References and Notes

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4. More comprehensive data on rates of increase and population densities noted in this study will be published elsewhere. These data indicate that more than 90 percent sterility was required before the natural rate of increase of this mosquito population was exceeded.
5. Rearing and sterilization of mosquitoes in this experiment required about 6 man-hours per day. The *C. p. quinquefasciatus* is easy to rear and we foresee no difficulty in producing very large numbers of mosquitoes.
6. We thank Dr. B. Smittle for technical assistance in the radioactive tagging study, Mr. M. Boston for supervising the rearing of the mosquitoes, and Dr. G. B. White [*Nature* **210**, 1372 (1966)] for suggesting the technique of sterilizing pupae with thiotepa.

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Intracranial Self-Stimulation and Wakefulness: Effect of Manipulating Ambient Brain Catecholamines

Abstract. *Rats were given disulfiram, an inhibitor of norepinephrine biosynthesis, to see if norepinephrine is a transmitter for motivation in electrical stimulation of the brain. Animals given the drug paused in bar pressing, appearing asleep or sedated; if replaced on the bar, they always resumed pressing at normal rates. The decrease in bar pressing may result from a direct or indirect effect of the drug on wakefulness rather than on reward.*

One can study the neurohumoral basis of integrative pathways in the central nervous system by observing drug-induced changes in behavior established and maintained by electrical stimulation of the brain (ESB) as the motivating stimulus. Wise and Stein (*1*) reported that the rate of bar pressing for rewarding brain stimulation was markedly reduced in rats treated with disulfiram, a potent inhibitor of norepinephrine (NE) synthesis. Assuming that magnitude of reward is correlated directly with rate, they inferred that NE was the neurotransmitter for reward pathways in intracranial self-stimulation (ICS) because diminution of NE led to diminution of rate. Using the work of Wise and Stein (*1*) and the theoretical position developed by Deutsch (*2*) as foundations, this laboratory tried to establish which of the two pathways in ICS (drive or reward) used catecholamines as neurotransmitters. Initially, I tried to confirm Wise and Stein's (*1*) conclusions. Tygon-insulated stainless steel monopolar electrodes were stereotaxically placed and permanently implanted in the medial forebrain bundle (MFB) [coordinates $A = 5.8$, $V = 2.25$, $L = 1.75$ (*3*)] of four adult male Simonsen Sprague-Dawley rats (350–450 g). The animals were then trained to stimulate themselves by pressing a bar in a modified Skinner apparatus in 1-hour daily practice sessions until stable performance was observed. Suitable current intensities were determined for each animal and chosen as the minimum which would sustain bar pressing. The stimulating apparatus was the same used by Deutsch (*4*), with the duration of pulse trains lasting either 0.15 or 0.8 second. The duration was varied only to permit more sensitive measurement of rate variation, but results were essentially the same for both durations. Disulfiram (100 mg/kg) (*5*), suspended in 1 ml of physiological saline with 0.03 percent Tween-80, was administered intraperitoneally. Because of its viscosity the suspension was administered either by syringe or with the aid of silicone tubes permanently implanted in the peritoneal cavity (*6*). Results were recorded by means of an event recorder and a digital counter.

Base-line rates of bar pressing for all animals were obtained from one or more sessions consisting of a maximum of 1½ hours of bar pressing per session. Rates were calculated every 5 minutes during each session. All base-line data obtained were used. Animals to be given disulfiram were run once for five continuous hours and injected at the end of the first hour; all data after the onset of observable symptoms were used, but no data were used if the animal did not evince the pharmacological symptoms of NE inhibition.

