parenteral administration, anyone conducting clinical research with these drugs should consider the possibility of long-term effects.

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11. B. Weiss and V. Laties, *J. Pharmacol. Exper. Therap.* **131**, 1 (1961). Male squirrel monkeys were restrained in a plexiglass chair equipped with a lever. Two electrodes, through which shock was applied, were strapped to the monkey's tail. The shock intensity was automatically programmed to increase from 0.01 ma to a maximum of 4.0 ma in 100 discrete steps. The shock intensity was increased one step every 5 seconds but, whenever the subjects made five responses on the lever, the shock was turned off for 5 seconds, and the intensity was decreased 20 steps. This sequence was continuously repeated throughout the testing sessions. Each subject was given one 2.5-hour session per week. The intensity of shock tolerated by the subjects was the intensity at which the subjects responded to terminate the shocks.
12. C. L. Scheckel, *J. Comp. Physiol. Psychol.* **59**, 415 (1965). Food-deprived subjects were individually placed in a large box containing three levers. Above each lever was a translucent disc (2.54 cm diameter) that could be illuminated with a red, green, or white light. Normally the discs were white, but every 3 minutes a sample stimulus was presented at the center disc. The sample was either a red or green light which remained on for a maximum of 10 seconds. The subjects were trained to press the lever under the sample, and this response started a delay interval during which all lights were turned off. After the delay, only the side lights came on; one side was red and the other green. The animal's task was to press the lever under the side disc that was the same color as the sample had been (match the sample). Correct matching responses were rewarded with a 190-mg banana pellet. Incorrect responses terminated the trial with no reward. The duration of the delay interval (the time that the subject had to "remember" the color of the sample) varied as a function of the animal's performance. At the start of each 4-hour session the delay was set at 0 seconds, but every time the subject made correct matching responses on two consecutive trials at one delay, the delay presented during the next trial was automatically increased by 10 seconds. Whenever a subject failed to match correctly or failed to respond to the sample, the delay interval in the next trial decreased 10 seconds. Thus, as the subject performed correctly the problem became more difficult, but errors or a failure to respond to the sample made the problem somewhat easier.
13. J. Sullivan and E. Boff, in preparation.
14. The hallucinations and panic states produced by racemic Δ^9-THC injected into monkeys correlate well with the effects of high doses of *l*-Δ^9-THC and marijuana smoked by man [H. Isbell, C. W. Gorodetzsky, D. Jasinski, U. Claussen, F. V. Spulak, F. Korte, *Psychopharmacologia* **11**, 184 (1967); M. H. Keeler, *Amer. J. Psychiat.* **124**, 674 (1967)].
15. Death occurred in two of five subjects that received 16 mg of Δ^9-THC per kilogram, in all five subjects at dosages of 32 mg/kg and in two of four subjects at dosages of 64 mg/kg. The exact cause of death is unknown, but was probably due to a combination of respiratory depression, heat loss, and decreased food and water intake.
16. All subjects in this procedure vomited within 1 hour after receiving Δ^9-THC, but Δ^8-THC did not cause emesis in any subject.
17. Supplied by Dr. T. Petrzilka, Eidgenosse Technischen Hochschule, Zürich.
18. We thank Drs. K. E. Fahrenholtz and R. W. Kierstead for making available the racemic drugs, J. W. Sullivan for technical assistance, and Dr. A. Brossi for encouragement.

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Alcohol Preference in the Rat: Reduction Following Depletion of Brain Serotonin

Abstract. *Preference for ethyl alcohol was significantly reduced or totally abolished in rats given orally* p-*chlorophenylalanine, a tryptophan hydroxylase inhibitor that selectively depletes brain serotonin. Some aversion to alcohol was observed while* p-*chlorophenylalanine was administered, but the rats' rejection of alcohol was even more marked after the drug was discontinued. Oral administration of* α-*methyl*-p-*tyrosine, a tyrosine hydroxylase inhibitor that depletes brain catecholamines, slightly reduced selection of alcohol, but preference returned to normal as soon as* α-*methyl*-p-*tyrosine was terminated.*

Repeated microinfusions of minute amounts of alcohol into the cerebral ventricles of unrestrained rats produce a dose-dependent preference for alcohol (*1*). This observation has led to the hypothesis that metabolic systems, in the limbic-forebrain structures lining the walls of the ventricles, are directly affected by the presence of alcohol, and that the biochemical state of these systems may underlie the aberrant drinking patterns observed in the chronic alcoholic (*2*).

