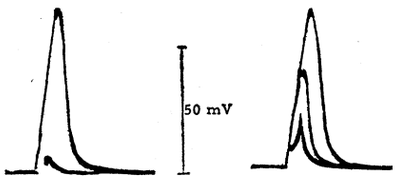

Fig. 1. Effect of clam poison on the mononodal action potential of a single nerve fiber preparation of the frog sciatic nerve (*Rana pipiens*). At $3 \times 10^{-10}M$ concentration (10^{-4} μg toxin per milliliter of solution), this toxin blocks conduction in 30 seconds. After return to Ringer's solution, conduction was restored within 1 minute (*p*H, 7.7; temperature, 23 °C).

known but not their structure. Knowledge of the mode of action of these toxins is rather limited, but it is known that some of them block conduction and neuromuscular transmission reversibly (*1*). On the suggestion of B. Jandorf we have tested, on the electrical activity of conducting membranes, the effects of two of the toxins (*2*): the clam poison, prepared according to the method of E. J. Schantz *et al.* (*3*), and the puffer-fish poison.

The action of both toxins was tested on Ranvier nodes of a single frog sciatic nerve fiber prepared according to the method of Staempfli (*4*), and on the single isolated electroplax of *Electrophorus electricus*, preparaed with the method developed by Schoffeniels (*5*). In the innervated membrane of the latter cell there are many synaptic junctions, although most of the surface area is conducting membrane; the two types of membranes can be readily distinguished by electrical characteristics.

On exposure of Ranvier nodes to either of the toxins in concentrations of about $3 \times 10^{-10}M$ (10^{-4} μg/ml), electrical activity was rapidly and reversibly blocked within 30 seconds (See Fig. 1). With higher or lower concentrations the period of time required changed correspondingly. When the electroplax was exposed to either of the two toxins, a marked difference in the effect was observed. Clam poison, in a concentration of 5×10^{-4} μg/ml, blocked the response to neural stimulation in 10 to 20 minutes. With higher toxin concentration (0.1 μg/ml) the inhibition occurred in seconds. A concentration of 0.2 μg/ml was required to block the response to direct stimulation. Puffer-fish poison, in a concentration of 0.025 μg/ml, blocked the response to both direct and indirect stimulation simultaneously. The presence of curare (50 μg/ml) did not change the concentrations required for blocking the response to direct stimulation. Inhibition by puffer-fish poison at concentrations of 0.1 to 0.25 μg/ml, recorded with intracellular electrodes, occurred without depolarization.

The extraordinary toxicity of the compounds, which is several orders of magnitude higher than that of the most toxic nerve gases, raises the interesting and challenging problem as to the underlying mechanism. The primary role of the acetylcholine system in the generation of bioelectric potentials prompts the question of whether that system may be affected by these toxins. A reaction of these toxins with any one of the members of the system is thus conceivable. However, the affinity of the toxins to acetylcholinesterase is so low that a reaction with this enzyme may be excluded as a casual factor (*6*). A few tentative tests by S. Ehrenpreis with the receptor were inconclusive; considerable modifications of techniques are required for analysis. Action on the storage protein leading to a release of the ester appears unlikely, since there seems to be no depolarization, and since the blocking effect persists as long as the toxins are present. Obviously, the toxins may react with entirely different constituents of the membrane. At present, no satisfactory explanation can be given of the underlying chemical reaction. But the data reported promise to provide biology with a new potent tool for the analysis of events associated with nerve activity (*7*).

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25 April 1960

Eating or Drinking Elicited by Direct Adrenergic or Cholinergic Stimulation of Hypothalamus

Abstract. A double cannula system, allowing repeated stimulation of central structures with crystalline chemicals, was developed. This technique was employed to study the effects of adrenergic and cholinergic stimulation of the lateral hypothalamus of rats. Drug-specific effects on the feeding and drinking mechanisms, respectively, were observed.

The exploration of the central nervous system by means of electrical stimulation has provided a wealth of information of great interest to physiologists and psychologists alike. The usefulness of this technique is limited, however, because the effects of stimulation are not restricted to synaptic junctions but affect fibers of passage, causing conduction in both normal and antidromic directions.

It has long been recognized that chemical stimulation avoids these problems, but the technique has in the past been plagued by the problem of uncontrolled spread, which raises a serious objection to the injection of chemicals in solution. Attempts to control for this factor by minimizing the injected quantities have apparently not been completely successful in preventing the escape of the fluid along the shank of the needle, following the path of least resistance.

Depositing chemicals in solid form has been shown to reduce this problem greatly (*1*), but this method has not allowed repeated stimulation of a selected locus. In the present study, a technique was developed which avoids this objection.

A double cannula system, consisting of two modified syringe needles, was permanently implanted unilaterally, by means of a stereotaxic instrument, into the lateral hypothalamus of each of 12 albino rats. Histological verification of the intended placements showed the tip of the cannula to be located in a circumscribed perifornical region at the same rostrocaudal coordinate as the ventromedial nucleus (see Fig. 1), an area corresponding to the ventral portion of Anand and Brobeck's "feeding area" of the lateral hypothalamus (*2*).

After 5 days of postoperative recuperation, the inner cannula was removed and minute amounts (1 to 5 μg) of crystalline chemicals were tapped into its tip before it was returned to its usual position. Successive treatments were administered to all animals in a counterbalanced order, with a minimum of 3 days between injections. Both food and water were freely available throughout the experiment. The food and water consumption of satiated rats was re-

corded for 1 hour immediately following stimulation and compared with the consumption in a comparable period immediately preceding the injection. Daily food and water consumption records were maintained.

