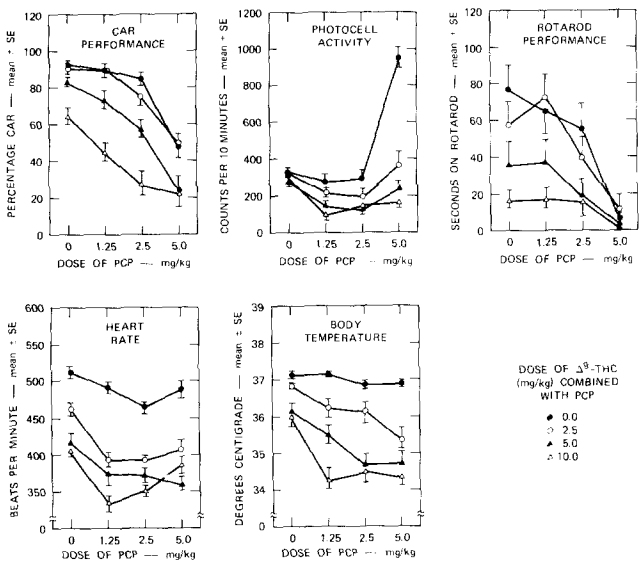

FIG. 1. Acute reciprocal dose-response interactions between Δ^9-THC and PCP.

experiment. Similarly, only the highest dose of PCP (5 mg/kg caused a significant impairment of CAR performance (t (258) = 6.0, $p<0.01$). When combined, a dose of 2.5 mg/kg Δ^9-THC and 2.5 mg/kg PCP caused a significant impairment (t (258) = 2.4, $p < 0.05$) as did a dose of 5.0 mg/kg Δ^9-THC and 1.25 mg/kg PCP (t (258) = 2.6, $p < 0.01$). With doses of 5 and 10 mg/kg Δ^9-THC, PCP caused clear, dose-related potentiation of impairment even though the two lower doses of PCP were without significant effects alone. The highest dose of PCP also caused a 21% decrease in escape responses (t (258) = 3.3 $p < 0.01$) and this effect was enhanced significantly by 5 (44%, t (258) = 3.4, $p < 0.01$) and 10 (58%, t (258) = 5.7 $p < 0.01$) mg/kg Δ^9-THC, although, alone, these doses were ineffective on this measure.

Photocell activity was not affected significantly by Δ^9-THC alone in this experiment, although we have generally observed depression at the highest dose [73]. Doses of 1.25 and 2.5 mg/kg PCP also were ineffective. However, a dose of 5.0 mg/kg PCP caused a threefold increase in counts (t (190) = 12.4, $p < 0.01$). The interaction term of the analysis of variance was significant (F (3,190) = 18.6, $p < 0.01$). At the lower doses of PCP, Δ^9-THC caused significant reductions in photocell activity (all ts (190) > 2.3, all ps < 0.05). Δ^9-THC antagonized the increase in counts caused by 5.0 mg/kg PCP. The combination of 5.0 mg/kg PCP and 10 mg/kg Δ^9-THC caused a significant decrease in counts (t (190) = 3.0, $p <$ 0.01).

Rotarod performance was impaired as a function of dose by both Δ^9-THC (F (3,189) = 11.6, $p < 0.01$) and PCP (F (3,189) = 15.1, $p < 0.01$). However, because of the large variability of this measure, only the highest dose of PCP reached statistical significance (t (189) = 5.0, $p < 0.01$). A dose of 5.0 mg/kg Δ^9-THC caused significant impairment (t (158) = 2.8, $p < 0.01$). The interaction was not significant (F (9,189) = 1.4, $p < 0.1$).

Δ^9-THC caused a dose-related decrease in heart rate (F (3,186) = 99.2, $p < 0.01$). The effect was significant at the lowest dose (t (186) = 3.4, $p < 0.01$). PCP also appeared to decrease heart rate (F (3,186) = 25.5, $p < 0.01$), but only the intermediate dose (2.5 mg/kg) was significantly effective when given alone (t (186) = 3.4, $p < 0.01$). Although the interaction term of the analysis of variance was not significant (F (9,186) = 1.5, $p < 0.1$), the bradycardia caused by every dose of Δ^9-THC was significantly increased by the lowest dose of PCP (all ts (186) $\geq$ 3.0, all ps < 0.01). The lowest dose of PCP was most effective in enhancing the bradycardia caused by 10 mg/kg of Δ^9-THC. This effect was attenuated with higher doses of PCP and at 5 mg/kg the combined effect was not different than the effect of Δ^9-THC alone. This reversal of effect of PCP on heart rate with increasing dose corresponds to the increase in photocell activity caused by 5 mg/kg.

Body temperature also was decreased as a function of dose of Δ^9-THC (F (3,190) = 68.6, $p < 0.01$) whereas PCP was ineffective alone. The interaction was significant (F (9,190) = 2.5, $p < 0.05$); PCP potentiated the hypothermia caused by all doses of Δ^9-THC.

Subacute treatment with Δ^9-THC. Figure 2 shows the acute dose-effect relationships of PCP alone and in combination with 10 mg/kg Δ^9-THC compared with the effects seen after subacute pretreatment with 10 mg/kg/day for six days. The effects of subacute treatment with both 10 mg/kg/day of Δ^9-THC and 2.5 mg/kg of PCP for all seven days are also shown.

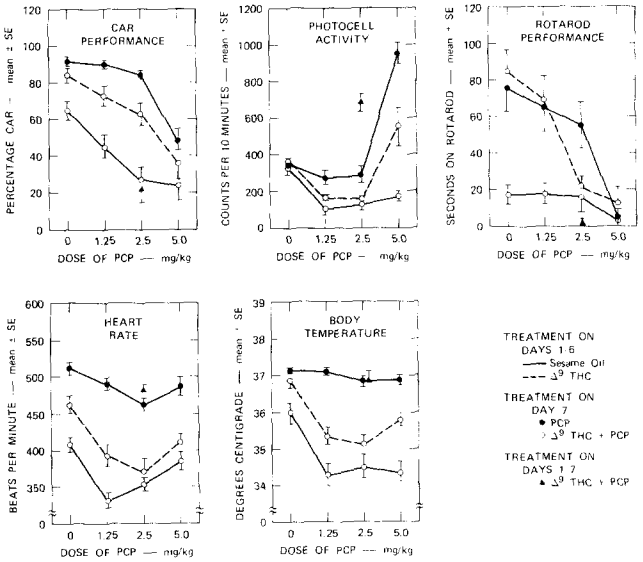

FIG. 2. Interactions between Δ^9-THC and PCP after subacute pretreatment with Δ^9-THC.

