rectly to either side when a single speaker sounded. We next postulate that if the leading pulse is contralateral to the intact hemisphere, then the tone pulse pair is perceived in the normal way, irrespective of training. However, if the leading pulse is contralateral to the damaged hemisphere, then the pulse pair is not perceived normally and the response tends to be to the side favored by training. This would not only explain the anomalous performance of cats with lesions on the right side to the LR pulse pair, but would also account for the apparently correct perception of the RL pulse pair by cats with lesions on the left side since both groups were trained to favor the right side.

To test these ideas we examined whether the effect of reciprocal training would cancel the effects of the original training. In the second experiment the same eight cats were trained in the reciprocal way shown schematically in Fig. 1B (R versus R + L). The results of learning the reciprocal task are shown in the second line of Table 1. Now it is the group of cats with lesions on the left side, previously severely impaired, which mastered the task in a few sessions, and the four cats with lesions on the right side failed to reach criterion in the allotted time (60 sessions). Thus, once again we encounter a symmetrical relation between the side on which the lesion occurred and the difficulty of the task. The extent to which the reciprocal training has reversed the favored side may be judged by the results of tests with "virtual left" and "virtual right" shown in the right half of Table 2. These results support the conclusion just drawn, that the pair of closely spaced tone pulses will be normally perceived if the leading member of the pair is opposite the intact hemisphere. The LR pair is judged to be on the left by cats with lesions on the left side and the RL pair is judged to be on the right by cats with lesions of the right auditory cortex. At the same time, the data support the conclusion that the locus of a pair of closely spaced tone pulses is not normally perceived when the leading sound is contralateral to the side of the lesion: The same cats, those with lesions of the left cortex, which before had apparently judged the RL pulse pair correctly now reveal a deficit in their perception of that pair; and the strong tendency of the group of cats with lesions on the right side to favor the right side (only 16 percent favor the left side) now has been overcome by the reciprocal training and these animals perform at a chance level of 53 percent.

Table 2. Virtual left and virtual right tests as a function of training and the side on which the lesion occurred. The proportions shown are the averaged proportions of responses to the side indicated by L or R.

Test	L versus L + R		R versus R + L	
	Left lesion	Right lesion	Left lesion	Right lesion
LR	0.89 L	0.16 L	0.92 L	0.53 L
RL	0.96 R	0.98 R	0.50 R*	0.78 R

* There were considerable individual differences, an indication of the variable effectiveness of the reciprocal training in reversing the earlier training.

Even though we do not wish to propose a single neural mechanism to account for our findings, there is a way of describing training effects and the role of lesion side in similar terms. Suppose that the training enhances the potency of stimuli on the side which must win out in competition with the opposite side. Suppose further that the lesion reduces the potency of the stimuli on the side contralateral to the lesion. Now these effects can work either in the same direction or in opposite directions. As an example of additive effects, we can cite the result of training cats with lesions on the left side to go left in the R + L test, and, as an example of antagonistic effects, we can cite the result of the same training procedure after a lesion is produced on the right side (see bottom line of Table 1). A similar interaction, that is, cooperation or competition, may be observed in the virtual right and virtual left tests. However, the training plays little or no role when the pair of stimuli is normally perceived, a condition which obtains when the leading sound is contralateral to the preserved auditory cortex. This result suggests that a unilateral lesion of the auditory cortex would disrupt the perception of the locus of pairs of pulses when the side of the leading pulse is ipsilateral to the preserved cortex, even in the untrained cat.

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Alcohol Dependence Produced in Mice by Inhalation of Ethanol: Grading the Withdrawal Reaction

Abstract. *Intoxicating blood levels of ethanol are maintained for several days in mice housed in an atmosphere of ethanol vapor. On removal from the alcohol, all the mice develop withdrawal signs. The signs can be graded to indicate the time course and intensity of the withdrawal reaction.*

Physical dependence is an aspect of drug addiction that lends itself well to analysis in quantitative terms, if an animal model is available. We describe here techniques for establishing and maintaining constant blood alcohol levels in small animals and for estimating the intensity of the subsequent withdrawal reaction (*1*). Such a model could be used to test the homeostat hypothesis (*2*)—the theory that adaptation to the continuous presence of a drug is the cause of both tolerance and physical dependence. A system under physiological control, such as an enzyme repressible by its product, could expand to offset the initial drug effect. On withdrawal, the inhibition would be removed and the overactivity of the expanded target system would be displayed.

