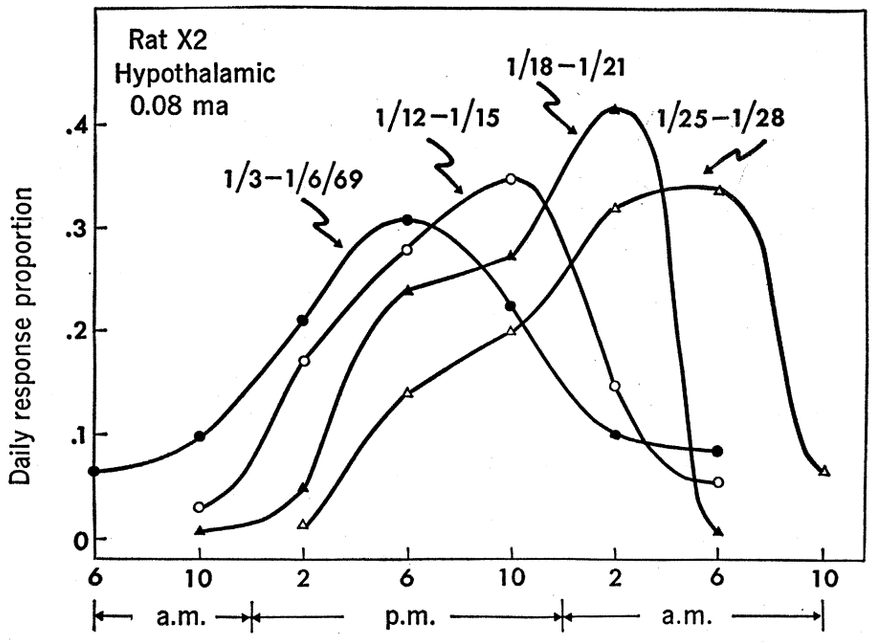

Fig. 3. Averaged daily response proportions (4-hour blocks) for sampled 3-day periods in the course of a month's testing with low-intensity hypothalamic stimulation, for rat X2.

variations in baseline response output). From 3 to 6 January 1969, peak response rate occurred at about 6 p.m.; when the behavior pattern was sampled 1 week later, the peak had shifted to the next 4-hour block, centered at 10 p.m. Subsequent samples revealed a continuing shift to later peak periods, reaching 6 a.m. near the end of January. The transition from peak activity to low activity appeared to become more abrupt as the month progressed. The data show that, on the average, peak response rate occurred about 20 minutes later each day; in 2.5 months, we would predict that the animal would "lose" about one objective day. A computer analysis of these data showed that the least-squares fit to a 24.3-hour trial period yields a ratio of 0.076 between the standard error of the amplitude and the amplitude itself (*15*). Such data support the conclusion that the operant behavior rhythm is not synchronized by an external clock corresponding to the 24-hour day. This result agrees with Aschoff's rule, which predicts that a nocturnal animal in constant light will show a circadian rhythm greater than 24 hours.

The present experiment demonstrates that the technique of intracranial self-stimulation can be profitably used to refine behavioral activity analyses. Indeed, Prescott (*16*) has shown that one index of general activity correlates positively with self-stimulation rate. Perhaps an oscillating temporal factor, related to physiological arousal states (*17*), modulates reinforcement strength. Our self-stimulation records may reflect rhythms in hypothalamic or limbic neural activity. Not only does the hypothalamus mediate strong positive reinforcement effects, but also its control over pituitary secretions (for example, adrenocorticotrophic hormone) is thought to underlie changes in general motor activity (*18*).

