held in "neutral" water during their early life history, (ii) they were released 4.7 km from the streams, and (iii) unexposed fish were recovered at several locations. We conclude, therefore, that fish treated with morpholine and p-alcohol used chemical cues to return respectively to streams treated with those substances. The fish learn (imprint to) the morpholine or p-alcohol during a short period of time during the smolt stage, and they retain these cues for 18 months without being again exposed to the chemicals. As this study was conducted in the field, it provides direct evidence that coho salmon use this mechanism for homing. These studies confirm the odor hypothesis for salmon homing.

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2. Recent studies indicate that coho salmon use chemical cues for homing. In four experiments with coho salmon (*3–6*) and three with steelhead trout (*5, 7*), significantly larger numbers of fish chemically imprinted to morpholine than untreated fish returned to a morpholine-scented stream. When morpholine was not added to the stream during the spawning migration, treated and nontreated salmon returned in equal low numbers. Behavioral experiments (*3–5*) show that morpholine-exposed fish tracked with ultrasonic transmitters stopped in an area scented with morpholine and passed through the same area when morpholine was not present. Electrophysiological studies (*8*) indicated a significant difference in the amplitude of the EEG response recorded from the olfactory bulb between morpholine-exposed and control salmon to morpholine. In addition, Jensen and Duncan (*9, 10*) report that coho salmon marked and held in a natural water supply for 48 hours while smolting returned specifically to that water to spawn.
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9. Jensen and Duncan (*10*) transplanted coho salmon smolts from their original hatchery on the Columbia River to a spring-fed fish-holding facility on the Snake River. The fish were held for 48 hours and then released into the Snake River. During the spawning migration, marked fish were recovered near the springwater discharge 0.8 km downstream from the release point but not from other locations. To determine if the fish were actually homing to the water in which they had been held as smolts, water from the holding facility was pumped through a floating trap. As a control, river water was pumped into the trap on alternate days. No fish entered the trap when river water was used, but 399 fish were captured during periods when springwater was used. Springwater from the fish-holding facility was thus the orienting stimulus; fish were able to learn the characteristics of this water within 2 days.
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12. During the smolt stage, salmon undergo physiological and behavioral changes (*5*). Coho salmon taken from their original home stream or hatchery prior to the smolt stage and transplanted to a second stream subsequently returned to the second stream to spawn (*9, 13*). This indicates that the smolt stage is a sensitive or critical period when fish learn the cues that identify their natal tributary or river of release. This learning process probably terminates soon after they become smolts. Peck (*13*), for example, transplanted coho salmon older than smolts and determined that large numbers strayed into other streams.
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15. The number of treated salmon recovered in their respective streams probably represents a good sample of the total number returning. About 0.5 to 5.0 percent of naturally produced fish or fish raised and released from a fish hatchery return to their home stream. The breakwater at Two Rivers presented greater difficulty compared to the Little Manitowoc River in terms of sampling. This may account for the difference in the total number of recoveries for each group. The reason for the difference in the number of recoveries within groups in 1974 and 1975 is that twice as many marked fish (5,000 versus 10,000) were released in each group in 1975.
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Residual Learning Deficit After Heavy Exposure to Cannabis or Alcohol in Rats

Abstract. *Acute oral administration of cannabis extract to rats (tetrahydrocannabinol dose, 10 milligrams per kilogram) impaired maze learning. The impairment was more marked after ten daily doses of the same size. After 1, 2, or 3 months' pretreatment with the same daily dose, followed by a 25-day drug-free period, no residual learning impairment was found. However, 6 months of daily administration of cannabis (tetrahydrocannabinol, 20 milligrams per kilogram) or alcohol (6 grams per kilogram) produced significant residual impairment of learning of maze and motor coordination tasks, 2 months or more after the last drug administration.*

It has long been recognized that chronic heavy use of alcohol may give rise to an organic brain syndrome characterized by slowing and interruption of mental processes, difficulty with abstract thought, and impairment of memory and learning ability (*1*). Walker and Freund (*2*) reported that rats kept on alcohol-containing diets for 6 to 12 months showed impaired avoidance learning when tested a month or more after the end of treatment.