The theory makes two predictions that are testable even without knowing what the target system is. (i) Physical dependence should develop over a period of hours or days (not years), in accordance with the time courses of known physiological regu-

latory mechanisms such as enzyme derepression or hormone effects on protein synthesis. (ii) The amount of expansion of the target system should be related to the drug concentration to which the system has been exposed. Thus we need a model in which the blood level of an addicting drug is held constant, to allow establishment of the new steady-state level of the target system. We also need a means of estimating the intensity of the withdrawal reaction, a measure of the amount of expansion of the target.

Animal models for alcohol dependence have only very recently been developed. Essig and Lam (*3*), Ellis and Pick (*4*), and Freund (*5*) have shown that development of physical dependence in animals is a surprisingly rapid process, as compared with the slow progress of alcoholism in man. The shortest reported times for producing withdrawal reactions are about 2 weeks in dogs and monkeys and 4 days in mice.

It is difficult to maintain high blood alcohol levels in mice by injections, because they eliminate alcohol very rapidly. Therefore, we administer the ethanol by inhalation. The mice are housed (with food and water supply) in a transparent plastic box to which ethanol vapor is continuously delivered in the air inflow. A Harvard infusion pump feeds absolute alcohol onto a filter paper wick in an Erlenmeyer flask. Air from a cylinder flows through the flask and the chamber at a constant rate, monitored by a Gilmont flowmeter. Flow rates of 2 to 3 liters of air per minute are used, which is more than adequate for the respiratory needs of 20 mice. The mice are removed twice a day for about 20 minutes in order to weigh them, to inject pyrazole (once daily), and to take blood samples. Blood alcohol is assayed enzymatically by the method of Lundquist (*6*) with use of duplicate 10-μl samples of tail blood, deproteinized with 0.09 ml of 3.4 percent perchloric acid. The pyrazole does not affect the enzymatic alcohol assay under these conditions. The ethanol vapor concentration in the chamber is determined twice a day. Air samples of 0.5 ml are injected through serum stoppers into the head space of tubes containing 3.0 ml of the reaction mixture (including enzyme) used for the alcohol assay. After 60 to 90 minutes at room temperature, the optical density at 340 nm is measured.

To obtain stable blood alcohol levels, we must use pyrazole, an inhibitor of alcohol dehydrogenase in vitro and of alcohol elimination in vivo (*7*). Although a constant blood level could theoretically be maintained by balancing pulmonary intake and hepatic elimination, this is very difficult in practice. Changes in respiratory rate or liver function quickly throw the system out of balance. If we completely inhibited enzymatic alcohol oxidation with pyrazole, alcohol would be taken in and excreted almost entirely by the lungs, and its level in the blood would be independent of respiratory rate. This would require toxic doses of pyrazole, however. The dose we use, 1 mmole/kg daily, inhibits alcohol elimination by 70 percent when a single dose is given along with an injection of ethanol. This dose suffices to stabilize blood alcohol levels in mice inhaling alcohol.

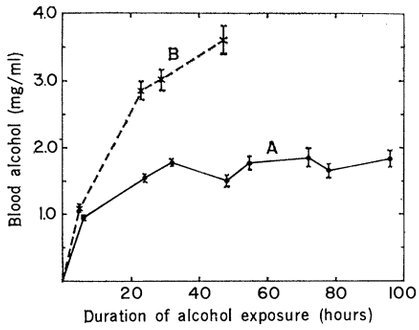

Fig. 1. Blood alcohol concentrations during exposure to ethanol vapor. The points represent blood alcohol (mean concentration and standard error) in two groups of seven mice. (Curve A) Mice exposed to a vapor concentration of 12 mg/liter for 4 days. Pyrazole (1 mmole/kg) was injected at 0, 24, 48, and 72 hours. (Curve B) Mice exposed to alcohol vapor at a concentration of 17 to 18 mg/liter for 2 days. Pyrazole (1 mmole/kg) was given at 0 and 24 hours. Blood samples were taken for alcohol assay each morning, before pyrazole was injected, and again 5 to 8 hours later.