To determine whether a neurochemical imbalance would either trigger or suppress an animal's preference for alcohol, compounds were chronically administered that alter endogenous substances in the brainstem regions involved in drinking and emotional behavior. In the experiment we report, the concentrations of monoamines were selectively lowered by either *p*-chlorophenylalanine (*p*CPA), a tryptophan hydroxylase inhibitor that substantially depletes brain serotonin (*3*), or α-methly-*p*-tyrosine (αM*p*T), which has a potent depletive action on brain catecholamines (*4*).

Adult male hooded rats of the Long-Evans strain, divided into three groups of six each, were maintained in individual cages and freely given Wayne Lab Blox throughout. Stable preference-aversion functions for alcohol (*5*) were obtained for each animal by offering water and a solution of alcohol simultaneously according to the two-choice, three-bottle, random-rotation method (*6*). Concentrations of alcohol were increased daily in the following sequence: 3, 4, 5, 6, 7, 9, 12, 15, 20, 25, and 30 percent. Thus alcohol preferences were validly measured over a broad range of concentrations for elimination of the drawbacks of a single-concentration method (*7*). The 11-day self-selection sequence was repeated three times: before, during, and after administration of saline vehicle to the first group, αM*p*T to the second, and *p*CPA to the third.

Each day of the 11-day drug period the compounds were given by the intragastric route and, to minimize the trauma of intubation, each rat was lightly sedated in an ether–ethyl chloride vapor chamber for 40 to 50 seconds before insertion of the esophageal tube. Alpha-methyl-*p*-tyrosine in normal

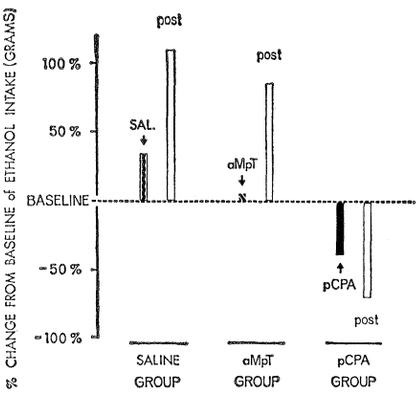

Fig. 1. Each bar represents the percentage shift (in total grams of alcohol selected), from individual base-line intakes before administration of the drug, during and after administration. The percentage increase in alcohol consumption is shown for the saline rats during (*SAL*) and after (*post*) control intubation, and for the aMpT rats during (*αMpT*) and after (*post*) drug administration. The percentage decrease in total alcohol selected is given for the *p*CPA rats during (p*CPA*) and after (*post*) administration of the drug.

saline was brought into suspension at *p*H 1.0 with 0.1*M* HCl, and the concentration of 13 mg/ml was raised to *p*H 4.5 with 0.1*M* NaOH; *p*CPA was similarly suspended in normal saline at a final *p*H of 4.5 and at 30 mg/ml.

Doses of *α*M*p*T at 130 mg/kg and *p*CPA at 300 mg/kg were given daily during the 11-day drug period to ensure maximum depletion of cerebral catecholamines and serotonin, respectively (*8*). As a control for the intubation procedure and for the volumes of the stomach loads of the drugs, 0.9-percent saline (*p*H 4.5) was given at 10 ml/kg to each rat in the control group. The 11-day alcohol-preference test periods, before, during, and after administration of the drug, were separated by 1 day on which all rats were offered water only.

The results clearly show that administration of *p*CPA markedly reduced preference for alcohol. Figure 1 shows for each group the percentage shift, in intake of alcohol, from the base line before the drug, both during and after the drug period. In the control (saline) group, alcohol preference increased during saline intubations as well as after administration, an effect typical of acclimation to alcohol (*9*). On the other hand, *α*M*p*T suppressed the usual increase in alcohol consumption due to acclimation during the drug period, but this effect disappeared as soon as the drug was discontinued. During treatment with *p*CPA, however, the preference for alcohol declined substantially, but the reduction was even more marked after termination of the drug (F, 18.44; 2/30 d.f.; $P < .01$).

