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Self-Stimulation of the Brain Used as a Screening Method for Tranquilizing Drugs

Behavioral effects of reserpine and chlorpromazine in animals and man have led to the search for an adequate screening method that would relate both to behavior in animals and site of action in the brain. Such a screening method is described here on the basis of the finding (*1*) that electric stimulation applied to specific hypothalamic and paleocortical structures of the rat brain has an effect on behavior tantamount to primary reward.

In these experiments (*2*), a bipolar electrode was chronically implanted in the brain of each animal. The pair stimulates only at the tip, and thus it affects only a small area of the brain. For testing, the animal was placed in a lever box (Skinner box), and an electric circuit was set up so that each bar-press produced a train of electric stimulation 0.6 sec in duration through the implanted electrode. The stimulus used was a 60-cy/sec sine wave of from 1 to 1.5 v applied through a resistance of about 10,000 ohms. In tests, the animal was never stimulated by the experimenter but was allowed to stimulate itself by pressing the lever.

The reinforcing value of an electrode placement is assessed in terms of the frequency of the lever-pressing response. When electrodes are placed in the anterior or middle hypothalamus, extremely high response rates can be achieved, often rising above 5000 responses per hour. When electrodes are placed in the region of the septal area or the amygdaloid complex, rates range from 200 to 2000 per hour. Rates of 200 responses per hour are also achieved from all structures of the rhinencephalic cortex. Other parts of the brain do not produce this positive reinforcing effect.

The size and anatomical differentiation of this "rewarding" system suggested that its parts might be differentially sensitive to neuropharmacological agents. Experiments were therefore designed to determine whether different agents would affect self-stimulation rates for some electrode loci more than for others.

In the series reported here, electrodes were implanted in the middle hypothalamus, the septal region of the forebrain, or the amygdaloid area. Each animal had an electrode pair in only one of these regions. Animals were allowed 4 days to recover from the operation and then were given 6 to 14 days of pretraining at self-stimulation in the test boxes. In training and tests, the animals were run for 80 minutes per day. The electric stimulus was the only reinforcing agent used in these experiments. Under these conditions, all animals showed day-to-day improvement during pretraining and achieved stable response prior to drug tests. The stable response rate of animals that were stimulated in the septal region of the forebrain or the amygdaloid area was about 500 per hour, and the stable response rate of animals that were stimulated in the middle hypothalamus was about 2500 per hour.

After stable rates had been achieved, drugs were introduced on the basis of a modified Latin square with crossing over of drugs between animals and control runs on intervening days to measure carry-over effects.

The preliminary series reported here consisted of a limited range of doses of reserpine, chlorpromazine, and pentobarbital. The effectiveness of a given dose was gauged by evaluating the response rate following drug administration as a percentage of the average response rate for all days when no drug was administered.

In three animals with electrodes placed ventromedially in the hypothalamus, reserpine at 1 mg/kg depressed response rates to 7 to 45 percent of normal. In two rats with electrodes implanted in the amygdala, the same dose of reserpine reduced the response rates to 1 and 22 percent of normal. In contrast, the response rates of four rats in which the electrodes were placed in the septal region of the forebrain were depressed to a mean of 75 percent of normal (range, 67 to 83 percent.) Thus, there was marked depression in rats that were stimulated in the hypothalamus or in the amygdala but only a minor depression produced in the rats that were stimulated in the septal region. Typical data are presented in Fig. 1.

