Neural Correlates of Food and Water Intake in the Rat

Abstract. *Adrenergic and cholinergic stimulation of several interrelated limbic and diencephalic areas causes the animal to eat and drink, respectively. The brain areas involved may be organized in terms of "Papez circuit" and, more generally, "Nauta circuit" neuroanatomy. However, it is proposed that separate synaptic nets, one for the control of hunger and one for the control of thirst, are deployed throughout the brain at all levels, including neocortical.*

Eating and drinking behavior is elicited by adrenergic or cholinergic stimulation of specific sites in the rat brain (*1*). Drinking is induced by cholinergic stimulation of a variety of neural areas. These areas may be organized in terms of circuitous neural systems proposed by Papez and Nauta (*2*). In contrast, adrenergic stimulation has elicited eating from a much more limited number of brain areas. Electrical stimulation and lesion studies, however, have indicated that eating and drinking must be mediated by similar neural systems (*3*). Experimental study with adrenergic and cholinergic stimulation has indicated that this is indeed the case. The findings demonstrate that both eating and drinking is elicited by adrenergic and cholinergic stimulation, respectively, of several interrelated limbic and diencephalic areas.

The experimental procedures were as follows. A hypodermic guide shaft was stereotaxically implanted into the brain of each of 55 adult albino and hooded male rats. The implants were directed at selected limbic and diencephalic sites. After surgery, subjects were placed in cages for recovery and adaptation, including handling by the experimenter; the same cages were used for the testing. The cages were designed to permit accurate measurement of food and water intake. Food and water were always available. Measurements of 24-hour eating and drinking values were begun on the 10th postoperative day. The length of the recovery period was determined by the stabilization of 24-hour eating and drinking values over a 4-day period and a healthy, active appearance of the animal.

On test days, the subjects were first given a preliminary test. Fresh food and water were presented approximately 2 hours before the preliminary test. One hour before the preliminary test the animals were handled and the implant tops were cleaned with a saline swab. Since handling and maintenance procedures can influence food and water intake, this method helped insure satiety conditions and the maintenance of a stable level of baseline responding during the preliminary test.

The preliminary test lasted 1 hour. The animals were picked up, and the implant needle wires manipulated. The subjects were then placed back in their cages. Food and water consumption was recorded at the end of the preliminary test hour.

The test hour immediately followed the preliminary test. A 30-gauge cannula was set on an implant connector and lowered to the target area. The cannula was either empty or contained noradrenaline or carbachol in solid form. The technique permits the introduction of 1 to 3 μg of crystalline substance per injection. Food and water consumption was measured at the end of the hour, and the behavior of the animals was observed at approximately 15-minute intervals from the time of stimulation.

Each animal, at a positive locus, received a series of eight test trials: four with noradrenaline, two with carbachol and two with the cannula empty. A minimum of 3 days intervened between successive test days. If the first few test trials indicated that the initial cannula setting was a negative locus for eating upon stimulation with noradrenaline, the cannula was reset 0.25 mm lower, and the stimulation series began over again at the more ventral locus. Generally, the cannula was not lowered more than 4 times or more than a total distance of 1.0 mm. No change in cannula setting was made if noradrenaline stimulation of the area induced the animal to eat. Control chemical tests on several initially positive subjects had indicated that application of NaCl, L-thyroxine, ephedrine, and *d*-amphetamine hydrochloride, as well as an empty cannula, were ineffective in eliciting food or water intake in the animal. These findings support other studies that indicate specificity of adrenaline and noradrenaline in eliciting food intake, and cholinergic stimulation in eliciting water intake in the animal (*1*).

At the end of testing the subjects were killed and the brains were sectioned and stained. The location of the implant and cannula track was determined with the aid of the DeGroot atlas (*4*). The end of the cannula track was considered to be the final stimulation site.