The mice are removed from the alcohol vapor chamber 24 hours after their last dose of pyrazole. The falling blood alcohol is then measured at intervals of 1½ or 2 hours. The grading system for assessing the severity of the withdrawal reaction is shown in Table 1. The mice are observed almost continuously for 14 hours, with scores recorded hourly. Thereafter, observations are made at longer intervals. The most useful sign is the convulsion elicited by holding the mouse up by the tail. The mouse arches his back, tightens his facial muscles into an abnormal grimace, and jerks or twirls violently. He may continue to convulse when replaced in the cage (score 4). This sign is easily graded, and withdrawal scores based on this sign alone correlate well with those computed from all seven signs (correlation coefficient = .94, $N = 55$). Scores for all signs are combined, and the mean score per mouse is recorded at each time point. Thus a plot of the intensity and time course of the withdrawal reaction can be drawn. Two or more observers participate in

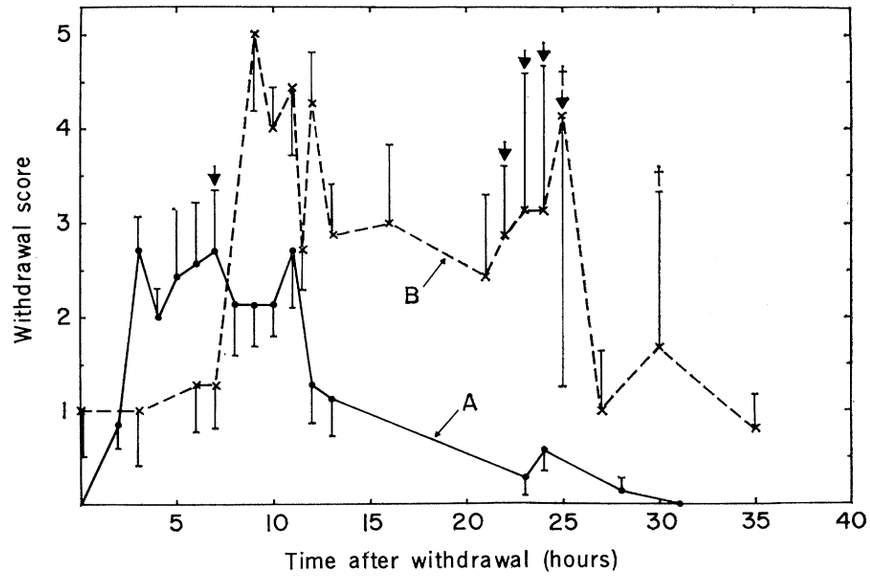

Fig. 2. Graded withdrawal signs after exposure to ethanol vapor. Curves A and B represent the same mice indicated by these curves in Fig. 1. The points are mean scores (see text for scoring system). Standard errors are shown as bars above or below the points. Times of death (†) and of spontaneous convulsions (↓) are indicated.

Table 1. Scoring system for graded withdrawal reaction. "Tail lift" (column 1) was defined as raising the tail over the back, one of the abnormal postures used in Freund's grading of withdrawal signs in mice (*5*). "Startle to noise" was elicited by a sharp tap on the cage.

Sign	Score	Minimum degree	Maximum degree
Lethargy	0– 4	Slow movement, dragging limbs	Coma
Tremor	0– 4	Twitching, occasional trembling	Continuous tremor
Tail lift	0– 4	One per hour	Four per hour
Startle to noise	0– 4	Twitch	Convulsion
Convulsion on handling	0– 4	Mild, tonic	Severe, clonic
Spontaneous convulsion	0–10	Mild, tonic; jumping	Three or more generalized tonic-clonic convulsions per hour
Death	10		Death

scoring each group of mice. Two observers sometimes record mean scores that differ by one point; usually they agree more closely.