Many clinical reports from India, North Africa, and elsewhere have referred to a similar "dementia" in long-term heavy users of hashish (*3*, p. 114). Clinical descriptions of a similar state have recently appeared in the North American and European literature, ranging from moderate impairment of verbal learning and recall (*4*) to a full clinical picture which in some cases was thought to indicate organic brain damage (*5*). Campbell *et al.* (*6*) described air encephalographic findings of enlargement of the cerebral ventricles and cortical atrophy in ten young patients who had used cannabis heavily in addition to smaller amounts of other drugs for at least 6 months.

The interpretation of these findings is complicated by the frequent presence of multiple drug use, malnutrition, infections, and other incidental factors, as well as by the difficulty of distinguishing between chronic intoxication and residual postintoxication effects. We have therefore examined the effects of cannabis, acutely and chronically, as well as those of chronic ethanol, on performance of learning tasks in rats, under conditions in which the confounding factors were excluded.

An ethanolic extract of preassayed marihuana leaf material was heated to convert all the tetrahydrocannabinolic acid to tetrahydrocannabinol (THC) (*7*). The THC content of the extract was assayed by gas-liquid chromatography (*7*), and the appropriate dose was then dissolved in 0.2 ml of olive oil for administration to the rats.

For the acute experiment, 18 animals were reduced to 80 percent of their free-feeding weight, and pretrained in the Rabinovitch-Rosvold modification of the Hebb-Williams closed-field maze (*8*). This test has been shown to be sensitive to cortical ablation and to drug-induced learning deficits.

Pretrained rats were tested on a series of 12 problems arranged in order of increasing difficulty. The score for each animal was the total number of errors on eight trials on each problem.

On test day 1 the rats were tested on the first four problems, and assigned to two equal groups matched on the basis of their scores. On test day 2 one group was treated with a dose of cannabis extract containing 10 mg of THC per kilogram, administered by stomach tube 1 hour prior to testing. The other group was treated with an equal volume of olive oil. Within the following 2 hours, each rat was tested on problems 5 to 8 of the series. This procedure was repeated on test day 3 with problems 9 to 12.

The marihuana-treated rats committed an average of 87.6 ± 9.0 errors on problems 5 to 12. The mean control score was 68.2 ± 4.8. This difference was significant on a one-tailed t-test ($P < .05$). This impairment agrees with the findings of Carlini and Kramer (*9*) on a different type of maze test.

For the short-term, subchronic experiment, five rats reduced in weight were treated with marihuana extract (THC, 10 mg/kg) daily for 14 days, while five others received olive oil. On days 6 to 14, all animals were given a training session, followed immediately by treatment. By day 14, all animals were trained to criterion. These animals were then tested on problems 1 to 12 over a 3-day period (days 15 to 17) from 1 to 3 hours *after* drug or placebo administration each day.

After 7 days the treated animals became very irritable shortly after treatment, as noted by Carlini *et al.* (*10*). They exhibited backward circling and licking behavior, and shrieked whenever handled. During testing they showed little interest in the problems, and moved very slowly, often stopping to lick the plexiglass floor of the maze. When they finally reached the food box, however, they ate avidly. Their error scores were very high as compared to controls: mean = 144 ± 18.6 (standard error of the mean) versus 77.6 ± 3.6, respectively; $P < .01$ by one-tailed t-test. The rats in this experiment did not become tolerant to the observed effects of the drug; instead the effect became progressively greater. This was in contrast to the tolerance to the anorexic effect of the drug that developed after 7 days of treatment, and to tolerance on other tests reported in the literature (*3*, p. 119; *11*). In view of the long half-life and high lipid solubility of THC, the apparent absence of tolerance in the maze tests might conceivably reflect drug accumulation in the body on the dosage schedule used.

For the first chronic experiment rats weighing 50 g were randomly divided into three groups of ten, each group containing five test animals and five control animals given placebo. Test animals received the standard daily dose of cannabis extract (THC, 10 mg/kg), while the control rats received an equal dose of olive oil. The three groups were treated similarly for 30, 60, and 90 days, respectively.