The differences between the values of the preliminary and experimental test for each 1-hour test trial for every subject were calculated. Table 1 presents the 1-hour test results for positive subjects. Preliminary test raw score values for combined subjects in Table 1 averaged 0.8 g and 1.0 ml of food and water intake, respectively. Negative loci (those at which noradrenaline stimulation elicited less than 3.0 g of food intake) were encountered in and about all target structures. This includes negative loci in and about the thalamic and hypothalamic parts of the third ventricle. Stimulation of anterior and lateral areas of the dorsal hippocampus was also negative.

Table 1 indicates that noradrenaline stimulation at limbic and diencephalic sites elicits food intake. The findings concerning water intake corroborate previous study (*5*). A minimum interval of 3 days between successive stimulation days was necessary for reliable responding to noradrenaline stimulation. An optimum chemical stimulation interval in the elicitation of eating may be on the order of 6 to 8 days. Short

Table 1. Mean differences between the test and preliminary test scores for empty-cannula control and experimental conditions for positive subjects across brain areas. (Differences between empty-cannula control and experimental conditions for both food and water values are statistically significant, $P > .01$.) Other areas are (i) arcuate nucleus, (ii) lateral habenular nucleus and habenulo-interpeduncular tract, (iii) periventricular hypothalamus and ventricle, and (iv) area in and about the paraventricular hypothalamic nucleus and ventricle, including the periventricular hypothalamus.

Area	Subjects (No.)	Eating (g) elicited by		Drinking (ml) elicited by	
		Empty cannula	Noradrenaline	Empty cannula	Carbachol
Dorsomedial hippocampus	6	0.5	3.1	0	3.6
Mammillary body	5	.7	3.6	0.2	8.9
Anterior thalamus	7	.3	3.9	.5	7.2
Cingulate gyrus	3	.2	2.1	1.0	6.0
Lateral septal nucleus	5	.8	2.5	0	7.9
Reuniens nucleus	2	.1	1.9	−0.1	10.4
Other	4	−0.2	2.6	−1.1	0.8

Table 2. Correlations of eating and drinking values at each locus of stimulation across brain areas. N, number of loci within each area for which paired scores (noradrenaline eliciting eating and carbachol eliciting drinking) are available. Correlations at the anterior thalamus and lateral septal nucleus are statistically significant, $P > .01$ and .05, respectively.

Area	Correlation	N
Hippocampus	+0.10	48
Mammillary body	+0.01	19
Anterior thalamus	−0.50	26
Cingulate gyrus	−0.13	43
Lateral septal nucleus	+0.53	19
Renuiens nucleus	+0.07	11

intervals, of 23 to 71 hours intervening between adrenergic stimulation, results in gradually increased preliminary test baseline eating values. A comparable effect concerning carbachol and drinking is not obtained under similar conditions. It is therefore possible that repetitive adrenergic stimulation of the brain represents an important conditioning process.

Changes in response category were noted when the cannulas were lowered at 0.25 mm intervals. This indicates that small distances may be critical in the rat brain in functional studies of nuclear areas or fiber systems. Changes in cannula length at intervals of greater than 0.25 mm may result in the by-pass of positive eating or drinking sites.

The highest mean difference eating value in response to noradrenaline stimulation occurred at the anterior thalamus. Considering the extent of brain area involved, and the relatively small number of positive subjects per brain area, comparative statements concerning food intake values caused by stimulation of the various areas does not seem practical. However, it is felt that the low value at the cingulate gyrus reflects uncertainty in coordinate definition of positive loci, as well as the extreme cannulation sensitivity displayed by these subjects.

Latencies were not systematically measured, but eating usually began 2 to 5 minutes after insertion of the noradrenaline-loaded cannula and continued for 15 to 45 minutes. Handling often initiated eating but the response was usually transient, seldom lasting for more than a few minutes. Time factors relevant to carbachol stimulation and drinking appeared to be the same as those previously reported (*5*).

Neural areas that control eating and drinking may also subserve control of physiological activity that support eating and drinking. For example, it is probable that a particular neuroendocrine regulation accompanies the expression of motivational states. Taken together, the findings of this and other studies indicate that similar brain areas are involved in feeding behavior and pituitary-thyroid function (*6*).