Clear tolerance to the effect of Δ^9-THC alone was apparent. Avoidance performance improved from an average of 65% CAR after acute treatment with 10 mg/kg Δ^9-THC to 84% CAR after subacute pretreatment with 10 mg/kg/day Δ^9-THC (t(401) = 2.3, $p < 0.05$) which was not different from control performance of 92% CAR (t (401) = 1.0, $p > 0.1$). The impairment in CAR performance caused by the acute combination of Δ^9-THC and PCP also was attenuated after subacute pretreatment with Δ^9-THC. Improved performance was seen with each dose of PCP after subacute pretreatment with Δ^9-THC. This effect was significant except for the highest dose of PCP (5 mg/kg) which caused marked impairment when given acutely alone. The impairment after subacute treatment with both 10 mg/kg/day Δ^9-THC and 2.5 mg/kg/day PCP was about the

same as that caused by the acute combination of both drugs at these doses. Since there was tolerance to Δ^9-THC, this result implies cumulative effects of PCP, a conclusion that will be supported further below.

There was no significant difference between the effects of acute and subacute treatment with 10 mg/kg of Δ^9-THC alone on photocell activity in this experiment. However, tolerance was indicated by the attenuated antagonism of the PCP-induced increase in counts caused by Δ^9-THC. In the Δ^9-THC-tolerant animals, 5 mg/kg PCP stimulated photocell activity (t (292) = 3.6, $p<0.01$), whereas the acute combination depressed activity. Further evidence for cumulative effects of PCP was seen in the group treated subacutely with both Δ^9-THC and PCP. In these animals a dose of 2.5 mg/kg PCP caused a twofold increase in counts (t (292) = 5.8, $p < 0.01$). Since the animals were presumably tolerant to Δ^9-THC the increase in counts can be attributed to subacute treatment with PCP. This dose of PCP was ineffective when given acutely alone.

Tolerance to the impairment of rotarod performance caused a dose-related impairment in performance that was greater than after acute treatment with PCP alone. Whereas an acute dose of 2.5 mg/kg PCP was ineffective alone, it caused a marked impairment when combined with Δ^9-THC after subacute treatment with the latter drug (t (290) = 4.0, $p < 0.01$). This result suggests that the tolerance to Δ^9-THC was not complete. The cumulative effects of 2.5 mg/kg/day PCP were again suggested in that performance by this group was poorer than the group treated subacutely with Δ^9-THC alone, although the difference was not significant (t (290) = 1.4, $p > 0.1$) due, presumably, to the floor effect.

There was significant tolerance to the bradycardia (t(284) = 2.8, $p < 0.01$) and hypothermia (t(284) = 2.8, $p < 0.01$) caused by 10 mg/kg Δ^9-THC. This tolerance generally extended to the drug's interaction with PCP on these measures. The effect of subacute treatment with both drugs on these measures is difficult to interpret. In the animals made tolerant to Δ^9-THC, an acute dose of 2.5 mg/kg PCP caused significant bradycardia (t(284) = 5.4, $p < 0.01$) and hypothermia (t (291) = 5.4, $p < 0.01$) compared with the animals treated subacutely with Δ^9-THC alone. After subacute treatment with both drugs, the combination was ineffective in changing heart rate and body temperature. Since PCP alone had little or no effect on these measures when given acutely, this result suggests tolerance to the interactive effects of PCP and Δ^9-THC rather than tolerance to the effects of PCP on these measures per se.

Subacute treatment with PCP. Figure 3 shows the results after subacute treatment with 2.5 mg/kg/day PCP for six days. The results for animals treated subacutely with both 10 mg/kg/day PCP are shown again for comparison.

The cumulative effects suggested above of PCP on avoidance performance, photocell activity, and rotarod performance were clear. Whereas an acute dose of 2.5 mg/kg PCP did not impair CAR performance, subacute treatment for seven days did (t (401) = 3.9, $p < 0.01$). The impairment caused by combinations of Δ^9-THC and PCP also was greater after subacute treatment with PCP than when both drugs were given acutely. This effect appeared to be caused by the increased effectiveness of PCP alone rather than an increase in the magnitude of the interaction, since the slope of the dose-effect curve for Δ^9-THC was not greater after subacute treatment with PCP than in acute combination.

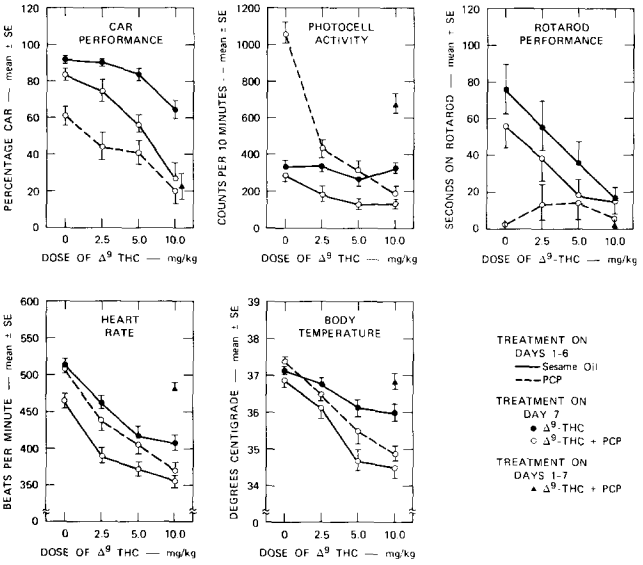

FIG. 3. Interactions between Δ^9-THC and PCP after subacute pretreatment with PCP.