The onset of drug effect was defined for purposes of data collection as the first pause in the animal's responding which exceeded the longest pause recorded during base line. During base-line recording, if a rat paused he voluntarily resumed bar pressing. After administration of the drug rats pausing longer than the maximum base-line pause never voluntarily resumed bar pressing; therefore we returned them to the bar according to an arbitrary criterion. Rats were permitted a pause of at least base-line maximum plus 5 seconds. For example, an animal which paused no longer than 60 seconds under control conditions would be allowed a pause of 65 seconds after treatment with the drug. This procedure differs from that of Wise and Stein (*1*), who sometimes handled the animal but did not return it to the bar. The procedure was introduced because it was suspected that the stops in bar pressing were due to the sedation reported by Wise and Stein (*1*), since the drugged animals were always found asleep during pauses. If the animals had gone to sleep because of diminishing returns from ICS, then replacing them on the bar should not have caused them to resume bar pressing. If, on the other hand, the animal had gone to sleep because of sedation and drowsiness, and stopped pressing the bar as a secondary result, then replacing it on the bar and so awakening it should have produced normal rates of bar pressing. Furthermore, even if the sedation and drowsiness were a result of drug toxicity or pain from peritoneal irritation, rather than a result of an effect on central NE pathways, it would still seem that the drug has no effect on reward that can be measured in Wise and Stein's (*1*) experimental design. The animals, however, seemed to show no discomfort at the time of the injection, and the symptoms they did evince were similar to those described for side effects of drugs antagonistic to NE (*7*).

Rates were thus measured to see whether Wise and Stein's (*1*) results with disulfiram could be obtained under our conditions. The numbers of criterion pauses for each condition were also compared. We expected that if reward were being diminished by NE inhibition, the response rate would be consistently depressed by the drug; if an animal paused in responding, it should not resume pressing the bar, either voluntarily or after being replaced on the bar. This particular observation is not reported by Wise and Stein (*1*); however, since measurement of rate is confounded by the rats' sedation and sleepiness in our study, as in Wise and Stein's (*1*), forcing the animal to resume bar pressing by replacement is a necessary control in order for one to determine completely the value of ESB to a drowsy animal. Resumption of bar pressing at a high rate after replacement on the bar would indicate that the animal does not find the stimulation less motivating than be-

Table 1. Mean rates of bar pressing for drug, drug minus pauses longer than 1 minute, and control are shown. The control rates and rates for drug with pauses deleted are almost identical, an indication that the manner of bar pressing in drugged and undrugged states is similar. This suggests that the rate decrease is due to pauses which are themselves due to sleep rather than to disinterest in ICS, since if the animals are awakened, they resume pressing.

Rat (No.)	Pulse-train duration (sec)	Block size (min)	Blocks (No.)		Σ time (min)		Means		Disulfiram without pauses (C)	A-B	A-C
			Control	Drug	Control	Drug	Control (A)	Disulfiram (B)			
427a	~.8	5	17	34	85	170	170	142	181	28	−11
	~.8	5	17	42	85	210	170	116	152	54	18
	~.15	5	7	21	35	105	359	263	347	96	12
414a	~.8	5	8	26	40	130	173	48	123	125	50
419a	~.15	5	3	3	15	15	761	430		331	
Pooled	~.8				Means		171	102	152	69	19
Pooled	~.15						359	263	347	213.5	12

fore treatment and that he stopped because he was sedated and sleepy.

The rates of bar pressing of treated rats are slower than control rates, unless the pauses greater than 60 seconds are deleted. Then the rates appear nearly identical, an indication that the animal's desire for ESB is not diminished by the drug. Mean rates of bar pressing for each animal, either treated or untreated, were obtained as follows (Table 1). For each animal, all of the base-line data were collated. These data consist of a series of rates calculated every 5 minutes during every session of the base-line condition. All of the 5-minute measurements for each individual animal were added (Σ time); the result was averaged to obtain the means. A similar procedure was used after the animal had been given the drug, except that only one session was used for derivation of each mean. Rat 427a had three sessions while on the drug, each tallied separately. He was subjected to two durations of pulse trains (of ESB) as a control for possible differences in drug effect dependent on ambient rate, which depends on the voltage of ESB. From his data, and from that on rats 414a and 419a, it does not appear that the duration makes any difference in the result. Thus, all the data from each rat were used to derive the means; column C of Table 1 was calculated by deleting pauses greater than the maximum ever observed during base line from data obtained during the drug condition, to illustrate that the decrease in rate of bar pressing was intermittent rather than constant. An intermittent decrease would be expected if the animal were sleeping sometimes; a constant, overall decrease would be expected if the animal experienced a decrease in reward value of ESB. Figure 1 shows the distribution of pauses for drug and control conditions, representing the probability of pausing for a particular length of time given the opportunity to pause that long. Logarithmic conversion permits comparison with the logarithmic graph of the Poisson distribution. Deviations from randomness for the drug and the control conditions are seen to be in opposite directions. There are more pauses of greater duration in the drug condition than in the control. Since treated animals were found asleep during pauses, whereas control animals never were, this figure lends support to the notion that the drug had no primary effect on ICS, but that the animals stopped pressing only because of sedation and sleep.

