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Neurohumoral Interaction in the Rat Amygdala after Central Chemical Stimulation

Abstract. *The interactions of chemically induced thirst and hunger with deprivation-induced hunger and thirst, respectively, were studied in the amygdala. The results suggest direct neurohumoral blocking at this locus, rather than mediation through activated circuits.*

The rat's amygdala shows a functional characteristic that makes it a particularly appropriate locus for investigation of the interaction of hunger and thirst by central chemical stimulation. Neither adrenergic nor cholinergic stimulation of the amygdala has significant effects on intake of food or water, respectively, in satiated animals (*1*). In suitably deprived animals, however, adrenergic stimulation greatly augments food intake and reduces water intake; and cholinergic stimulation greatly augments water intake and reduces food intake. Thus the amygdaloid "thirst" and "hunger" circuits in the rat display a modulating, but not initiating, function. They are apparently not functional unless the animal has been appropriately deprived for a sufficiently long time.

For this reason we chose the amygdala as the locus of stimulation in this investigative study of the interactions of adrenergically induced "hunger" with deprivation-induced thirst, and of cholinergically induced "thirst" with deprivation-induced hunger. Grossman (*2*, *3*) originally hypothesised a direct neurohumoral blocking effect in at least partial explanation of the depression of consummatory behavior (drinking, for example) by some other, chemically enhanced drive (adrenergic "hunger," for example). However, on the basis of his findings in the septal area (*4*), he later proposed the alternative hypothesis that the observed depression of consummatory behavior was mediated by inhibitory circuits activated, if indirectly, by the chemical stimulation. As a test of this hypothesis we predicted a greater depression of consummatory behavior after amygdaloid chemical stimulation in deprived rats (that is, where the amygdaloid circuits have been sensitized by deprivation) than in satiated rats (that is, where the amygdaloid circuits are apparently not responsive to chemical stimulation).

Of the two experiments that tested the hypothesis, one failed to establish the predicted increase in adrenergic depression of drinking in food-deprived rats over that in food-satiated rats. Thus we felt that our results gave more, if limited, support to Grossman's original hypothesis of a direct neurohumoral blocking effect at the locus of stimulation. The second experiment failed to replicate the cholinergic depression of eating behavior and thus did not contribute to decision between the two hypotheses.

Eleven male albino rats (Sydney University Wistar strain; 70 to 120 days old at the time of operation) were cannulated during the experiments. Eight were used at any one time; some replacements were necessitated by sickness or death.

Throughout each experiment the subjects were kept singly in test cages designed to permit accurate measurement of intake of food and water. Availability of water was automatically controlled by an electric motor; food supply was manually controlled. Cannulas were made from modified gauge-19 needle tubing, cut to the appropriate length, and sealed at the end with silicone rubber that permitted repeated injections but acted as a barrier to external infection. Injection needles were modified gauge-26 hypodermic needles attached by polyethylene tubing to an Agla micrometer syringe. The cannulas were implanted bilaterally by means of a stereotaxic device; the intended locus was the amygdaloid cortical nucleus. A subject was allowed to recuperate for at least 7 days after the operation before it was placed in the experimental squad.

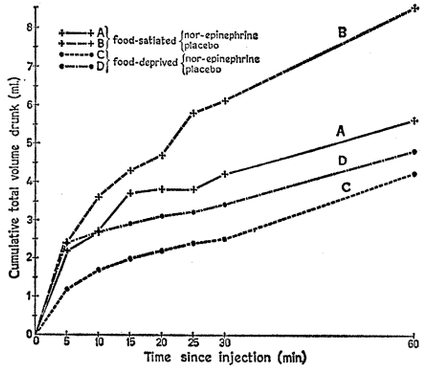

Fig. 1. Amounts of water drunk after injection, versus time; four different conditions of treatment (*A*–*D*).

For study of the interaction of chemically induced hunger and deprivation-induced thirst, all subjects were deprived of water for 3 hours, and half were food-satiated and half were food-deprived for 3 hours. They were injected with either norepinephrine ($648 \times 10^{-4}M$, made isotonic to 0.9 percent saline by addition of NaCl) or placebo (0.9 percent saline) (*5*). Orders of treatment were counterbalanced over subjects, allowing each subject to be used as its own control. Subjects had 1-hour access to water but no access to food. This procedure avoided possible confounding of a behavioral interaction with the predicted effect, a procedural error that may have occurred in Grossman's two later studies (*1*, *4*) in both of which the subject had only a limited time in which to satisfy both hunger and thirst, while one of these conditions was enhanced by chemical stimulation.

The results appear in Table 1. In addition to recording the total volume drunk during the 60-minute test period, we made cumulative measurements of water intake at 5-minute intervals for 30 minutes after injection; these time curves for water intake are graphed in Fig. 1. Analysis of these results, with *t*-tests (*6*), showed significant differences in mean water intake (i) between food-deprived and food-satiated subjects —to be expected; and (ii) between adrenergically stimulated and placebo-injected subjects [t (7) = 3.37, $P < .02$], so that Grossman's finding is confirmed (*1*). However, no significant difference was found in adrenergic depression of water intake between food-satiated and food-deprived subjects.