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3. Rat B33 was run on the variable interval (V I) 30-second schedule in an earlier experiment in which a 60-hz sine brain-stimulation waveform was used (peak-to-peak current was specified). This animal received a liquid diet of diluted Carnation condensed milk, and food intake was monitored by a photocell circuit at the drinking tube. It is interesting to note that feeding occurred only during the animal's active bar-pressing period; during quiet periods, the animal mainly groomed and slept.
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20 March 1970

Alcohol Aversion in the Rat: Behavioral Assessment of Noxious Drug Effects

Abstract. *Injections of* p-*chlorophenylalanine or* n-*butyraldoxime given after rats were first given a 10-minute drinking test with saccharin or ethanol solutions produced a learned aversion to these solutions. These findings suggest that the reduced self-selection of alcohol (preference) resulting from the administration of these drugs, reported by others, is not specifically alcohol-related. The technique described offers a sensitive procedure for the assessment of unpleasant effects of drugs.*

There is considerable research interest in drugs which have the effect of reducing the intake of ethanol (*1*), although there is little understanding of the mechanism by which such a reduction occurs. Drugs such as disulfiram or *n*-butyraldoxime (*n*BAO), which are aldehyde dehydrogenase inhibitors, are

presumed to cause a decrease in ethanol consumption because of the increased concentrations of acetaldehyde which circulate in the blood after ethanol is consumed (*1*, *2*). On the other hand, *p*-chlorophenylalanine (*p*CPA), which depletes brain serotonin, and α-methyl-*p*-tyrosine, which depletes brain catecholamines, also significantly reduce the selection of ethanol by rats; in the case of *p*CPA, the marked ethanol rejection persists after drug administration is discontinued (*3*).

In studies of the influence of a drug on ethanol intake (*1*), the ethanol intake usually has been measured before, during, and after drug administration. However, the finding of reduced selection or intake of ethanol during or after drug administration may be mistakenly interpreted to be a result of some specific interaction of the drug with ethanol. A major difficulty of this interpretation is that rats readily learn to avoid ingesting any distinctive substance that has been associated with toxic effects (*4*), and, if a rat becomes progressively sicker while ingesting a particular dietary substance, the rat will develop a strong aversion to that substance (*5*). Because the rat learns food aversions associated with such toxic effects, it may be that drugs that have been shown to result in decreased ethanol intake actually produced a nonspecific conditioned aversion to ethanol because ethanol intake was associated with the unpleasant effects of the drug.

The following experiments were performed to test two of the drugs most recently reported to cause a decrease in ethanol intake and to determine whether these drugs would produce a nonspecific learned aversion if they were administered in association with the drinking of ethanol or of any other distinctive solution. Saccharin was chosen as such a distinctive solution, since presumably its intake bears no relation to ethanol intake nor is its disposition affected by drugs that modify the interaction of the organism with ethanol.

The subjects were 60 male 250-g Wistar rats. Each rat was deprived of water for 1 day, and, beginning the next day and continuing for 4 days, each rat was placed in a test cage (18 by 24 by 28 cm) where it was offered as its sole water supply a daily single bottle of tap water for 10 minutes. The rats were randomly assigned to ten groups of six rats each. On day 5 rats in groups 1 to 4 were given a 10-minute drinking test with 6 percent (weight-volume) ethanol, and rats in groups 5 to 8 were given a 10-minute drinking test with 0.25 percent sodium saccharin. Immediately after the tests the rats were returned to their home cages; 5 minutes later they were given intraperitoneal injections (2 ml per 100 g) according to the following scheme: rats in groups 1 and 5 were injected with *p*CPA (1.0 mmole/kg in 0.15*M* NaCl); rats in groups 2 and 6 were injected with *n*BAO (0.5 mmole/kg in 0.15*M* NaCl); rats in groups 3 and 7 were injected with 0.15*M* LiCl; and rats in groups 4 and 8, control groups, were injected with 0.15*M* NaCl. These dosages of *p*CPA and *n*BAO were chosen because they were substantially less than those reported to reduce ethanol intake (*2*, *3*). Some rats were injected with LiCl because this compound is very effective in producing learned aversions (*6*), and we could therefore compare the amount of learned aversions shown by other groups of rats to that of the groups of rats injected with LiCl.