Whereas acute administration of 2.5 mg/kg PCP did not affect photocell activity, subacute treatment caused over a threefold increase in counts (t (292) = 11.6 $p < 0.01$). This effect was even greater than that caused by acute administration of 5 mg/kg PCP (see Fig. 1) although the difference did not reach significance (t (292) = 1.8, $0.1 > p > 0.05$). Δ^9-THC antagonized this cumulative effect of PCP as a function of dose. The highest dose of Δ^9-THC caused significant depression of photocell activity in such PCP-pretreated animals (t (292) = 2.2, $p < 0.05$).

Similarly, whereas 2.5 mg/kg PCP failed to impair rotarod performance significantly when given acutely, subacute treatment caused total loss of the ability of the animals to stay on the rotarod (t (290) = 5.4, $p < 0.01$). There was a tendency (not significant) for the lower doses of Δ^9-THC to offset this impairment, presumably by antagonizing the hyperactivity caused by subacute treatment with PCP.

Acute administration of 2.5 mg/kg PCP caused a significant reduction in heart rate and the dose-related bradycardia caused by Δ^9-THC was displaced downward accordingly. Apparent tolerance to this effect of PCP was accompanied by a shift of the dose-effect curve for Δ^9-THC and PCP towards that of Δ^9-THC alone. A similar effect was seen for body temperature, although PCP did not significantly affect this measure alone either acutely or subacutely.

EXPERIMENT 2

In the previous experiment Δ^9-THC was administered 2 hr and PCP 30 min before testing. These times were chosen on the basis of our estimation of when both drugs would reach approximately peak pharmacologically effective levels at the beginning of testing by the oral and IP routes of administration, respectively. Δ^9-THC dissolved in sesame oil is absorbed slowly from the gastrointestinal tract and levels of total radioactivity derived from ^{14}C-Δ^9-THC do not reach a peak in plasma and brain until 2 to 4 hr after administration, after which they decline slowly [73]. On the other hand, PCP is absorbed rapidly after IP adminis-

tration and reaches peak levels within 30 min (see Experiment 5). However, it is possible that Δ^9-THC and PCP interact metabolically to alter their respective pharmacokinetics such that these predictions would not remain valid. Therefore, in Experiment 2 the times of administration of the two drugs were varied and the effects on CAR performance were determined.

Method

The type of subjects, apparatus, and procedures were the same as described in Experiment 1, except that the animals were left undisturbed during the six-day interval between training and testing (i.e., they were not given sesame oil or drug during this time, and, therefore, they were not handled as in Experiment 1). Drug or placebo administration on the test day was according to the design shown in the results section.

Results

Table 2 summarizes the results of this experiment for total CAR during the first 30 trials and the last 30 trials separately to observe any early or late effects. When Δ^9-THC was given 30 min before the test the impairment did not reach significance during the first 30 trials (t (164) = 1.9, $0.1 < p < 0.05$), but was significant during the last 30 trials (t (164) = 2.9, $p < 0.01$) indicating that the peak effect had not occurred by 30 min. When Δ^9-THC was given two hr before the test, marked impairment was seen during the first 30 trials. When 2.5 mg/kg PCP was given 30 min before the test, there was a trend (not significant) toward impairment during the first 30 trials (t (164) = 1.9, $0.1 > p > 0.05$) with no effect during the last 30 trials. There were no effects on CAR performance when PCP was given 2 hr before the test. The administration of Δ^9-THC 2 hr and PCP 30 min before the test (as in Experiment 1) caused marked impairment throughout the test and was accompanied by a 78 and 47% reduction in escape responses during the first and last 30 trials, respectively. When the order of administration was reversed the impairment was about the same as that caused by Δ^9-THC alone given 30 min before the test. Similarly, when both drugs were given 2 hr before the test, the impairment was the same as that caused by Δ^9-THC given alone 2 hr before the test. However, when both drugs were given 30 min before the test, the impairment was greater than what would be expected from the simple addition of the separate effects of each drug given alone at that time. Since PCP is the faster acting drug by these routes of administration, this result suggests that the low levels of Δ^9-THC present at this time were sufficient to cause the observed interaction.

TABLE 2

ACUTE EFFECTS OF Δ^9-THC AND/OR PCP ON PERCENTAGE AVOIDANCE AS A FUNCTION OF TIME OF ADMINISTRATION

Treatment*			Trials 1–30	Trials 31–60
2 hr†	30 min†	N	Mean SE	Mean SE
SO	SAL	10	88 ± 1.6	93 ± 1.6
SO	Δ^9-THC	9	69 ± 6.3‡	64 ± 10.1§
Δ^9-THC	SAL	10	47 ± 6.1§	65 ± 9.6§
SO	PCP	9	69 ± 6.0‡	83 ± 8.1
PCP	SAL	10	81 ± 2.5	93 ± 1.4
Δ^9-THC	PCP	11	5 ± 2.7§	14 ± 4.5§
PCP	Δ^9-THC	11	65 ± 6.8‡	49 ± 9.5§
Δ^9-THC+PCP	SAL	11	41 ± 9.7§	58 ± 9.4§
SO	Δ^9-THC+PCP	10	11 ± 4.9§	18 ± 9.6§

*Sesame oil (SO) was administered intragastrically (IG, 2 ml/kg); saline (SAL) was given IP (2 ml/kg); Δ^9-THC (10 mg/kg) was given IG in SO; PCP (2.5 mg/kg) was given IP in SAL.
†Time before testing.
‡$p<0.05$.
§$p<0.01$.
Compared with controls by t-test.

EXPERIMENT 3

The increase in photocell activity caused by PCP in Experiment 1 is in agreement with the results of others who used a jiggle cage with rats and an actophotometer with mice [11]. However, these procedures do not adequately measure the quality of the drug-induced changes in locomotor behavior. Because PCP is a depressant in other species [11], the apparent excitatory effect in rats and mice and its antogonism by Δ^9-THC is of special interest. Chen *et al.* [11] noted that "ataxia was clearly noticeable at higher dosages," and that "the animals ran around the cage in a manner resembling somewhat that induced by desoxyephedrine [p. 242]." We directly observed a few animals treated with PCP and noted that their increased activity appeared to resemble blind, compulsive forward locomotion that was often ataxic. The animals would move in a straight line and literally run into any barrier present (e.g., a wall) after which they would change direction and repeat the sequence. In order to verify and extend these casual observations and to see how these effects interacted with Δ^9-THC, we conducted this experiment using an open field procedure.