My data indicate that although Wise and Stein's (*1*) finding that treatment with disulfiram decreases the rate of bar pressing by rats and increases drowsiness and sedation is replicable, the inference that reward has been affected by the drug may well be confounded by the sedation and drowsiness of the animal. The decrease in rate of bar pressing caused by disulfiram appears to result from an increase in frequency and duration of pauses in responding; the animals were always found asleep during pauses; replacement on the bar was always sufficient to induce resumption of self-stimulation. Since the animals were responding at threshold current intensity, a decrease in reward should have caused them to stop bar pressing, whether forced to remain alert or not. This was not the case. If kept awake by handling and replacement on the bar, the animals pressed the bar at a rate which did not differ consistently from the control rate. Changes in behavior were used as indicators of sedation and sleepiness. The electroencephalograph (EEG) was not used because, although the EEG has been used extensively and with some reliability to study sleep and wakefulness in the undrugged state, many drugs cause a complete dissociation of the EEG and ongoing behavior (*8*, *9*). Since the present dispute concerns what the animal was doing, it was deemed better to observe it than to obtain a measure which might not be correlated with its actions because of the presence of the drug. Augmentation rather than inhibition of NE function by administration of chlorphentermine hydrochloride (*10*), a sympathomimetic amine with central effects, but without those activity-increasing side effects produced by its close relative, amphetamine (*11*), led to no consistent change in rate of bar pressing. If increasing functional NE did increase reward, then the rate of bar pressing should have increased.

It appears that NE is not the neurotransmitter for reward in ICS, for

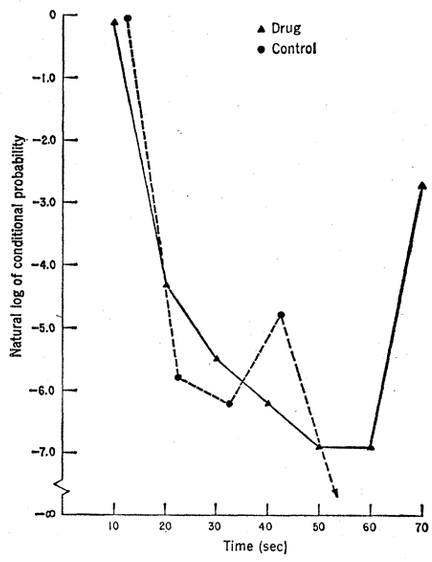

Fig. 1. The conditional probability of making a pause of a particular duration with and without drug, for one animal. The probabilities are corrected for (made conditional on) total amount of time available for pausing. Although the number of brief pauses remains approximately the same for both cases, the number of pauses exceeding the 1 minute limit is greater in the drug condition. Since the animals were found asleep during pauses, and since there are no such pauses in the control condition, this figure supports the view that NE inhibition led to slumber.

neither decreases nor increases in its concentration in the brain consistently affect the rate of bar pressing at threshold, given that the animal is kept awake; results of studies which claim to show this effect (*1*, *3*) are best accounted for by variations in wakefulness and activity level, either as a result of direct drug action or as a secondary result of toxicity.

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Cataracts Produced in Rats by Yogurt

Abstract. *Rats kept on an exclusive diet of yogurt avidly ate the yogurt, grew at a normal rate, were normally active, mated, conceived, and gave birth to normal, healthy litters. However, all of the rats developed cataracts. Cataracts appeared in young rats 2 to 3 months, and in adult rats 4 to 6 months, after initiation of the yogurt diet. Cataracts first manifested themselves in small vacuoles at the periphery of the lens and then in small striae extending toward the center of the lens. These striae progressively became longer, more coarse, and numerous until they coalesced, finally forming a mature white lens. The high content of galactose in commercially available yogurt could account in full for appearance of cataracts in 100 percent of the experimental animals. The cataracts appear to be the same as those produced by diets with a high content of galactose.*

The observation that an exclusive diet of commercially prepared yogurt produces cataracts in rats was made (*1*) during experiments to elucidate factors responsible for the production in man of benign paroxysmal peritonitis (*2*).