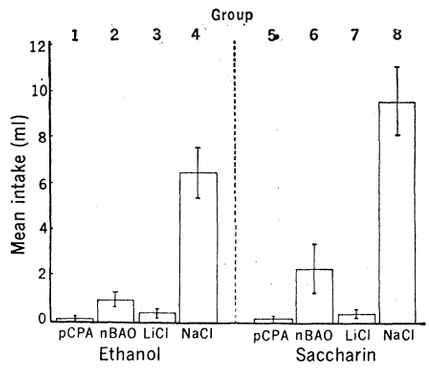

Fig. 1. Mean intake (in milliliters), with standard error of the mean, of 6 percent ethanol and of 0.25 percent saccharin in drinking test of day 8.

Two additional groups of rats, those in groups 9 and 10, were included to determine whether saccharin or ethanol aversion would occur in response to drug effects alone without any prior conditioning. On day 5, rats in groups 9 and 10 were not given a drinking test but did receive intraperitoneal injections (2 ml per 100 g) of *p*CPA (1.0 mmole/kg in 0.15*M* NaCl).

On days 6 and 7 all rats were given a 10-minute test with water; on day 8 rats in groups 1 to 4 and 9 were given a 10-minute test with 6 percent ethanol and rats in groups 5 to 8 and 10 were given a 10-minute test with 0.25 percent saccharin. The tests with water on days 6 and 7 were included to ensure that all rats were drinking normally so that any decreased intake on day 8 would therefore be attributable to a learned aversion to the test solutions.

The mean water intake for all groups on days 4, 6, and 7 was 9.5, 8.9, and 10.0 ml, respectively, and there were no significant differences among groups on any of these days. On day 5, the mean intake of 6 percent ethanol for rats in groups 1 to 4 was 4.2 ml and the mean intake of saccharin solution for rats in groups 5 to 8 was 7.7 ml with no significant differences among the rats receiving ethanol or saccharin. The fact that the intake over 10 minutes of 6 percent ethanol and 0.25 percent saccharin was less than the intake over 10 minutes of water reflects, in part, the rats' initial hesitancy toward these solutions. In tests with other Wistar rats we have found that on first exposure their intake of 6 percent ethanol or of 0.25 percent saccharin was depressed relative to their intake on subsequent tests.

The mean intake of test solutions on the final test day, day 8, is presented in Fig. 1. It is clear that rats in all groups, except those in the control groups, acquired a strong aversion to the test solutions. The rats learned an aversion both to saccharin and to ethanol, and every rat of the experimental groups, without exception, drank much less of the test solution on day 8 than on day 5. In contrast, the rats in the control groups all increased their intake. The learned aversions to the saccharin and to the ethanol displayed by the rats on day 8 were clearly a result of the treatments given on day 5. Rats in groups 9 and 10, which were given *p*CPA but neither ethanol nor saccharin solution on day 5, did not show any aversion to these substances on day 8; the mean intake of ethanol or saccharin solution of rats in groups 9 and 10 was 3.5 and 5.0 ml, respectively, amounts which, although low, are not significantly different from the amounts drunk by rats in the other groups in their first test with these solutions (probabilities $> .05$).

We used the same procedure to assess the nonspecific toxic effects in rats of pyrazole at a dosage which inhibits in vivo ethanol oxidation by about 90 percent (*7*); unlike *n*-butyraldoxime, pyrazole does not inhibit aldehyde dehydrogenase. Intraperitoneal injections (2 ml per 100 g) of pyrazole (3.0 mmole/kg in 0.15*M* NaCl) were given 5 minutes after 12 rats had been tested with sac-

charin solution for 10 minutes; all rats exhibited a complete saccharin aversion when they were tested 3 days later. The mean intake of saccharin solution was 7.6 ml in the first test and 0.2 ml in the second test. No attempt was made to test whether pyrazole would produce an alcohol aversion since the saccharin aversion was so conclusive.

The results clearly reveal that rats avoid ingesting solutions that are associated with the effects produced by *p*CPA, *n*BAO, pyrazole, or lithium chloride. Although the single dose of LiCl might have been sufficient to produce transient sluggishness and diarrhea in the rat, there were no obvious symptoms produced by the injections of the other drugs. The technique of assessing learned taste aversions as used in these experiments would appear to have widespread generality for the assessment of unpleasant or toxic drug effects not otherwise perceived by the investigator or revealed in the course of many routine toxicological evaluations. Since the rat appears to learn taste aversions quickly, the degree of "unpleasantness" of a drug may be determined on the basis of whether the drug produces such a learned aversion. Thus the behavior of the animal may yield a more sensitive bioassay than other toxicological or pharmacological procedures.