The relationship may even be specific: neural loci at which chemical stimulation elicits feeding may be loci concerned in the associated regulation of pituitary-thyroid function. Thus, adrenergic stimulation of appropriate brain loci may elicit feeding and affect pituitary-thyroid function. Possibly, these neural loci represent isomorphic, complementary, neuroendocrine, and neurobehavioral regulatory systems. The brain areas concerned may be systematized in terms of "Papez circuit" and, more generally, "Nauta circuit" neuroanatomy. In view of these findings, and those of other studies, it is apparent that the "Nauta circuit" provides a heuristic definition of the neural substrate underlying motivational and emotional states (*2, 7*).

It was of interest to know if degree of eating and drinking under noradrenaline and carbachol stimulation, respectively, are related. In order to find this out, noradrenaline food intake values at each locus of stimulation, within each brain area, were correlated with the corresponding carbachol water intake values. This was done for all loci within the various areas tested, whether or not these loci were considered positive or negative. The correlations are presented in Table 2. Strong correlations were found at the anterior thalamus and lateral septal areas, while negligible or near zero correlations occurred at hippocampus, mammillary body, cingulate gyrus, and reuniens brain areas. The correlations at anterior thalamus and lateral septal areas are not simply a function of overlap effects from stimulation of dual response (eating and drinking) loci, because dual response loci occur at all brain areas at which data were correlated (Table 3).

The correlations at lateral septal and anterior thalamus brain areas may reflect specialized structural and functional characteristics of the neural substrate within these areas. The positive correlation at the lateral septal area may indicate a mechanism for matching of food and water intake levels and maintaining eating and drinking until these levels are met. A negative correlation at the anterior thalamus may indicate reciprocal inhibition between food and water intake systems: one function may be maintained while the other is inhibited. A mechanism of this type could function to sustain eating or drinking and to regulate shifts of behavior.

In the course of the experiment, 184 loci were stimulated with both noradrenaline and carbachol. Table 3 shows that 94 percent of the loci were distributed within four categories of response: (i) loci which were negative for eating and drinking; (ii) loci at which carbachol stimulation elicited drinking; (iii) loci at which noradrenaline stimulation elicited eating and carbachol elicited drinking; and (iv) loci at which

Table 3. Type of response exhibited by single loci each of which has been stimulated, at least once, by both carbachol and noradrenaline. A chi-square test of the total percentage distribution was statistically significant with $P < .001$. Abbreviations for brain areas were taken from the DeGroot rat brain atlas. Code for type of response: 1, negative; 2, carbachol elicited drinking; 3, noradrenaline elicited eating and carbachol elicited drinking; 4, noradrenaline elicited eating; ("Other," 5–9) 5, carbachol elicited eating and drinking; 6, carbachol elicited eating and drinking; noradrenaline elicited eating; 7, carbachol elicited eating; 8, carbachol and noradrenaline elicited eating; 9, carbachol and noradrenaline elicited drinking.

Areas	Type of response (No. of loci)				
	1	2	3	4	5–9
"Papez circuit"					
Dorsal hippocampus	31	7	5	2	
Mammillary body	3	5	3	1	5
Anterior thalamus	0	7	7	1	
Cingulate gyrus	18	11	4	2	2
Percent	46	26	17	5	
Other areas					
Lateral septal	11	3	3	2	
Reuniens	1	1	2		
Corpus callosum area	1	2			
Ventricle and posterior commisure	7				
Habenula	6		2		
Fornix-fimbria	1	5	1	1	
Stria medullaris	1				
Lateral habenula, stria medullaris, and lateral thalamus	1				
Paratenial and paraventricular	3				
Medialdorsal	3				
Rhomboid	1				
Paraventricular hypothalamus and third ventricle	1	2		1	1
Periventricular hypothalamus and third ventricle		1		1	
Periventricular hypothalamus	1				
Arcuate nucleus				1	
Ventral midline areas involving posterior mammillary nucleus anteriorly and interpeduncular nucleus posteriorly		1			2
Percent	41	34	11	8	
Total	81	54	27	12	10
Percent	44	29	15	6	