Method

The animals and drug design were the same as described in Experiment 1, except that only one dose each of Δ^9-THC (10 mg/kg) and PCP (2.5 mg/kg) were used. A 61-cm^2 arena with 46-cm-high walls was used to observe exploratory behavior. The interior of the arena was painted flat black and white lines divided the floor into a grid of 7.6-cm squares. A 25-W light was suspended 91 cm above the center of the arena. The arena was located in a sound-attenuated semidarkened room. At the appropriate time after dosing each animal was brought into the room, placed in one corner of the arena, and observed for 5 min. Locomotion was measured by tracing the animal's path on a scaled-down drawing of the arena and the number of lines crossed was counted. The separate incidence and cumulative duration of rearing and grooming were also recorded on manually-operated, solid state electronic counters and timers. The animal was removed and the number of boli dropped and number of separate urine spots were counted and recorded.

Results

The results are summarized in Table 3. The number of separate rears and grooming bouts are not shown, since they were highly correlated with their respective cumulative durations. Acute administration of 10 mg/kg Δ^9-THC did not affect locomotion significantly as measured by the number of lines crossed, but it markedly suppressed rearing and grooming. Acute administration of 2.5 mg/kg PCP, on

TABLE 3

INTERACTION BETWEEN Δ^9-THC AND PCP: EFFECTS ON OPEN-FIELD BEHAVIOR

Days	Treatment* Day 7		Lines Crossed		Time Rearing (sec)		Time Grooming (sec)		Number of Boli		Number of Urine Spots	
1–6	2 hr†	30 min†	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SO	SO	SAL	129	9.1	25.4	2.8	91.3	12.8	1.75	0.50	0.88	0.26
SO	Δ^9-THC	SAL	146	26.2	4.7	1.6§	6.3	2.6§	1.69	0.44	2.94	0.40§
SO	SO	PCP	288	26.3	20.5	5.1	9.3	4.0§	2.00	0.56	3.62	0.53§
SO	Δ^9-THC	PCP	40	10.6§	0.2	0.1§	0.0	0.0§	0.03	0.03§	2.47	0.29‡
Δ^9-THC	Δ^9-THC	SAL	174	14.7	13.7	1.7§	89.4	12.3	1.91	0.56	2.50	0.43‡
Δ^9-THC	Δ^9-THC	PCP	177	23.1	0.9	0.5§	0.4	0.3§	0.75	0.39	2.88	0.41§
PCP	SO	PCP	374	20.4§	33.1	4.4‡	27.0	5.9§	2.38	0.60	2.81	0.49§
PCP	Δ^9-THC	PCP	64	15.8‡	0.0	0.0§	0.0	0.0§	0.19	0.19§	2.50	0.75‡
F-ratio			32.8		22.4		33.5		3.9		2.8	
p			<0.01		<0.001		<0.001		<0.01		<0.05	

*Sesame oil (SO) was given IG (2 ml/kg); saline was given IP (2 ml/kg); Δ^9-THC (10 mg/kg) was given in SO; PCP (2.5 mg/kg) was given IP in SAL. There were 16 rats in each group.
†Time before testing.
‡$p<0.05$.
§$p<0.01$.
Compared with controls by t-test.

the other hand, caused over a twofold increase in lines crossed. As noted earlier, the locomotion was characterized by ataxic forward movement with no apparent awareness of the walls as barriers. Rearing time was not affected, but grooming time was markedly suppressed. The acute combination of Δ^9-THC and PCP virtually eliminated all behavior in the arena.

After subacute treatment with 10 mg/kg/day Δ^9-THC, there was a trend toward increased locomotion (not significant) and significant tolerance to the suppressant effects of the drug on rearing (t (120) = 2.4, $p < 0.05$) and grooming (t (120) = 8.6, $p < 0.01$). Rearing was still suppressed below that of controls indicating only partial tolerance to this effect, whereas grooming was not different from that of controls. PCP given to such Δ^9-THC-tolerant rats caused complete suppression of rearing and grooming, but it did not alter locomotion from that seen after subacute treatment with Δ^9-THC alone. On the other hand, subacute treatment with PCP caused a greater increase in locomotion than that caused by acute treatment (t (120) = 3.1, $p < 0.01$), further indicating this drug's cumulative or sensitizing effect. This increase in locomotion, which was accompanied by a significant increase in rearing as well, appeared to displace grooming which was still depressed and not significantly different from that caused by acute administration. When Δ^9-THC was given to such PCP-pretreated rats, the result was the same as after acute administration of both drugs; namely, almost complete suppression of all behavior in the arena.

There were no significant effects of either drug alone on defecation in the arena. However, when combined, there was marked suppression. On the other hand, both drugs caused significant increases in urination, whether given acutely alone or in combination or after subacute pretreatment with either. There were no significant differences among the groups in this regard to indicate interaction, tolerance, or sensitization to this effect.

EXPERIMENT 4

A number of workers have shown that Δ^9-THC alone will modify operant responding for food and water controlled by several schedules of reinforcement in rats [19, 29, 32, 37, 46], pigeons [19, 25, 32], and primates [30, 31, 38], and that tolerance develops rapidly to these effects [62], depending on dosing schedule [20] and route of administration [1]. The direction of the effect is dependent on the dose of Δ^9-THC and the schedule of reinforcement. Low doses of Δ^9-THC have been reported to increase response rates on FI schedules, whereas higher doses typically suppress responding [37]. Response rates generally are decreased as a function of dose on FR and VI schedules.

The effects of PCP on schedule-controlled behavior is not well documented. However, Wenger recently reported that PCP and ketamine increased response rates at low doses and decreased response rates at high doses during the FI component of a multiple schedule in pigeons [86] and mice [87]; only a dose-related decrease in response rates was seen in the FR-30 component. Response rates also were suppressed by 2 mg/kg of PCP in a VI-60 schedule in rats, and this effect was augmented by 0.5 mg/kg Δ^9-THC that was ineffective alone [67]. Thus, Δ^9-THC and PCP appear to have similar effects on these behaviors and, when combined, the effect is enhanced. To verify this effect and to determine whether it would be modified by pretreatment with either Δ^9-THC or PCP, we conducted this experiment using an FR-10 schedule.