The animals were then withdrawn from treatment for 2 weeks. During the second week they were coded randomly, and reduced to 80 percent of free-feeding weight. After a pretraining period of 8 to 10 days they were tested on problems 1 to 12, over a period of 4 days (beginning 25 days after the end of drug treatment). In order to test for retention of learning, the 60- and 90-day groups were retested 2 weeks after the initial testing.

The scores over 12 problems for these rats are shown in Table 1. Analysis of variance showed that although there were higher scores with increasing age ($P < .05$) there was no significant difference between the scores of the treated and control animals. Retesting of groups 2 and 3, 2 weeks after the initial testing, gave means ranging from 48.2 ± 3.5 to 54.8 ± 5.8, which indicated no signifi-

Table 1. Scores in Hebb-Williams maze learning by rats, 1 month after end of chronic treatment with cannabis extract or ethanol. N.S., difference not significant.

Treatment	Duration (days)	N	Total errors	Runs to criterion
Control	30	5	81.6 ± 15.1	
Cannabis (THC, 10 mg/kg)	30	5	89.6 ± 12.2 (N.S.)	
Control	60	5	108.4 ± 10.9	
Cannabis (THC, 10 mg/kg)	60	5	96.2 ± 9.2 (N.S.)	
Control	90	5	113.6 ± 9.8	
Cannabis (THC, 10 mg/kg)	90	5	124.8 ± 6.1 (N.S.)	
Control*	180	8	87.4 ± 6.4	93.7 ± 6.0
Cannabis (THC, 20 mg/kg)	180	8	106.5 ± 5.1 ($P < .025$)	104.3 ± 2.9 ($P < .075$)
Ethanol (6 g/kg)	180	8	102.4 ± 5.3 ($P < .05$)	108.8 ± 4.3 ($P < .05$)

*This experiment was done at a different time from the preceding ones, with rats of a different stock and using a different food reward. Comparisons can be made only within, and not between, experiments.

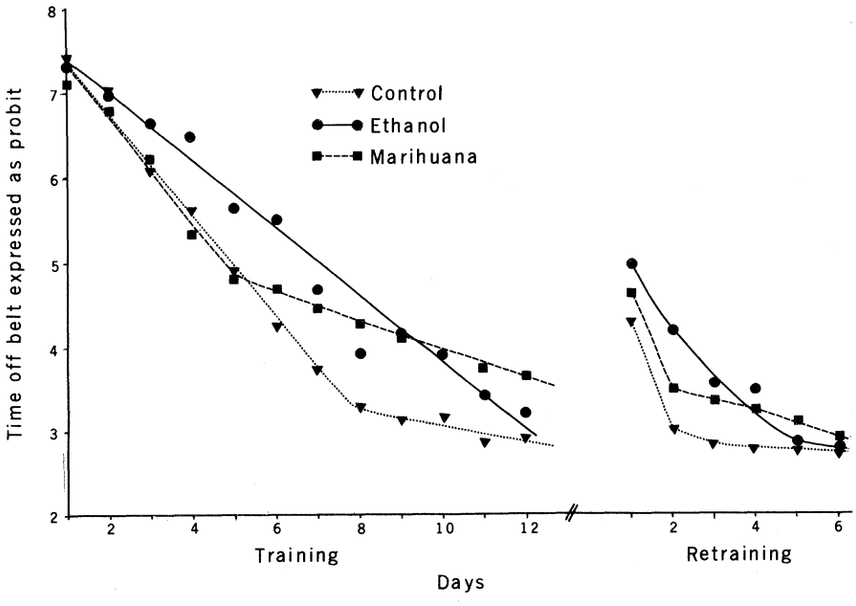

Fig. 1. Probit transformation of respective group mean scores for 6-month treatment groups on successive training days during learning of a motor coordination test. Ordinate is maximum time off belt (seconds) during a single 2-minute trial. Initial training began approximately 2 months after the end of chronic drug treatments. The interval between the first training period and retraining was 2 months.

cant differences due to treatment or duration.

A second chronic experiment was carried out, using heavier cannabis exposure and a second test of learning ability in addition to the maze. In the light of reported evidence of ethanol-induced learning impairment (*2*), an ethanol group was added as a further check on the sensitivity of our testing procedures.