In the previous experiments which reported decreased ethanol intake after the administration of *n*BAO (*2*) or *p*CPA (*3*), the drugs were administered daily and ethanol was continuously available to the animals. With such a procedure, it is not possible to specify the conditioning contingencies and it is for this reason that we used a one-trial drinking-injection test. However, it seems likely that continuous access to ethanol with daily drug administration would have allowed adequate opportunity for the establishment of aversive conditioning since aversive conditioning will occur even when the temporal delay is long (*6*, *8*). Animals avoid previously acceptable and familiar solutions if their ingestion is followed by sickness (*6*), and animals learn to avoid a diet which after prolonged intake has made them sick (*5*).

When one assesses any of these drugs, such as *p*CPA, *n*BAO, or pyrazole, for potential alcohol aversion effects, one must determine whether at lower drug dosages the unpleasant effects result only from the interaction of the drug with alcohol or whether they are effects of the drug per se, revealed, for example, by testing with a solution of saccharin. Such a differentiation is necessary before one may conclude that a drug acts specifically to cause a learned aversion to alcohol. Since the substantially lower dosages used in these experiments were clearly sufficient to cause a learned aversion to solutions other than alcohol, we conclude that the effects on the self-selection of alcohol previously reported for these drugs (*2*, *3*) are based, not on their specific effects with relation to alcohol, but rather on their character as noxious agents.

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Frequency-Specific Relation between Hippocampal Theta Rhythm, Behavior, and Amobarbital Action

Abstract. *The frequency of the hippocampal theta rhythm in freely moving rats varies predictably in relation to behavior in a simple learning situation. The theta rhythm may be driven by electrical stimulation of the medial septal area at frequencies within the theta range. The threshold for septal driving is lowest at that frequency which the rat displays in response to frustrative nonreward; the driving threshold is selectively raised at this frequency by sodium amobarbital. It is suggested that the behavioral effects of amobarbital are due to a disruption of the theta frequency normally displayed in response to nonreward.*

Hippocampal lesions (*1*), septal lesions (*2*), and injections of sodium amobarbital (*3*) have similar effects on behavior in learning situations involving reward and the omission of expected reward ("frustrative nonreward," *4*). Behavioral responses to reward are unaffected, whereas behavioral responses to nonreward are seriously impaired. The similarity in the patterns of dysfunction produced by these three treatments prompted us to seek a common mechanism of action. One possibility is that all three treatments affect behavior by disrupting the hippocampal theta rhythm, which is affected by barbiturate drugs (*5*). We therefore investigated the relationship between the hippocampal theta rhythm and the occurrence of reward and frustrative nonreward, and the effects of sodium amobarbital on septal driving (*5*) of the hippocampal theta rhythm. Our results suggest that (i) reward and frustrative nonreward produce different frequencies of theta rhythm, and (ii) the driving thresholds for these frequencies are differentially affected by amobarbital.

A bipolar recording electrode aimed at the hippocampus and a bipolar stimulating electrode aimed at the septum were stereotoxically implanted in male Sprague-Dawley rats (~ 400 g). Coordinates, with skull flat from lambda to bregma, were: hippocampal, 6 mm posterior to bregma, 2 mm lateral, 5 mm deep (from the surface of the skull); septal, 1 mm anterior; midline, 5.5 mm deep. Electrode placements were examined histologically at the end of the experiments. The recording electrodes were all located in the posterior dorso-medial hippocampus, and the stimulating electrodes were in the medial septum, on, or very close to, the midline.

The electrodes consisted of two Teflon-covered stainless-steel wires (200 μm diameter) twisted together and exposed only at the tips. The tips were adjacent to each other in the septal placement and separated by 2 mm vertically in the hippocampal place-