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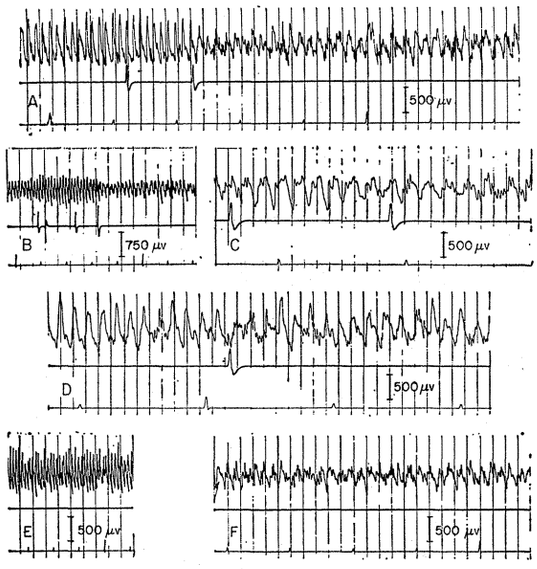
## Frequency-Specific Relation between Hippocampal Theta Rhythm, Behavior, and Amobarbital Action

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-



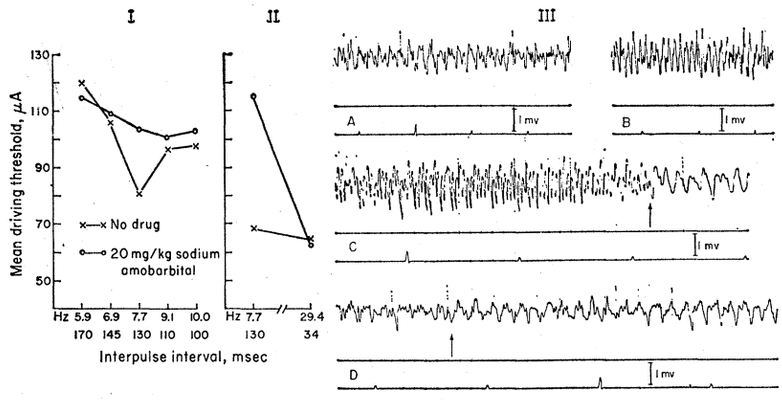




Fig. 1 (left). Hippocampal activity in free-moving rats under various conditions. Top channel: hippocampus. Middle channel: breaking of photobeam. Bottom channel: time in seconds. (A) Rat No. 15: Eighth rewarded trial. Water delivered at second mark on middle channel. Note 10-hz theta rhythm changing to lower-amplitude 6-hz theta overlaid with faster waves when goal is reached. (B) Rat No. 287: Complete rewarded trial on slower paper speed. Note 8-hz theta in startbox (up to first mark on middle channel) followed by burst of high-amplitude 9-hz theta as rat traverses runway and reaches goal (final mark), when this pattern is replaced by low-amplitude 6-hz theta. (C) Rat No. 443: Third trial of extinction, paper speed 50 mm/sec. Note anticipatory slowing of theta rhythm and reduction in amplitude between penultimate photobeam (first mark) and reaching goal (second mark), and preservation of low-frequency theta in goalbox in spite of nonreward. (D) Rat No. 15: Eighth trial of extinction. Compare with A. Note reduction in (mark on middle channel) theta frequency and amplitude before goal is attained and increase in theta frequency and amplitude after it is attained. There is a transient appearance of reward-type theta for about 0.3 second after the goal is reached, followed by theta at about 8.5 hz. (E) Rat No. 15: Steady theta at about 8 hz during exploratory behavior in runway, before injection of amobarbital. (F) Rat No. 15: Still in runway, 15 minutes after sodium amobarbital (20 mg/kg) is injected intraperitoneally. Note reduction in theta frequency (to about 6 hz) and amplitude, as well as increase in fast waves. Fig. 2 (right). (I and II) Effect of amobarbital on threshold for septal driving of hippocampal theta rhythm as a function of driving frequency. (III) Examples of driven hippocampal response. Bottom channel: time in seconds. (A) Rat No. 2. Driving current: 5.9 hz, 175 μa. (B) Rat No. 2. Driving current: 10.0 hz, 150 μa. (C) Rat No. 15. Driving current: 29.4 hz, 150 μa. Arrow marks termination of stimulation. (D) Rat No. 497. Driving current: 7.7 hz, 60 μa. Arrow marks start of stimulation.