Method

Apparatus. The test chamber consisted of a circular, clear Plexiglas tube 28 cm in diameter and 32 cm in height. The floor was constructed of radial 0.08-cm-diameter spokes. A pole was located in the center of the chamber, and two 7-½-W lights and a 18-cm loudspeaker were

mounted in the ceiling. A 5-cm circular bar (1.3 cm in diameter) protruded 1.3 cm into the chamber and was located 4.3 cm above the floor. This bar served as the operant manipuladum. A small receptacle was located just to the right of the bar to receive the reinforcements (45-mg Noyes rat pellets) which were delivered by a flexible tube from a Foringer pellet dispenser. The chamber was housed inside a larger 76-cm^3 sound-attenuated box that had an air-circulating system. Six of these units were operated simultaneously by solid-state programming and recording equipment located in an adjoining room.

Procedure. The animals were placed on a 23-hr food deprivation schedule and maintained at about 80% of their free-feeding body weight. They received supplemental feeding in their home cages after a training session and on nontraining days. Water was available ad lib in the home cage. Each animal was individually shaped to press the bar on a continuous reinforcement schedule. Once shaped, the animal was allowed 80 to 90 self-initiated reinforcements on this schedule. Daily 30-min sessions were continued during which the FR was gradually increased until an FR-10 was attained. The FR was increased by one-step increments following successful completion of 25 reinforcements at each step at a rate of 1 to 2 responses per second. The animals were stabilized on the FR-10 for at least four days. On the next day (baseline), all animals were given sesame oil (2 ml/kg, IG) two hours and saline (2 ml/kg, IP) 30 min before a 40-min baseline test session. The animals were ranked on the basis of total number of rewards received during the baseline session and assigned to one of five groups. Assignment was such that the average number of rewards received did not vary among groups by more than three. The next day the acute effects of Δ^9-THC and/or PCP were evaluated. Sesame oil (2 ml/kg, IG) or Δ^9-THC (5 mg/kg in sesame oil, IG) was given 2 hr and saline (2 ml/kg, IP) or PCP (2.5 mg/kg in saline, IP) was given 30 min before the 40-min test session. Two groups were given both Δ^9-THC and PCP. On Days 3 through 8, separate groups were given sesame oil, Δ^9-THC or PCP according to each group's treatment on Day 2. Of the two groups that received both Δ^9-THC and PCP on Day 2, one received Δ^9-THC subacutely and the other received PCP subacutely. No training or testing occurred during this subacute drug treatment phase, although the food-deprivation schedule was maintained. On Day 9, all animals were treated the same as on Day 2 and given a 40-min test session to determine the effects of the intervening subacute treatment. The experiment was conducted in two replications of 20 animals in each replication. One control animal was eliminated because of poor baseline performance.

Results

Figure 4 summarizes the results in terms of rewards per minute received over the entire 40-min session on each of the three test days. The interaction term of the analysis of variance was significant ($F(8,68) = 7.44$, $p < 0.001$). Comparisons of baseline performance showed that there were no differences among the five groups before any drugs were administered (all ts < 1.0). Also, there were no differences in control performance among the three test sessions showing that the response was well retained over the 6-day drug treatment period during which time the animals were not tested.

Acute oral administration of 5 mg/kg Δ^9-THC did not affect response rates, whereas 2.5 mg/kg PCP reduced

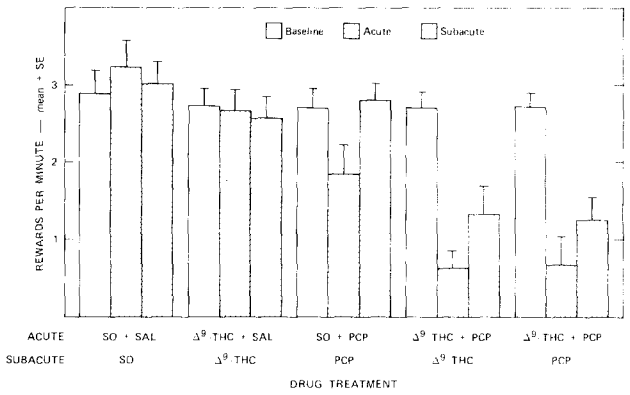

FIG. 4. Effects of Δ^9-THC and/or PCP on FR-10 performance.

response rates by about 27% ($t(68) = 2.9$, $p < 0.01$ compared with baseline); their acute combination reduced response rates by about 76% in both groups (ts $(68) \geq 6.72$, ps < 0.01). The differences between PCP alone and the combination of Δ^9-THC and PCP were also significant (ts $(102) \geq 3.1$, ps < 0.01).

Subacute treatment with Δ^9-THC also did not alter response rates significantly from baseline or control performance. The reduction in response rate caused by acute administration of PCP was significantly attenuated after subacute treatment with PCP ($t(68) = 3.2$, $p < 0.01$). Subacute treatment with either Δ^9-THC or PCP slightly attenuated the acute interaction of both drugs. However, response rates in these groups were still significantly lower than baseline and response rates of animals treated subacutely with either Δ^9-THC or PCP alone.

EXPERIMENT 5

The metabolism, distribution, and pharmacokinetics of ^{14}C-Δ^9-THC and ^{3}H-Δ^9-THC have been studied in a number of species [2, 3, 33, 40, 47, 52, 76, 78], including man [53,54]. Δ^9-THC is rapidly metabolized by the liver [47,68] and at least one of its metabolites, 11-OH-Δ^9-THC, is pharmacologically active [15]. Excretion in the rat is mainly in the feces as polar metabolites [2,47] and enterohepatic circulation has been established [47]. Elimination of total radioactivity from the circulation appears to be biphasic with an initial, rapid half-life of the order of min [3,56] or hr [68,77] and a slower phase of the order of days [2,53]. Because of its lipophilic nature, Δ^9-THC and its metabolites are preferentially taken up by fat [48] and bound to plasma lipoproteins [84]. They are preferentially concentrated in the particulate subcellular fractions of brain homogenates, especially in the nerve-ending membranes [16,59]. Accumulation, especially in fat, but also in plasma and brain, with repeated doses has been reported [48,61]. The accumulated compounds appear to be primarily nonextractable, polar metabolites of Δ^9-THC [61]. Attempts to explain the tolerance that develops to the effects of Δ^9-THC in terms of altered metabolism, distribution, or pharmacokinetics have not been successful [22, 51, 61, 77].