The second experiment employed the converse procedure, involving cholinergic stimulation with all subjects de-

Table 1. Mean intake of water by variously treated rats (see Fig. 1). The drug used was norepinephrine and the placebo was saline. Conditions of treatment (*A–D*) are defined in Fig. 1.

Treatment		Intake (ml)
Food	Injection	
As desired	Drug (*A*)	6.4
As desired	Placebo (*B*)	8.3
None	Drug (*C*)	4.1
None	Placebo (*D*)	5.0

prived of food—half of them deprived of water and the other half satiated with water for any one trial. We could not replicate Grossman's finding of cholinergic depression of intake of food (*1*). The most plausible explanation of this failure is in the fact that we used solutions of carbachol as our stimuli, whereas Grossman employed crystals; thus he delivered a considerably larger quantity of chemical to the locus of stimulation. Grossman has demonstrated in the hypothalamus (*3*) that, when chemicals are used as blocking agents (as by us), relatively high dosages are needed, and even then the blocking effect may not necessarily be complete.

Functional checks of the accuracy of placements were made during the experiments. Appropriately deprived subjects were tested, by cholinergic or adrenergic stimulation, for the now well-established increases in drinking or eating, respectively (*1*). Of seven subjects checked with both carbachol and norepinephrine, four showed positive responses to both chemicals, two responded to norepinephrine only, and one responded to carbachol only. Differences in response possibly reflected minor variations in placement. The other four subjects all gave positive responses to carbachol. Ultimately all subjects were killed and the brains were removed, sectioned, and stained. The locus of stimulation was then determined with the de Groot atlas. Each subject had at least one cannula tip placed in the immediate vicinity of the amygdaloid cortical nucleus.

Thus Grossman's suggestion that the hunger-thirst interaction in the amygdala is mediated by activated circuits is not supported by the results. His alternative hypothesis of direct neurohumoral blocking seems more likely. The nature of such a mechanism is not yet known, but presumably it would be analogous, and perhaps similar, to the lateral inhibition demonstrated peripherally in receptors, as has been suggested (*2*).

An alternative explanation of our results may be that the adrenergic amygdaloid circuit can modulate ongoing, deprivation-induced, consummatory behavior, but not initiate such behavior in the satiated animal; but the circuit is still functional in the satiated animal and thus can stimulate inhibitory circuits. This explanation would preserve Grossman's interacting-circuit hypothesis, but evidence of such a generality of function for the amygdaloid circuits is not yet forthcoming.

On the other hand, the mechanism we posulate, whereby the synaptic release of a specific neurohumor from activated neurons would serve both to stimulate the next segment of that specific circuit and to inhibit adjacent, possibly antagonistic, circuits, would display the functional type of role postulated by Coury (*7*) for the overlapping of neurobehavioral systems; it could be an important contributor to changes in the "central set" of the organism (*8*) and to the integration of ongoing drive states (*9*). Of course, interaction by other activated circuits in the central nervous system (in the septal area, for example) also would not thus be precluded from contributing to the hunger-thirst interaction, although the amygdala is apparently not involved in such a mechanism; current thought in this field tends to support the notion of multiple mechanisms underlying the thirst and hunger drives (*9*).

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Junctional Physiology and Motor Nerve Distribution in the Fast Adductor Muscle of the Scallop

Abstract. *Electrical recordings from single muscle cells in the fast portion of the scallop adductor have revealed a multiterminal distribution of motor nerves. All motor junctions appear to be of the fast designation, and several nerve fibers supply each muscle cell. The muscle fibers, by virtue of common innervation, are grouped into functional motor units. The pattern of innervation in scallops thus shares functional similarities with the motor distribution to skeletal muscle fibers of both vertebrates and arthropods.*

The electrophysiology of molluscan muscle is not well known. Although there have been some extracellular recordings from cephalopods (*1*) and bivalves (*2*), intracellular recordings from single muscle fibers have, so far, been confined to only one, rather unusual, muscle type—the anterior byssal retractor of the mussel *Mytilus* (*3*). A growing number of neurophysiological investigations deal with molluscan material in an effort to elucidate the manner in which the central nervous system programs behavior in these diverse forms. It is thus important to establish comprehensive descriptions of molluscan neuromuscular mechanisms. I have studied the neuromuscular physiology of the fast portion of the adductor muscle in the scallops *Aequipecten gibbus* (from Panacea, Florida) and *Aequipecten irradians* (from Woods Hole, Massachusetts). Electrical responses from the two species appeared to be similar.

The fast portion of the scallop adductor receives up to four major nerve branches from tracts on each side of the parietovisceral ganglion. These radiate over the surface of the muscle and branch profusely. However, the individual adductor nerves are small and usually hard to identify in living preparations; therefore, muscle responses were