None of the animals ever consumed food or water in measurable quantities during the prestimulation period. The injection of epinephrine or norepinephrine resulted in highly significant ($p < .01$) food consumption beginning 5 to 10 minutes after stimulation and persisting with variable intensity for 20 to 40 minutes. Food consumption averaged 3.0 gm under epinephrine and 4.3 gm under norepinephrine.

The injection of acetylcholine (capped by physostigmine) or carbachol into the identical loci in the same animals resulted in highly significant drinking ($p < .01$), the latency, duration, and magnitude of the effect being comparable to those obtained for eating after the injection of adrenergic substances. Water consumption averaged 7.4 ml after the injection of acetylcholine and 12.8 ml after the injection of carbachol, this difference being highly significant ($p < .01$). There was no significant food consumption after cholinergic stimulation (see Fig. 2).

The injection of adrenergic substances resulted in significantly less water intake than cholinergic stimulation ($p < .01$). Since in all but one animal the drinking occurred only after a considerable amount of dry food had been consumed, water consumption seemed to be secondary to the food intake rather than a direct consequence of stimulation. To establish further the specificity of the adrenergic effect, norepinephrine was deposited in the lateral hypothalamus of six food- and water-satiated animals, which were then placed in observation cages containing only water. For 30 minutes after the injection none of the animals consumed measurable quantities of water, though

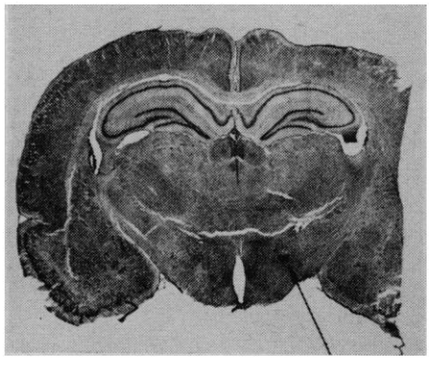

Fig. 1. End of needle tract in the right perifornical region of the rat brain. Stimulation at this point, as well as at loci slightly more medial and ventral, produced the effects described in the text.

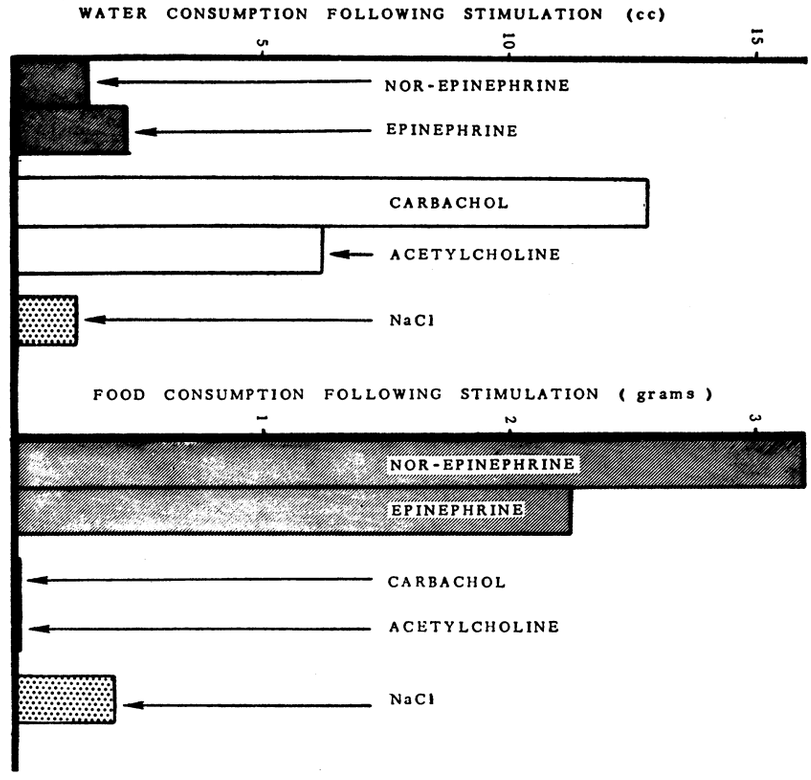

Fig. 2. Food and water intake during 1 hour following stimulation. (The intake during a comparable control period was zero in all cases and is not shown.)

four of them repeatedly sampled the drinking tube very briefly. Food was then introduced, and all animals ate almost immediately, though total food consumption was lower than that normally observed, since the food was introduced only toward the end of the period previously established as the duration of the adrenergic effect.

In order to control for the effect of osmotic stimulation, comparable amounts of NaCl were deposited in all the animals. No significant food or water intake was observed. In order to control for general excitation effects, strychnine in comparable quantities was deposited in six animals which also showed the above-described effects of adrenergic and cholinergic stimulation. No consumatory behavior was observed following this stimulation.

The daily consumption records indicate that the amount of food or water consumed during the 1-hour period after stimulation, totaling as much as 40 percent of the animal's normal daily intake, appeared to be consumed above and beyond the normal daily intake. Because of the variability of these records, no statistical evaluation of this effect can be presented, but the conclusion is supported, at least for eating, by the consistent weight gain observed on the day following adrenergic stimulation.

A control for the specificity of the localization of the observed effects was obtained in a preliminary study designed to yield optimal stereotaxic coordinates for the study reported here. It was found that very small deviations from the optimal position, shown in Fig. 1, sufficed to eliminate the effects completely.

The results of this investigation indicate that (i) cell concentrations active in the regulation of both food and water intake are present in the lateral hypothalamus; (ii) cell concentrations exerting this control appear to be highly localized but not clearly separate from each other, since stimulation of "identical" loci in the same animal can evoke both forms of behavior; and (iii) the feeding mechanism appears to be selectively activated by adrenergic stimulation, while the drinking mechanisms appear to respond selectively to cholinergic stimulation (*3*).

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