PCP is metabolized in most species to the mono-4-hydroxy piperidine derivative and is excreted as conjugates in the urine [69]. This rapid metabolism probably accounts for the short duration of action of PCP, since its

metabolites have relatively weak pharmacologic activity.

The purpose of this experiment was to provide information about the time course of total radioactivity in plasma and brain derived from ^{14}C-Δ^9-THC and ^{3}H-PCP that might be related to, or help explain, the behavioral and pharmacological interactions between Δ^9-THC and PCP.

Method

Animals and treatment. Male, Fischer-strain rats of the same age and weight as used in the other experiments were used in these experiments. They were treated daily for six days with sesame oil (2 ml/kg, IG), Δ^9-THC (10 mg/kg in sesame oil, IG), or PCP (5 mg/kg in saline, IP). On the seventh day separate groups were treated with ^{14}C-Δ^9-THC (10 mg/kg, specific activity = 40 μCi/kg, IG), and 90 min later they were injected IP with 2 ml/kg of saline or 5 mg/kg PCP. 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. Other animals were treated the same and sacrificed at these times for determination of total radioactivity in whole brain. In separate experiments animals were treated the same as described above for six days and on the seventh day they were given sesame oil (2 ml/kg, IG) or Δ^9-THC (10 mg/kg in sesame oil, IG) followed 90 min later by 5 mg/kg of ^{3}H-PCP (specific activity = 70 μCi/kg, IP). Blood was sampled serially in these animals by periocular puncture or they were sacrificed for brain analyses 0.5, 1.0, 2.5, 6.5, or 22.5 hr after administration of ^{3}H-PCP. All animals were treated on the seventh day between 0800 and 1000.

Determination of total 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 carefully notched and broken and placed in a counting vial containing 10 ml of Oxifluor-H_2O™ (New England Nuclear). Each whole brain was homogenized in 3 volumes of distilled water. Total radioactivity was determined in a 0.1 ml aliquot of the homogenate in the same counting system as for plasma.

Total radioactivity was determined using a Beckman Model LS250 liquid scintillation system. The cpm were converted to μg/ml of plasma or μg/g of brain of Δ^9-THC or PCP equivalents, which include the parent compounds and its radioactive metabolites. The radioactivity from known amounts of ^{14}C-Δ^9-THC and ^{3}H-PCP was determined and was used as standards for these conversions.

Results

Effects of PCP on ^{14}C-Δ^9-THC. The time course of total radioactivity derived from acute oral administration of ^{14}C-Δ^9-THC in plasma and brain is shown in the top panels of Fig. 5 along with the acute and subacute effects of PCP on this time course. Levels of total radioactivity reached a maximum in both tissues between 2 and 4 hr after administration and declined in a biphasic way thereafter. Levels in plasma were approximately twice those in brain after 1 hr reflecting the slower distribution process by this route of administration. When tissue equilibrium was reached after 2 hr, the levels in plasma and brain were the same and continued to be so over the next 22 hr within the limits of experimental variation. Neither acute nor subacute treatment with PCP significantly altered the time course of total radioactivity in plasma, but it appeared to retard the

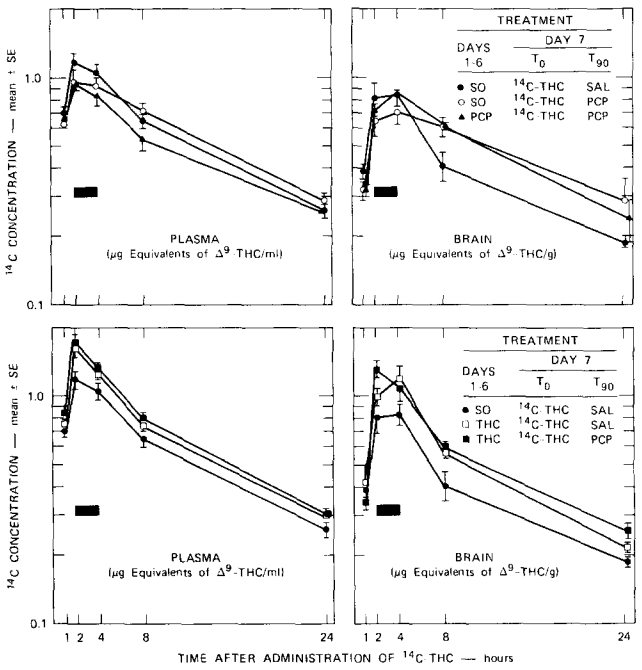

FIG. 5. Effects of acute and subacute treatment with PCP and subacute treatment with Δ^9-THC on the time course of total radioactivity in plasma and brain derived from ^{14}C-Δ^9-THC. Bars correspond to time when behavioral and pharmacological tests were done.

disappearance of total radioactivity from brain. However, the magnitudes of the differences were small and only marginally significant at 8 hours (t (8) = 2.2, $p < 0.1$).

The bottom panels of Fig. 5 show that the total radioactivity from an acute dose of ^{14}C-Δ^9-THC reached significantly higher levels in both plasma and brain after subacute treatment with Δ^9-THC than with sesame oil. However, there were no differences in the rates of disappearance after maximum levels were reached. PCP did not change the time course of total radioactivity in these Δ^9-THC-pretreated animals.

Effects of Δ^9-THC on ^{3}H-PCP. Maximum levels of total radioactivity derived from the IP administration of ^{3}H-PCP were attained within 30 min in both plasma and brain (Fig. 6). Disappearance was rapid initially with estimated half-lives of 1.9 and 2.4 hr, in plasma and brain, respectively. Thereafter, disappearance proceeded much more slowly with half-lives estimated from only the last 2 point of 60.8 and 37.2 hr. The disappearance of total radioactivity from brain was about twice as fast as from plasma during this second phase.