Of fundamental importance is the response of individual animals to *p*CPA, especially for rats possessing an apparent phenotype for alcohol preference. Figure 2 illustrates the alcohol preference-aversion curves for three representative rats during each of the 11-day preference sequences. During the base-line period (Fig. 2, top) the three animals' preference functions were similar, although the saline (control) rat did not drink alcohol until higher concentrations were offered. During the drug period (Fig. 2, middle) the *p*CPA rat decreased its intake of alcohol, whereas the other rats' intakes persisted. After the drug was discontinued (Fig. 2, bottom), the *p*CPA animal rejected alcohol at virtually every concentration, but the saline and *α*M*p*T rats continued to prefer alcohol at baseline or even higher concentrations.

Although the reasons for the long-term action of *p*CPA in reducing preference for alcohol are not clear, the mechanism responsible for this effect may be highly local depletion of serotonin from one or more of the limbic structures that comprise the drinking-emotional "neural circuit" (*10*). This interpretation is necessarily limited by the fact that parallel serotonin and catecholamine levels have not been determined in our *p*CPA, *α*M*p*T, and control animals. There is some evidence that alcohol reduces the content of serotonin in brain tissue but this idea is not yet firmly established (*11*). Since *p*CPA depletes serotonin, and if alcohol has a similar effect, the *p*CPA rats may have rejected alcohol because its intake would have only further lowered already-depleted levels of serotonin. Thus aversion to alcohol may have reflected the animals' attempts to conserve the remaining stores of serotonin.

It is also possible that variation in the concentration of tryptophan, a precursor to synthesis of serotonin, or of another precursor may in some way alter the action of alcohol on certain structures of the central nervous system. Moreover, the effects of *p*CPA on other metabolic systems within the brainstem (and even the liver) cannot be disregarded, since levels of other substrates, including vitamins, coenzyme

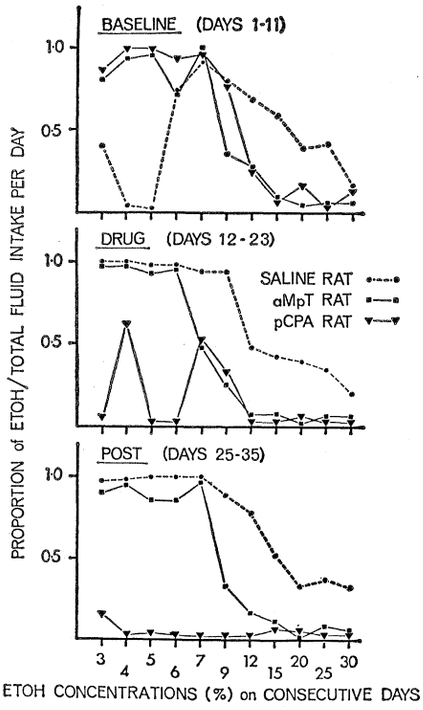

Fig. 2. Preference for alcohol (*ETOH*) or water (expressed as the alcohol proportion of total intake of fluids) by three representative rats. Alcohol concentrations were increased on each of 11 days (abscissa) before (*Baseline*), during (*Drug*), and after (*Post*) daily intragastric loadings with 0.9-percent saline at 10 ml/kg, *α*M*p*T at 130 mg/kg, or *p*CPA at 300 mg/kg.

factors, or other amines, also could be affected by *p*CPA. The long-lasting action of *p*CPA appears not to be due to interference with caloric need or extracellular fluid balance (*12*), because, during the test period following administration of *p*CPA, intakes of food and fluids were identical with those of the base line.

In any event the pharmacological action of *p*CPA that occurs primarily after the drug is discontinued indicates that a metabolic system has been drastically and perhaps permanently altered. Astonishingly, another 11-day preference-test sequence, 1 month after termination of administration of *p*CPA, revealed that the same aversion to alcohol persisted. Since whole-brain serotonin values return to normal within 16 days after administration of *p*CPA (*3*), an entirely different biochemical system may thus be involved in the resultant rejection of alcohol after administration of *p*CPA. This notion can be verified only by assays of levels of serotonin after administration of *p*CPA, in view of the fact that our rats re-

ceived the drug for 11 consecutive days.