Twenty-four male rats weighing about 120 g were divided randomly into control, cannabis-treated, and ethanol-treated groups. All animals had free access to rat chow for the duration of the experiment. For 6 months the cannabis group was treated daily with cannabis extract in olive oil at a dose equivalent to 20 mg of THC per kilogram of body weight.

The ethanol animals were intubated daily with a 25 percent solution of ethanol in water. The initial dose of 2 g/kg was gradually increased to 6 g/kg over 2 weeks, and this dose was continued for the balance of the 6-month period. The control group was treated with a sucrose solution equal in calories to the daily dose of ethanol. The cannabis and control groups showed equal weight gain over the 6-month period, while the alcohol-treated rats gained 50 to 100 g less than the others. Ideally, a second control group receiving daily intubation with 0.2 ml of olive oil should have been included, but it was not possible to carry four groups through the maze-training simultaneously. Since it is highly improbable that 0.2 ml of a normal dietary lipid would have any effect on the performances under study, the use of the single sucrose control group seems adequate.

After 6 months, all treatment was stopped, and the surviving animals were allowed to recover from drug effects for 1 month. Each rat was coded, and then reduced to 80 percent of free feeding weight. After a 20-day pretraining period, rats were tested on the same 12 problems used in the earlier experiments. Two separate scoring criteria were used. One was the number of runs needed to reach a criterion of three out of four correct trials for each problem, to a maximum of 20 runs per problem. The other was the total error score as calculated previously. The ethanol group performed significantly worse than the controls by both criteria, and the marihuana extract group showed significant impairment in the error score and marginal impairment in runs-to-criterion score (Table 1).

The same rats were then immediately started on a second and independent learning task, the moving belt test, which has previously been used as a sensitive measure of alcohol impairment (*12*). If the animal puts one or more paws off the moving belt, it receives a small electric shock from the grids on either side of the belt, and simultaneously activates a cumulative timer. The error score is expressed as the number of seconds spent off the belt, during a standard 2-minute trial. The rats were given three trials per day for up to 12 consecutive days. As each rat reached criterion score (no more than 1.2 seconds, or 1 percent of the total time, off the belt) it was eliminated from further training to prevent overtraining. The rats were then left for 2 months without any training, and were retested after this period.

A probit transformation of the mean daily scores yielded linear learning graphs over the training period (Fig. 1). The slopes of the ethanol and control groups are significantly different ($P < .05$). There was no obvious ataxia which could account for the difference. The initial learning in the marihuana group appeared to be identical with that of the controls, mainly because of two rapid learners in the marihuana group which heavily influenced the mean score. The group as a whole, however, showed significantly poorer scores than the controls from day 8 to day 12.

In the retraining phase, the ethanol curve was again significantly different from that of the controls. Although the scores of the marihuana group are higher than those of the controls, the large variance in this group prevented any conclusions from this phase of the experiment.

Thus, long-term treatment with a high dose of alcohol produced residual impairment of learning on both cognitive and motor tasks. This confirms and extends the findings on Walker and Freund (*2*). The same duration of treatment with the highest dose of cannabis extract caused comparable residual effects. The study was later repeated with slightly larger numbers ($N = 10$ per group) and older rats (100 g initial weight); the results were closely similar to the present ones, but with smaller variation in the cannabis group and greater statistical significance. (A report on the latter findings, together with electroencephalographic and histological observations on the same rats, is in preparation.)

It should be noted that no residual impairment was found after THC dosage of 10 mg/kg daily for 3 months, so that a very high level of cumulative exposure seems necessary. The effective dose (20 mg/kg) cannot be applied literally to humans for two major reasons. The first is that rodents are much more resistant than larger species to most drug effects on the central nervous system. The lowest intravenous dose of THC reported to produce significant effects on electroencephalographic recordings is 1 mg/kg in rats and 0.5 mg/kg in rabbits (*13*). The second is that much larger doses of cannabis are required by mouth than by intravenous or intrapulmonary administration to produce comparable effects (*14*). In fact, the cannabis-administered animals were visibly intoxicated for only about 4 hours after each dose, gained weight normally, and were in good general health throughout the experiment. The 6 g/kg dose of ethanol would also be lethal in humans, yet even though it produced some general impairment of health in the rats it was fairly well tolerated.

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