Acute treatment with Δ^9-THC did not significantly affect the disappearance curves in plasma. However, subacute pretreatment with Δ^9-THC appeared to prolong the initial disappearance phase in both plasma and brain and to accelerate the slow phase in brain ($t_{1/2}$ = 19.1 hr). There was a tendency for the initial disappearance phase also to be prolonged in brain after acute treatment with Δ^9-THC.

After subacute treatment with PCP total radioactivity was slightly lower in plasma and higher in brain after 30

min than in sesame oil-treated controls (lower panels of Fig. 6). The respective plasma:brain ratios were 1.36 and 0.96 at this time. The initial disappearance also was slightly prolonged in plasma after subacute pretreatment with PCP, but not in brain where the disappearance appeared to be more rapid. Acute administration of Δ^9-THC also appeared to accelerate the disappearance of total radioactivity from brain.

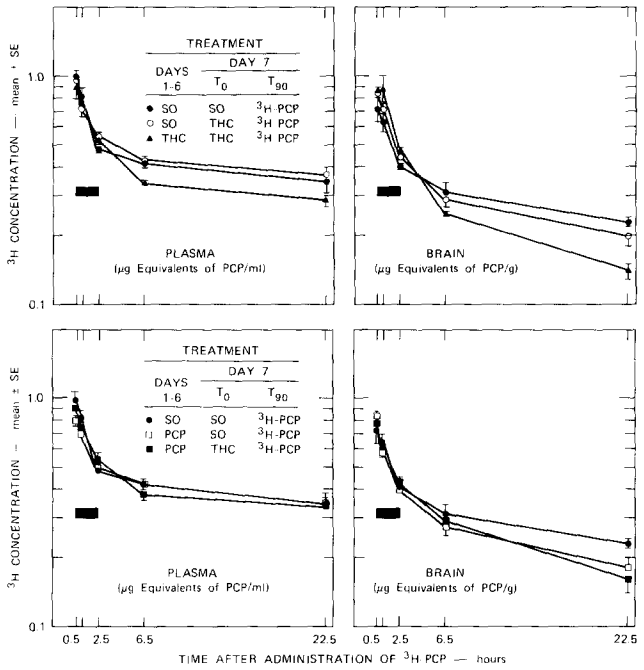

FIG. 6. Effects of acute and subacute treatment with Δ^9-THC and subacute treatment with PCP on the time course of total radioactivity in plasma and brain derived from ^{3}H-PCP. Bars correspond to time when behavioral and pharmacological tests were done.

DISCUSSION

The results of Experiment 1 provide an extensive evaluation of Δ^9-THC and PCP alone and in combination as a function of the respective doses of each drug and after subacute treatment with selected doses of either or both drugs on a number of behavioral and physiological measures. The results for acute administration of Δ^9-THC alone are in general agreement with other studies that show that this drug impairs performance [21, 24, 41, 44, 45, 75, 85], causes bradycardia in rats [65] as compared with tachycardia in humans [24,43], and is hypothermic [6,25]. They also confirm the development of tolerance to Δ^9-THC [1, 6, 20, 22, 62, 85] using an experimental design tailored to demonstrate pharmacological tolerance that was not confounded with behavioral tolerance (c.f. [10]). These results also confirm previous findings [11,23] of acute PCP-induced stimulation of photocell activity in rats. The stimulation of activity caused by PCP was of an antaxic, compulsive nature [11], which we confirmed in Experiment 3 by direct observation. This effect is related to and may account for the impairment of CAR and rotarod performance that we observed after a dose of 5 mg/kg of PCP. The general lack of effect of low doses of PCP on heart rate and body temperature is also in agreement with others [11, 12, 23]. These results also show that some of the effects of PCP are increased after repeated administration; a dose of PCP (2.5 mg/kg) that was ineffective when given acutely caused stimulation of activity and impairment of CAR and rotarod performance after subacute treatment equivalent to an acute dose of 5.0 mg/kg.

The interactions between Δ^9-THC and PCP can be summarized as an enhancement of any depressant properties of Δ^9-THC by PCP and antagonism of any stimulant properties of PCP by Δ^9-THC. We have found this result to be characteristic of the interactions between Δ^9-THC and a number of other drugs, including stimulants, depressants, hallucinogens, and tranquilizers [72,73]. However, the predominant interactive effect depends upon the respective doses of the two drugs, the history of pretreatment with either or both drugs, and the particular measure used.

The results of the second experiment were in general agreement with the results of Experiment 1 in demonstrating that Δ^9-THC will interact with PCP to cause greater impairment of CAR performance than that caused by either drug alone. Moreover, the magnitude of the interaction depended upon the pharmacokinetics of the two drugs and the effective levels of each drug at the time of testing. Thus, PCP was ineffective alone when administered 2 hr before testing and the interaction with Δ^9-THC was minimal when Δ^9-THC was given 2 hr or 30 min before testing. Similarly, since Δ^9-THC is absorbed slowly after oral administration, a greater impairment of CAR performance was seen when this drug was given 2 hr before the test than when it was given 30 min before the test and the combined effect of both drugs was greatest when Δ^9-THC was given 2 hr and PCP was given 30 min before the test. This result was predicted on the basis of the expected pharmacokinetics of the two drugs by these routes of administration, unless the pharmacokinetics were changed by the interaction. It was not expected that the interaction would be as great as was observed when both drugs were given 30 min before the test. However, since 5 mg/kg Δ^9-THC was sufficient to interact with 2.5 mg/kg PCP to cause greater impairment than either drug alone in Experiment 1, the result seen in Experiment 2 with 10 mg/kg Δ^9-THC given together with 2.5 mg/kg PCP can be easily reconciled in terms of effective levels at the time of testing. The plasma levels of total radioactivity derived from 10 mg/kg ^{14}C-Δ^9-THC are about the same after 1 hr as the levels derived from 5 mg/kg ^{14}C-Δ^9-THC after 2 hr [73].