Chlorpromazine (2.5 mg/kg) depressed response rates in rats that were

SELF STIMULATION IN FOREBRAIN AND HYPOTHALAMUS AS AFFECTED BY RESERPINE Ⓡ, CHLORPROMAZINE Ⓒ, AND PENTOBARBITAL SODIUM Ⓐ

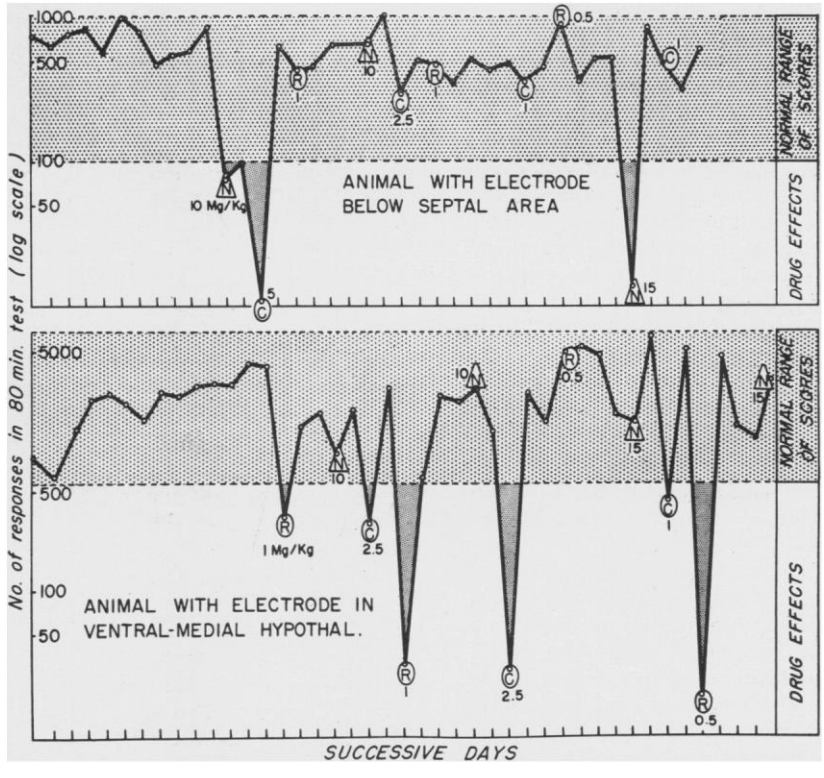

Fig. 1. The number of responses in 80-minute test periods plotted for each day of the experiment for two representative rats. The top graph presents data for a typical rat with an electrode implanted in the region below the septal area. Reserpine at 1 mg/kg and 0.5 mg/kg and chlorpromazine at 2.5 mg/kg and 1 mg/kg produce no major change in response rate; pentobarbital at 10 mg/kg slightly depresses responding on first, but not on second, administration. The lower graph presents data for a typical rat with an electrode placed in the posterior ventromedial hypothalamus. Doses of reserpine (1 mg/kg) and chlorpromazine (2.5 mg/kg) produce sharp falls in response rates; pentobarbital has little effect. Both rats were responding for a 1 v, 60 cy/sec sine wave stimulus.

stimulated in the hypothalamus to 0 to 11 percent of normal (Fig. 1). The response rates of two rats stimulated in the amygdala were depressed to 1 and 17 percent of normal. With the same dose, the response rates of six rats stimulated in the septum were depressed to a mean of 38 percent of normal (range, 0 to 77 percent). Three of the animals had scores of 50 percent or higher following administration of the drug. Thus, chlorpromazine appears to have selective effects similar to those of reserpine, but the effects are more variable.

Smaller doses of reserpine and chlorpromazine depressed the response rates of rats stimulated in the hypothalamus but rarely altered response rates in rats stimulated in the septal region.

Pentobarbital at doses of 10 mg/kg did not have similar selective depressant effects, although one aberrant animal showed a depressant effect. At doses of 15 mg/kg, marked motor depression made it difficult to assess the data; animals stimulated in the hypothalamus, however, have been seen to give high response rates even with this extreme dose.

Increasing sensitivity to reserpine on successive administrations at 1 or 0.5 mg/kg was also found in these experiments. This is illustrated in Fig. 1 by the greater depression caused by the second 1-mg and the second 0.5-mg dose for the rat stimulated in the hypothalamus.

From these preliminary studies it appears that, in the rat, the rate of self-stimulation through electrodes implanted deep in the brain may be used as a behavioral screening method to distinguish tranquilizing agents from other central nervous system depressants, and possibly also from each other. Reserpine and chlorpromazine, at doses without observable side effects, have been shown to depress selectively at certain brain sites, thus distinguishing them from pentobarbital, which has no selective effects. At doses of the tranquilizing agents large enough to produce gross changes in spontaneous motor activity, selectivity between animals stimulated in the septal region and the hypothalamus is no longer observed.

These observations are being extended by the use of other electrode placements and a wider dose range of these and other agents. Such techniques should lend insight into selective sites of action of tranquilizing agents. Studies now in progress relating primary drives to the various parts of the "rewarding" system may provide a basis for interpreting the differential drug effects.