The rats were housed in individual cages (*3*) with a revolving drum, a cyclometer, a nonspillable food cup, and a graduated inverted 100-ml water bottle. Fresh yogurt was offered each day in food cups used for our stock diet. Guards placed over these cups prevented spillage. Several kinds of commercially available yogurt were used, all prepared in essentially the same way.

Records of the number of revolutions of the drum, and of food and water intake and of vaginal smears, were taken daily. At weekly intervals, the rats were weighed and inspected for condition of hair, skin, and teeth.

In our initial experiments, four rats were given access to stock diet, yogurt, and water in separate containers to determine whether rats would freely eat yogurt when also given access to a stock diet. All four rats ingested large amounts of yogurt and greatly reduced their intake of the stock diet. They drank very little water. They all grew at a normal rate, were normally active, and remained in apparently excellent health.

Then, four rats were kept on an exclusive diet of yogurt and water. They ate large amounts of yogurt, grew normally, and drank only small amounts of water. Estrous cycles in all four were regular. When mated, three rats conceived and gave birth to normal, healthy litters which were nursed and cared for until time of weaning. Two of these rats later gave birth to normal litters. These observations thus indicated that yogurt alone appeared to be a complete diet.

However, after these four rats had been observed for several months, it was noted by chance that all had developed bilateral mature cataracts. The changes in the lenses had escaped notice until then, because we had not been especially looking for them. Only when the cataracts were mature were they discovered. To our knowledge, no rat of the 40-year old colony had ever developed a cataract spontaneously.

We next designed studies to determine (i) rapidity of development of lens changes after initiation of the yogurt diet; (ii) frequency of occurrence of lens changes; (iii) age predilection, if any, in the rat for the development of these changes; (iv) the clinical evolution of these changes; and (v) clinical features of these cataracts compared with those produced by other experimental methods. We also hoped to determine what factor in the diet of yogurt produced the lens changes.

Twenty-six rats, 34 to 221 days old at onset of the diet, were used. Our observations made on six of these young animals (three males and three females) will be described in some detail as they are typical of the results obtained with

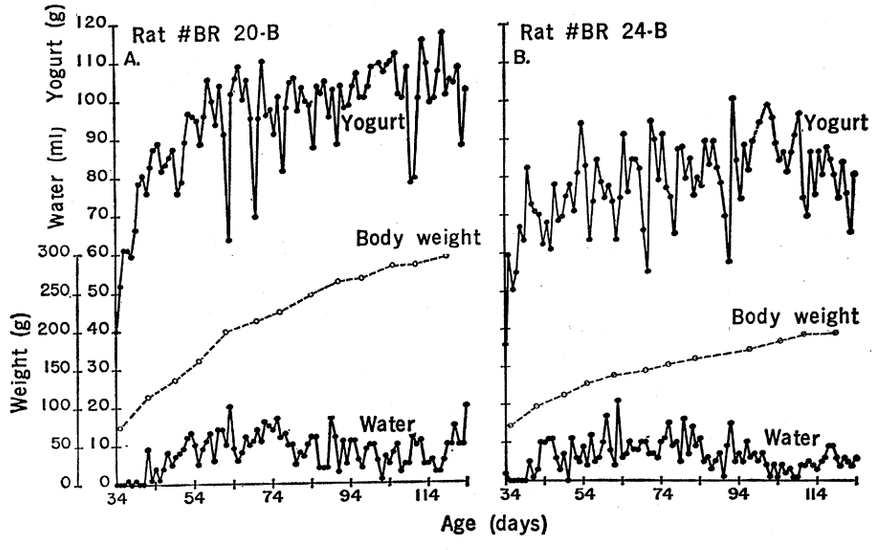

Fig. 1. Typical records of daily intake of yogurt and water over 90 days for a male (A) and a female (B).