The two experiments shown in Figs. 1 and 2 illustrate the uses of this technique. Swiss-Webster male mice (34 to 44 g) were used. With air alcohol concentrations of 12 mg/liter (and daily pyrazole at 1 mmole/kg), the blood alcohol stayed at 1.5 to 1.8 mg/ml for 4 days (Fig. 1, curve A). It took 24 hours to reach this level. Vapor concentrations of 17 to 18 mg/liter, with the same pyrazole dose, resulted in a rise of blood alcohol levels to 3.6 mg/ml after 2 days (Fig. 1, curve B). During the alcohol exposure, the mice were lethargic and ataxic, and they were limp when handled. They did not show convulsions (spontaneous or elicited by handling), abnormal posture, or startle responses, as were seen during the withdrawal reaction.

Figure 2 shows the withdrawal reaction in the same mice. The maximum intensity of the syndrome occurred approximately at the time that alcohol disappeared from the blood. This was at about 4.5 and 9 hours in experiments A and B, respectively, with an alcohol elimination rate of 0.40 mg/ml in both. The syndrome regressed slowly over the next 24 hours. The overall intensity of the reaction is represented by the area under the curve (score × hours). Areas under the curves for individual mice were calculated from the scores and time intervals. The mean areas and standard errors were computed for each experiment: 34.0 ± 4.7 for experiment A; 66.4 ± 13.5 for experiment B. The difference is significant ($P < .05$). The higher blood alcohol level achieved in experiment B thus led to a more severe withdrawal reaction, although the duration of that experiment was shorter and the total alcohol intake was less than in experiment A. (The total alcohol exposure was computed as the area under the blood alcohol curves for individual mice, in [milligrams per milliliter] × hours. The mean area was 148.9 ± 5.7 in experiment A and 118.9 ± 5.4 in experiment B.)

Because pyrazole is toxic (*8*), we are concerned about its contribution to the withdrawal reaction. Mice lose weight during the alcohol-pyrazole treatment. There was a 10 percent loss of body weight in experiment A and a 14 percent loss in experiment B. Either alcohol or pyrazole alone at these doses caused some weight loss. To assess the role of pyrazole, we subjected two groups of mice to comparable amounts of alcohol (blood levels of 1.8 mg/ml for 3 days) with and without pyrazole, using different vapor concentrations. Typical withdrawal signs were seen in both groups. After administration of pyrazole alone for several days, no such signs were seen.

The vast literature on voluntary consumption of alcohol by laboratory animals shows that animals do not consume enough ethanol in the drinking water to produce physical dependence. Indeed, they rarely achieve intoxicating blood alcohol levels at all. Our simple technique allows sufficient alcohol intake for production of physical dependence. It is much more convenient and less stressful than intravenous infusion, and it can be used for large numbers of animals. We have here shown that physical dependence to ethanol in mice can develop in as short a time as 2 days. The time scale is thus similar to that of known physiological regulatory processes, which we postulate to be the mechanism of physical dependence. The techniques described here have been used in a study of the possible relationship between alcohol dependence and opiate dependence in mice (*9*).

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Alcohol Dependence and Opiate Dependence: Lack of Relationship in Mice

Abstract. *According to a recently proposed hypothesis, physical dependence upon alcohol is due to the formation of an endogenous opiate. We tested the hypothesis by determining whether or not ethanol-dependent mice would show typical opiate-dependent behavior (withdrawal jumping syndrome) when challenged with the opiate antagonist naloxone. Our results do not support the hypothesis.*

It has been known since 1963 (*1*) that mammalian liver converts dopamine to tetrahydropapaveroline (THP), a benzylisoquinoline alkaloid that is an intermediate in morphine biosynthesis in the opium poppy (*2*). The further