ment. The electrodes were led out to a plug assembly and secured to the skull by means of screws and dental cement. The animal was grounded by means of a stainless-steel wire wrapped round the screws in the skull. Movement artifacts were eliminated by means of a source-follower, consisting basically of two field-effects transistors (Type 2N5405, Texas Instruments), cemented in the cable connecting the animal to a Grass 79 polygraph (filters set at 3 and 100 hz) and a Tektronix 502A dual-beam CRO.

Hippocampal records were taken as thirsty rats learned to run down a straight alley for a water reward. The 6-inch-wide alley consisted of an 8-inch-long startbox, a 4-foot-long runway, and an 11-inch goalbox; side-opening lucite doors separated these three sections from one another. Marks were automatically made on a second channel of the polygraph when the experimenter opened the start-door and when the rat broke photobeams located 7 inches from the startbox, 7 inches before the goalbox, and right at the end of the goalbox. Breaking this last photobeam automatically delivered water to a small cup fixed in the far goalbox wall. Some subjects were run in this apparatus with reward on every trial and were subsequently extinguished; that is, they were given no further rewards. Others were exposed to a schedule in which rewarded and nonrewarded trials were randomly mixed. The interval between trials was approximately 1 minute. Subjects were detained in the startbox for 5 seconds before the experimenter opened the startbox door and for 5 seconds in the goalbox after breaking the final photobeam. Drinking usually lasted 2 to 3 seconds.

Observations of the hippocampal theta rhythm recorded under these conditions, and also when the subject was first allowed to explore the novel alley, revealed the following consistencies, both within individual rats and between rats. During exposure to a novel environment the observed theta rhythm was usually in the 7.5 to 8.5 hz range. This frequency was also observed when the well-trained animal was waiting in the startbox for a trial to begin. Immediately on the opening of the startbox door, the well-trained animal showed a burst of higher-amplitude theta waves (8 to 10 hz), this burst usually being of maximum amplitude as the rat started to run down the runway. When the rat was consuming the water reward the electroencephalogram (EEG) had a mixed pattern containing lower-amplitude theta waves (6 to 7.5 hz) overlaid with some higher frequencies; in agreement with the records presented by Routtenberg (*6*), theta waves were always clearly present. On many occasions, this mixed pattern replaced the high-frequency theta burst just before the animal broke the final photobeam and therefore before any water could be consumed. The effect of nonreward was to replace this mixed pattern with the same 7 to 8 hz theta waves observed during exposure to a novel environment. However, on the first few trials of extinction, the mixed pattern persisted as the animal tried to drink from the now-empty water cup. With hippocampal placements 2 mm lateral from the midline (though not with more lateral placements), these regularities were clear enough to be visible on the EEG, and no more complex analysis was necessary (Fig. 1). They are in striking agreement with the frequency shifts seen by Elazar and Adey (*7*), who used computer techniques to analyze the hippocampal EEG in cats, even though the frequency range cov-

ered by the theta rhythm in the cat is lower (3 to 7 hz).

To get a quantitative evaluation of the effects of reward and nonreward on theta frequency, we used an automatic wave-count device (pass-band, 3 to 50 hz) constructed in the laboratory. This counted the number of times per second the hippocampal record rose above the zero-volt base line set by the resting position of the EEG pen. It was set into operation when the rat crossed the final photobeam in the runway and printed out the wave-count for each of the 5 seconds the animal remained in the goalbox. The mean goalbox wave-count for seven rats during an initial series of rewarded trials was 7.03 (S.D. = 0.64); on a subsequent series of nonrewarded trials it was 7.70 (S.D. = 0.77) the difference being significant at $P = .01$ ($t$ for correlated means = 5.45, d.f. = 6). Similar differences between rewarded and nonrewarded trials appeared in animals trained on a sequence of mixed reward and nonreward.