The results of Experiment 3 confirm those of Experiment 1 and others [11], in further demonstrating an increase in locomotor activity caused by PCP that was enhanced by subacute treatment. They extend those results in showing that this effect is characterized by compulsive, ataxic forward movement accompanied by a reduction in grooming behavior with no change or an increase in rearing. The results for acute administration of Δ^9-THC also agree with those of others [25, 58, 64, 80] who have studied the effects of this drug on open-field behavior, and who have reported relatively greater suppressant effects on rearing and grooming than on locomotion. The acute combination of Δ^9-THC and PCP caused almost complete suppression of all behavior in the arena. Subacute pretreatment with Δ^9-THC abolished this interactive effect on locomotion, but not rearing and grooming, whereas no changes were seen on any of the three measures after subacute treatment with PCP.

Others have reported that Δ^9-THC inhibits defecation in rats with a high initial index [25] and inhibits gastric motility [5, 6, 13]. This effect was not reflected in decreased defecation in the arena in this experiment, perhaps because the control rates were low. However, the combination of Δ^9-THC and PCP, which does not inhibit gastric motility alone [11], significantly suppressed defecation. Both drugs caused an increase in urination, but there was no interaction between the two on this measure. PCP has been reported to increase urine output in hydrated rats [12] and cannabis has been reported to have a similar effect in man [4,7].

The lack of any significant effect of a dose of 5 mg/kg Δ^9-THC on operant responding under an FR-10 schedule of reinforcement in Experiment 4 agrees with the results of others [29,32] using similar doses and schedules. Moreover, the reduction in response rates caused by acute administration of 2.5 mg/kg PCP is similar to that caused by 2 mg/kg in rats working on a VI-60 schedule [67]. The increased suppression caused by the acute combination of Δ^9-THC, which was ineffective alone, and PCP also confirms this interaction [67].

In view of our earlier observations that PCP has cumulative effects in other test systems, we were surprised to see, what appeared to be, tolerance to its effects on response suppression under the FR-10 schedule. We do not think that the results were fortuitous, since the experiment was conducted in two replications and the apparent tolerance was seen in both replications. Moreover, the interaction between Δ^9-THC and PCP was attenuated slightly after subacute treatment with PCP. We cannot offer any explanation for these disparate effects of PCP in the various test systems we used at this time. Subacute treatment with Δ^9-THC also attenuated the interaction between Δ^9-THC and PCP. This result is indicative of tolerance to Δ^9-THC even though the acute administration of this dose was below the effective level on this test.

The pharmacokinetic experiments were done using ^{14}C-Δ^9-THC dissolved in sesame oil and administered intragastrically to provide data that might help explain the results of our behavioral and pharmacological studies. A direct comparison of our data with others generally is not possible since most investigators have used other vehicles and other routes of administration for studying the metabolic fate of Δ^9-THC. One study [60], however, compared the time course and distribution of total radioactivity derived from ^{14}C-Δ^9-THC in various vehicles and by all the major routes of administration in mice. The curve for total radioactivity derived from ^{14}C-Δ^9-THC in corn oil given orally was essentially flat over a 16-hour interval in this species. Our results in the rat, which have been replicated many times in our laboratory [73], indicate that total radioactivity reaches a peak in this species in both plasma and brain between 2 and 4 hours after oral administration in sesame oil and declines slowly thereafter. This time of peak total radioactivity corresponds to the time period over which the drug is most active pharmacologically by this route. These results were obtained in animals that had continuous access to food and water throughout the experiment. We have found that fasting results in slower absorption and that peak levels of radioactivity are not reached until 8 hours after administration in such fasted animals [in preparation].

Subacute treatment with Δ^9-THC caused the levels of total radioactivity derived from ^{14}C-Δ^9-THC to reach higher levels in both plasma and brain than subacute treatment with sesame oil. This effect is also reliable and has been repeated many times in our laboratory [73]. Others [61] did not find any differences in the time course of total radioactivity (or the radioactivity in various organic extracts) derived from ^{3}H-Δ^9-THC in the plasma or brains of tolerant and nontolerant pigeons when the drug was administered intramuscularly. The difference in routes of administration may provide a clue as to the mechanism involved in our results. Since acute administration of Δ^9-THC inhibits gastric motility [5, 6, 13] and there is tolerance to this effect, and since Δ^9-THC is absorbed more readily in fed than fasted animals, it is possible that these phenomena interact to allow more rapid absorption from the gastrointestinal tract in our tolerant rats. Another possibility is that since Δ^9-THC and its metabolites accumulate with repeated administration [48,61], the increase we see in total radioactivity may be the result of an isotope dilution effect. Thus, as the newly introduced labeled material is diluted with the accumulated cold material, less of the label is lost by elimination and an apparent increase in Δ^9-THC and its metabolites is seen. Finally, storage sites in fat, for example, may become saturated after subacute treatment with the result that there is a shift in distribution of the newly-introduced ^{14}C-Δ^9-THC. In any event, whether or not this increase in total radioactivity is in any way related to the tolerance that develops to Δ^9-THC cannot be answered with the available data.

The effects of acute and subacute treatment with PCP on the time course of total radioactivity derived from ^{14}C-Δ^9-THC were negligible and could not be related to their combined interactive functional effects. Although there was some evidence that PCP retarded the disappearance of total radioactivity from brain, the effect was small and only marginally significant at 8 hr. Moreover, even if reliable, these changes occurred at a time much later than when their pronounced behavioral interactive effects were seen.

The rapid absorption of PCP after IP administration was indicated by the fact that peak levels were already reached within 30 min. Total radioactivity derived from ^{3}H-PCP disappeared rapidly at first and at a slow rate thereafter. These results agree with those of Ober et al. [69], who reported that 60% of the ^{14}C from IV doses of ^{14}C-PCP appeared in the urine of rhesus monkeys in 12 hours and about 75% in 8 days. Disappearance was more rapid from brain than plasma and treatment with Δ^9-THC appeared to accelerate this disappearance. However, there was no significant relationship between the levels of total radioactivity during the time of behavioral testing that could account for the functional interaction between PCP and Δ^9-THC.

The results of these experiments, while providing valuable information about the time courses of total radioactivity derived from ^{14}C-Δ^9-THC and ^{3}H-PCP and how they are affected by each other, do not suggest any metabolic explanation for the behavioral and pharmacological interactions we have seen between these two drugs. It is possible that such metabolic effects would be revealed by examining the levels of the parent compounds and metabolites in some detail. However, other explanations in terms of central mechanisms are just as likely.

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