Whether or not treatment with *p*CPA may be applied to the problem of human alcoholism is unknown, especially because of the difficulty inherent in postulation of an animal analogue to the human disease state (*13*). It would be premature to infer that *p*CPA would have some value in ameliorating the causal factors related to human imbibition, including social, psychological, and possible metabolic defects associated with the abnormal intake of alcohol. However, our findings do suggest that restoration of normal neurochemical function in an organism that drinks alcohol excessively, regardless of the etiology of an aberrant drinking pattern, may now be within the realm of possibility.

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Conditioned Reinforcement in the Goldfish

Abstract. *Goldfish were trained to press a lever on a 10:1 fixed-ratio schedule of reinforcement. They were extinguished under three conditions. Responding was followed by (i) solenoid noise and water delivery formerly associated with food reinforcement, (ii) solenoid noise only, or (iii) nothing. The number of extinction responses was largest in condition 1, less in condition 2, and smallest in condition 3, thus providing evidence for conditioned reinforcement in goldfish.*

There has been a resurgence of interest in the comparative study of conditioning. Bitterman (*1*), who contributed much in this area, suggested that the role of the brain can be effectively studied by comparing learning in different species. Therefore, the process of conditioned reinforcement in the fish has been investigated to see whether the fish, like the rat, can be controlled by conditioned reinforcement.

A conditioned reinforcement is a stimulus which acquires its reinforcing attribute through the process of conditioning, whereas a primary reinforcement, such as food, does not depend on conditioning history. Conditioned reinforcement is a central concept in many theories of behavior (*2*); it allows many kinds of stimuli to control the behavior of animals.

In our experiments, six goldfish (*Carrasius auratus*, 12 to 16 cm long), were conditioned in the following manner. Each fish was housed individually in a 10-liter tank with a filter. During the experiment a target was placed inside the tank. The response consisted of striking the target and displacing it approximately 0.3 cm; the displacement of the target closed the switch and activated a worm-dispenser that dropped tubifex worms into the tank right over the target (*3*). Each time a worm was dispensed, there was a noise produced by the solenoid, and the water and worm were dropped from an eye dropper into the tank. The noise of the solenoid was used as one potential conditioned reinforcer, and the combination of noise and water was used as the other potential conditioned reinforcer.

The fish were initially fed about 75 worms each in their home tanks every 2 days for 2 weeks. After they had learned to strike the target, the fish were reinforced continuously, and then they were gradually brought up in ratio so that ultimately they had to make ten responses for every worm. Each fish was given 75 reinforcements per session; each experiment took place every 2 days at approximately the same time of day. All fish responded on the 10 to 1 schedule by the third session. After seven sessions of conditioning, the fish were put on extinction. Two fish were extinguished with every tenth response followed by the solenoid noise and the delivery of water through the eye dropper; two fish had every tenth response followed by the solenoid noise only; the last two fish received no feedback at all, that is, neither the solenoid noise nor the water delivery. The extinction periods were continued until the fish reached a criterion of 10 minutes of no response. Thirty minutes after the end of each extinction period (each extinction period lasted 45 minutes) the fish were fed 75 worms. The extinction sessions occurred every 2 days.

The results showed the following: the two fish receiving noise and water after every tenth response in extinction took 25 and 26 sessions to reach criterion; the two fish receiving only noise after every tenth response required 9 and 17 sessions to reach criterion; and the two fish receiving neither noise nor water took 15 and 4 sessions to reach criterion. The relatively rapid and immediate drop in response rate in the group receiving no feedback at all for responding (Figs. 1 and 2) differs from

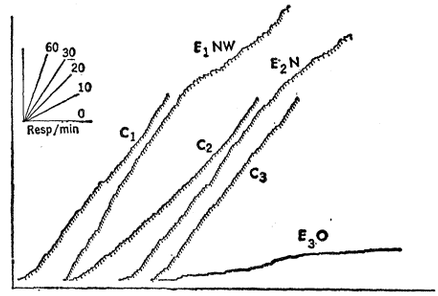

Fig. 1. Cumulative number of responses for one fish in the course of the last conditioning session before extinction and the first extinction session. The subscripts refer to the order in which the fish was run through the three different conditions. *C*, conditioning; *E*, extinction; *N*, noise; *NW*, noise and water; *O*, nothing. The downward "blips" indicate the receipt of food under the *C* condition, the noise under the *EN* condition, and the noise and water under the *ENW* condition.