That these small but highly consistent frequency shifts have some functional significance is strongly suggested by the results of a second experiment in which we examined the effects of sodium amobarbital on the threshold for septal driving of the hippocampal theta rhythm. Stimulation (pulse duration, 0.5 msec) was applied to the septal electrode by a constant-current square-wave generator constructed in the laboratory. In agreement with observations on rabbits reviewed by Stumpf (*5*), low-frequency stimulation drove the hippocampal theta rhythm, as judged by synchrony between the stimulus trace and the hippocampal EEG displayed on the cathode ray oscilloscope. Stimulation at 1-hz steps within the range 6 to 10 hz caused a driven wave to appear in the hippocampus at the corresponding frequency (Fig. 2). An excellent driven response could also be obtained at 30 hz (Fig. 2). Using the runway already described, and a dose of 20 mg/kg injected intraperitoneally, we determined the effect of sodium amobarbital on the threshold for septal driving of the hippocampus at approximately 2-minute intervals for 10 to 40 minutes after injection, the period over which the behavioral effects of this drug have usually been noted (*3*). Control experiments with saline injections showed that these had no effect on driving thresholds.

The effects of amobarbital on driving thresholds in two groups of five rats tested, in one case, at approximately 1-hz steps over the range 5.9 to 10.0 hz and, in the other, at 7.7 and 29.4 hz are shown in Fig. 2 as a function of driving frequency. Within the theta range, the threshold for a driven response at 7.7 hz is considerably lower than at any other frequency investigated ($P < .001$). Amobarbital completely eliminates the advantage of this frequency for driving within the theta range. This selective effect of amobarbital on 7.7 hz is further emphasized by the fact that the drug fails to alter the threshold for driving 29.4 hz, a frequency that can be driven at about the same threshold as 7.7 hz when no drug is given. Over the two groups of rats taken together, amobarbital raised the 7.7-hz driving threshold by about 70 percent ($P < .01$). These results of the driving experiment are consistent with the changes produced in hippocampal electrical activity by amobarbital: there is a shift from the dominant 7.5 to 8.5 hz theta of the normal record to a lower-frequency theta of about 6 to 7 hz together with an increase in fast-wave activity [(*5*) and Fig. 1)].

Thus, the effects of amobarbital on septal driving of the theta rhythm are disproportionately great precisely for that frequency (7.5 to 8.5 hz) which is spontaneously displayed in the hippocampal record when the rat is exposed to novelty or frustrative nonreward, while frequencies spontaneously displayed when the rat is running toward an expected reward or consuming a reward are unaffected. These results suggest that a hippocampal theta rhythm at a frequency of about 7.7 hz plays an important role in the control of behavioral responses to frustrative nonreward, and that injections of amobarbital (*3*), medial septal lesions (*2*), and hippocampal lesions (*1*) all affect this kind of behavior by disrupting the hippocampal theta rhythm.

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## Antarctic Pelecypod Faunal Peculiarities

The living antarctic pelecypod fauna does not fit all of the postulates on cold-water marine bivalve faunas as proposed by Stehli *et al.* (*1*). Some of their assertions concerning the general characteristics of cold-water marine pelecypod faunas are these: (i) Cold-water pelecypod faunas virtually lack endemic families and consist of families that are cosmopolitan in distribution. Unfortunately, the authors do not state what they consider an endemic family to be. (ii) Cosmopolitan families, which comprise nearly all of the cold-water pelecypod faunas, are relatively old and include few families that evolved within the past 50 million years. In other words Stehli *et al.* claim that, at least as a general rule, endemic families are young families and cosmopolitan families are old families. This is simply the Willis theory of age and area, which was proposed in 1922 and which was ably reviewed by Lotka (*2*). Applied to living pelecypod families, this is generally true, but there are some exceptions such as trigoniids and astartids. Also, a few old families are today confined to warm water, such as the pteriids and the pinnids.

Among shallow-water marine pelecypod faunas, that of the antarctic has several peculiar aspects. There is an uncommonly high percentage of small-sized species. Sixty-one percent of the antarctic pelecypod species are no more than 10.0 mm in height or length; the fauna with the next highest percentage of small-sized species is the South Australian with 38 percent (*3*). The antarctic has an uncommonly low percentage of infaunal species—59 percent as compared to the arctic, which is about average with 73 percent (*4*).