noradrenaline elicited eating (*8*). The remaining 6 percent of the responses covers five different response types and are grouped into a single "other" category (response types five through nine). In most instances, however, eating is related to adrenergic, and drinking to cholinergic, stimulation. It should be noted that combined dual response and noradrenaline single response type percentages (response types three and four) indicate that only 21 percent of the loci tested may be positive to noradrenaline stimulation. Salmoiraghi and Bloom (*9*) suggest that noradrenaline and acetylcholine may function as neurotransmitters at mammalian central nervous system synapses. Therefore, the behavioral effects reported here may normally be mediated by noradrenaline and acetylcholine functioning as neurotransmitters within the central nervous system.

As indicated in Table 3, however, exceptions to complete differentiation do occur; there are mixed effects at 6 percent of the loci tested. Most of this variability may be attributed to the fact that carbachol occasionally elicits eating. This has been observed at the lateral hypothalamus (*5*). The elicitation of drinking by noradrenaline stimulation, however, is extremely rare. This chemical elicited drinking at only one of the 184 loci tested. The "mixed response" variability falls within two brain regions, hypothalamus and cingulate gyrus. Thus, the two regions may share a common function.

The loci, represented by the first four response types, may be classified as negative, dual response, or single response types. However, only 15 percent of the loci tested were of the dual response type. It is likely, then, that the negative and single response loci type indicate the presence of functional systems, other than those related to eating or drinking.

Table 3 shows one other important relationship: the total percentage distribution between the first four categories of response seem proportional to each other. Thus, the twofold significance of the existence of categories of loci that have a possible quantitative relationship between them becomes clear. First, it indicates that there are shifts in chemical receptor densities across a range of neural tissue, and that this shift is in the form of a density gradient. Second, it indicates that these shifts in receptor densities are the basis of a distribution of overlapped neurobehavioral systems within "Nauta circuit" brain areas. Citing the hippocampus as an example, a careful lamina and pyramidal field analysis should reveal zones of chemically receptive neurons whose organization would reflect structural and functional characteristics specific to the various motivational and emotional states. Findings within this limbic structure may be generalized, on neuroanatomical grounds, to other "Nauta circuit" areas.

It seems unlikely that neurobehavioral systems could have widespread limbic, diencephalic, and midbrain representation without involvement of neocortical areas. In fact, frontal cortical areas may be involved in the control of food and water intake (*10*). There may be a continuity between "Nauta circuit" brain areas and neocortex, and an integrated structural and functional characterization of neurobehavioral systems may be possible.

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Short-Term Memory, Parsing, and the Primate Frontal Cortex

Abstract. *Removal of the frontal cortex of primates resulted earlier in a psychological deficit usually classified in terms of short-term memory. This classification is based on impairment in performance of delayed-response or alternation-type tasks. We report an experiment in which the classical 5-second-delay right-left-right-left (R-L-R-L) alternation task was modified by placing a 15-second interval between each R-L couplet: R-L . . . R-L . . . R-L . . . . This modification made it possible for monkeys with frontal lesions, which had failed the classical task, to perform with very few errors. The result suggests that proper division, parsing of the stream of stimuli to which the organism is subjected, is a more important variable in the mechanism of short-term memory than is the maintenance of a neural trace per se.*

Interest in the problem of short-term memory has recently revived. Psychologists have become adept at manipulating verbal learning (*1*), and biologists have used intracerebral injection of drugs to good advantage (*2*). Meanwhile, a time-honored approach to the problem has apparently lagged; that is to say, very few advances in understanding have recently come from the use of primates with frontal lesions. An opportunity seems to have been neglected, since a lesion of the frontal eugranular isocortex inflicts a very specific psychological loss that has been regularly characterized as a deficiency in short-