Specificity of carbachol in the elicitation of drinking*

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Two studies were reported in which rats with hypothalamic cannulae were injected with carbachol. All animals exhibited drinking following injection. In the first study, this was demonstrated both prior to and following a series of test sessions, and in the second study, at the end only. During the test sessions themselves, only food was available. Animals displayed no tendency to increase their food intake upon carbachol administration during the 16 test sessions. This was contrasted with the ease with which such shifts have been demonstrated with electrical stimulation and suggests a specificity of function that can be detected with chemical but not electrical stimulation.

It is a well-documented fact that electrical or chemical stimulation of hypothalamic sites can produce consummatory behavior. The most common explanation of this phenomenon is that the stimulation energizes motivational mechanisms that are normally involved in the mediation of these responses. Areas from which feeding can be evoked by stimulation, accordingly, are conceived of as active under conditions of normal deprivation, and this activity represents the neural concommitant of hunger. Valenstein, Cox, & Kakolewski (1969) have described a series of recent studies from which they have concluded, on the contrary, that these responses are not reflections of normal motivational states but instead constitute the discharge of sensitized neural substrates "...underlying well-established response patterns [p. 29]." One source for this conclusion is the fact that the various evokable responses seem to be, in a great degree, modifiable. Thus, given the proper conditions, Ss who show one response upon stimulation can almost invariably be induced to perform at least one or more other responses that compete favorably with the initial behavior in subsequent stimulation tests.

This response plasticity during electrical stimulation stands in apparent contrast to the specificity of behavior elicited by chemical stimulation (Grossman, 1962a, b). The question to which the present study is addressed is whether or not the response elicited by chemical stimulation can be subjected to the same manipulations as Valenstein has demonstrated to be effective with electrical stimulation.

SERIES A

Subjects and Apparatus

The Ss were nine male albino rats approximately 160 days old at the time of operation. They were housed individually throughout the experiment in a 48 x 24 x 24 in. high plywood box partitioned into 12 7½ x 12 in. compartments.

When appropriate, food (powdered Purina Chow) was available in aluminum cups attached to the floor of each compartment. Richter tubes were mounted on the side of each compartment to supply water to each rat. To assess the effects of injection on gnawing, each animal was provided with a pinewood block.

Chemical stimulation was accomplished with a Sage infusion pump on which was mounted an 0.25-cc syringe. A length of polyethylene tubing (PE 10) attached to the syringe terminated in a 30-ga stainless steel cannula. For stimulation this injection cannula was inserted through a permanently implanted 23-ga guide cannula. Both the injection and guide cannulae were imbedded in threaded cannula holders (Plastic Products). The pump gears and associated tubing were adjusted for an injection rate of approximately 3 microliters/min.

The entire apparatus, including animal quarters, was located in a 9 x 8 ft sound-attenuated room that was continuously illuminated.

Surgery

While under ether anesthesia, each S was implanted with a guide cannula. The surgical procedures, with slight modification for cannulae, have been described previously (Goldstein, 1967). The cannulae were aimed at the lateral hypothalamus at the anteroposterior level of the ventromedial hypothalamic nucleus.

Procedure

Following implantation a minimum of 2 weeks recuperation was allowed before the first stimulation test. During this recovery period the Ss were housed in the individual compartments with food, water, and gnawing block freely available, and were handled for 10 min every day. In order to establish baseline behavior after recovery, the rats were subjected to a 9-day series of saline and handling control tests. (All tests during and after this series were conducted on alternate days; the designation "days" will hereafter imply *test* days rather than consecutive days.) On the first five of these control days, each animal was taken out of its cage and injected with 1 microliter of physiological saline. On the following 4 days, each animal was handled for an equivalent time period but was not injected. Food and water levels were recorded prior to injection or handling. After the appropriate manipulations, the S was returned to its compartment and water intake was recorded after delays of 15, 30, and 60 min; food intake was recorded at 30 and 60 min only. This procedure was continued throughout the following drug tests.

Upon completion of the control phase, the Ss underwent a series of 17 injection days on each of which 1 microliter of 0.8 micrograms/microliter of carbachol (carbamylcholine chloride) was injected. The goal objects available to the rats followed a sequence similar to that employed by Valenstein, Cox, & Kakolewski (1968a) for electrical stimulation. That is, on the first day, food, water, and gnawing block were all available. Since drinking was the only response observed during the first stimulation test, the water was removed following the next 15 injections and replaced 15-30 min after each test hour. Food intake was recorded as usual, and gnawing, if any, was noted. On the 17th test day water was once more available along with the food and wood block.

Results

For data analysis the total food and water intakes in the test hours were employed, although the carbachol effect usually terminated within 30-40 min. No gnawing was observed throughout the experiment.