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2. These studies were aided by contracts NR 110-402 [Nonr-233(33)] and NR 144-102 [Nonr-233(32)] between the Office of Naval Research, Department of the Navy, and the University of California, and by Eli Lilly and Company ("Neuropharmacology of anesthetics and excitant drugs").

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Heat Denaturation of Serum Albumin in Presence of Perfluorooctanoic Acid

Based on studies of the interaction between bovine serum albumin (BSA) and perfluorooctanoic acid (PF) ($C_7F_{15}COOH$) (*1*) and the initial observation that PF may prevent the heat coagulation of BSA (*2*) similar to the studies of Ballou *et al.* (*3*), an investigation was undertaken of the physical-chemical and immunochemical properties of BSA-PF complexes heated under controlled conditions (*4*).

Solutions of 0.33-percent BSA in the presence of varying amounts of PF in acetate buffers of 0.1-ionic strength at *p*H 5.44 or 5.72 were autoclaved at 105°C and 15-lb pressure in thin-walled, sealed, 10-ml ampoules for 20, 30, 40, 50, 60, and 120 minutes, respectively. The solutions were stored in a refrigerator for 1 week, after which it was observed that only those systems composed of a minimum of 266 moles of PF per mole of BSA remained clear. For the immunochemical reactions with calibrated rabbit anti-BSA sera, the various solutions were adjusted to *p*H 7 to 7.5 without appreciable change in protein concentration.

Sedimentation patterns were obtained at two rotor speeds—namely, 42,040 and 56,100 rev/min. Typical sedimentation constants, corrected for adiabatic expansion of the rotor, are assembeld in Table 1. From the variation of the sedimentation constants as a function of the gravitational field and of heating time, it may be concluded that heated BSA and PF form complexes of micellar nature. Because many types of micelles could exist —each with its own critical micelle concentration—one would expect that the area under each sedimenting peak would decrease with increasing sedimentation time and with change of concentration across the boundary. These area losses are different from those observed by Brand (*5*), inasmuch as they cannot be accounted for by the sedimentation of large aggregates at low speeds, and inasmuch as they become more pronounced as heating time is increased.

The reversibility of PF binding in heated samples was investigated by exhaustively dialyzing these solutions against 20 volumes of buffer and following their behavior at periodic intervals, both in the ultracentrifuge and by immunochemical analysis. The ultracentrifugation study (Table 1) indicated that micelles still remained, for area losses were observed in all dialyzed heated solutions. The *p*H of the dialyzates was raised to 7.4 in order to utilize electrostatic repulsion to dissociate complexes, but even this procedure did not reduce area losses, nor did we observe a component the sedimentation of which was equal to that of native BSA at the same concentration. When the *p*H of the dialyzates was low-

Table 1. Sedimentation constants of BSA-PF complexes. Area losses were observed in autoclaved samples. The numbers in parentheses represent average relative proportions (percentage) of total schlieren areas.

Experimental conditions	Sedimentation constants, $S_{20,w}$							
*p*H 5.44, mole ratio PF to BSA = 293/1								
Unheated, 56,100 rev/min	14.50	(84.3)	17.81	(15.7)				
Autoclaved 20 min, 56,100 rev/min	15.58	(37.5)	27.79	(50.0)	82.5	(12.5)		
Autoclaved 20 min, 42,040 rev/min	16.46	(36.6)	23.78	(63.4)				
*p*H 5.72, mole ratio = 468/1, 56,100 rev/min								
Unheated	10.47	(32.5)	13.95	(67.5)				
Autoclaved 30 min	7.35	(9.0)	10.98	(50.4)	14.74	(32.3)	20.82	(8.3)
Autoclaved 60 min	10.69	(57.6)	14.64	(42.4)				
*p*H 5.72, mole ratio = 366/1, 56,100 rev/min								
Autoclaved 120 min	11.13	(39.2)	15.14	(45.9)	18.95	(14.9)		
Autoclaved 120 min, dialyzed 3 times, *p*H 5.72	5.76	(44.4)	14.60	(55.6)				
Autoclaved 120 min, dialyzed 5 times, *p*H 7.41	4.55	(67.2)	10.72	(32.8)				
BSA control	4.05	(100)						