The first point necessary to ascertain was whether the individual Ss displayed consummatory behavior upon stimulation. This was established by comparing the food and water intake following injection to the baseline intakes in the control tests. (Since there was no differential effect on intake of handling alone compared to handling plus saline injection, the data for these 9 control days were pooled.) A mean and standard deviation were calculated for each rat's food intake and another for each rat's water intake on the control days. The food and water intakes of each S on the first carbachol day were then expressed as deviation scores from its own control means. In these terms, every rat demonstrated a significant increase in water intake ($p < .01$ in every case),

whereas none showed an increase in food consumption ($p > .05$ for every S). For the group as a whole, the water intake increased from 3.9 ml under control conditions to 13.6 ml in the hour following carbachol injection.

Following carbachol administration on the subsequent food-only days, every animal showed a somewhat *reduced* food intake. On the final carbachol day (food and water both available), the food and water intakes were essentially the same as on the initial carbachol day. Thus, carbachol increased water intake but did not induce feeding despite repeated exposure to food in the absence of water.

SERIES B

As Valenstein, Cox, & Kakolewski (1970) have indicated, the amount of experience an animal has had with a goal object can play a role in the preference subsequently displayed for that object. In the present context it is conceivable that the exposure to water on the first carbachol test might have biased the probability that feeding would emerge in the succeeding tests. This was controlled for in a second series. Ss were four male albino rats approximately 160 days old at the time of operation. The apparatus, surgery, and procedure were identical to those in Series A with the single exception that the first carbachol day was omitted (when both water and food were presented in the first series). Thus, the last day of this series was the first on which water as well as food was available after carbachol injection.

Some time after the second series was completed, all Ss were sacrificed, then perfused with isotonic saline and formalin. Cannula localization was accomplished, following slicing, by microscopic examination of the unstained sections (100 microns).

Results

The data of Series B were treated in the same manner as that of the first series and with the same results. The water intake of each S in the final carbachol test was significantly greater than on its precarbachol control days ($p < .01$ in every case). The food intake of individual rats on the last 5 food-only days again revealed a universal reduction compared to baseline consumption. Thus, every animal in both series showed a reduction in food intake upon carbachol injection. In fact, this inhibition was statistically significant in five of the nine Ss ($p < .05$ in each case).

The histological results verified the assumption that the inner cannula in all cases extended into the target area. In the DeGroot (1959) system this corresponded to 4.8 to 5.2A, 1.8 to 2.2 laterally, and approximately −2.3 in the horizontal plane.

DISCUSSION

The results support the hypothesis of a functional chemical specificity in the rat hypothalamus for drinking behavior. For both groups of animals, carbachol evoked drinking upon the first test exposure to water. Nevertheless, repeated injections of carbachol in the absence of water did not elicit eating or gnawing responses, whether or not the first drinking test preceded or followed the eating/gnawing tests. The contrasting ease of transition from stimulus-bound drinking to stimulus-bound eating with electrical stimulation suggests that we are dealing here with a different order of events. In fact, the present evidence suggesting that food intake is *depressed* by carbachol is reminiscent of the reduction in eating commonly observed during normal thirst (e.g., Kutscher, 1969). Also suggestive of normal thirst was the fact that two animals given carbachol subsequent to the second series drank as readily from a dish of water as they had previously from the tube. Again by way of contrast, stimulus-bound eating (Valenstein, Cox, & Kakolewski, 1968b) and drinking (Valenstein, Kakolewski, & Cox, 1968a) induced by electrical stimulation do not generalize along a dimension indicative of an underlying need.

With respect to the suggestion (Valenstein et al, 1970) that chemical stimulation might exert its effect by potentiating responses that already have a high probability (such as drinking in the rat) rather than evoking these responses, the selective nature of the chemical would still remain as a problem to resolve. Recent data by Smith, King, & Hoebel (1970), furthermore, would seem to argue against this alternative. In addition to its pertinence in this regard, the latter's evidence also serves as a partial response to the challenge issued by Valenstein et al (1970) to extend the concept of specific pharmacological coding "... to include a similar coding of the neural circuits underlying gnawing, copulation, grooming, stalking-attack ..." (p. 24). It should be mentioned that Smith et al also observed eating by their carbachol-injected animals, a fact that would appear to necessitate some qualification of a strict specificity notion. However, the long latency to first kill, the absence of drinking in their study, and the differences in injection parameters (dose size and form) suggest caution in directly analogizing their results to those reported here.

Given that the interpretation of chemically evoked responses is not without its controversies (Routtenberg, 1967; Fisher & Levitt, 1967), one should hasten to add that electrical excitation is by no means a physiological stimulus. Moreover, if we assume the existence of interlaced but nevertheless specific circuits, the problem of predicting the response to simultaneous activation of these circuits becomes paramount. Thus, where under normal circumstances thirst and hunger are reciprocally related, electrical stimulation evokes both states (and possibly others as well) simultaneously. Under these circumstances it would be surprising if the evoked consummatory responses mimicked the normally elicited behavior in all respects.

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