result of physical exertion is occupied by changes in metabolism of biogenic monoamines [2, 5, 7-10]. The noradrenalin concentration in the brain as a rule falls under these circumstances [4, 9] whereas the serotonin level, on the contrary, rises [2, 5, 8]. It has been shown that under these conditions the formation and fixation of temporary connections are disturbed [4], and this probably played an essential role also in the development of the disturbances described in this paper.

## LITERATURE CITED


1. N. R. Boiko, in: Problems in Experimental and Clinical Gerontology [in Russian], Kiev (1968), pp. 123-124.
2. G. N. Kassil', I. L. Vaisfel'd, É. Sh. Matlina, et al., Humoral-Hormonal Mechanisms of Regulation of Functions during Athletic Activity [in Russian], Moscow (1978).
3. Yu. S. Dmitriev, Zh. Vyssh. Nerv. Deyat., No. 1, 51 (1975).
4. R. I. Kruglikov, Neurochemical Mechanisms of Learning and Memory [in Russian], Moscow (1981).
5. É. Sh. Matlina and G. N. Kassil', Usp. Fiziol. Nauk, No. 2, 13 (1976).
6. G. I. Mil'shtein, Zh. Vyssh. Nerv. Deyat., No. 4, 505 (1960).
7. K. I. Pogodaev and N. F. Turova, Biochemistry of the Brain in Fatigue and Exhaustion [in Russian], Moscow (1972).
8. J. D. Barchas and D. X. Freedman, Biochem. Pharmacol., 12, 1232 (1963).
9. E. T. M. Ruthker, M. Ackenheie, and N. Matussek, Arzneimittel-Forsch., 16, 261 (1966).
10. E. A. Stone, J. Neurochem., 21, 589 (1973).


# MICROIONTOPHORETIC ANALYSIS OF RETICULAR FORMATION NEURONS IN FOOD-MOTIVATED ANIMALS


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The concept of the pacemaker role of hypothalamic centers in the formation of the principal biological motivations was formulated by Anokhin [2]. Further investigation of this problem showed that food motivated excitation, arising in the hypothalamic structures, spreads to several regions of the cortex and basal ganglia [5, 6]. The presence of two-way morphological and functional connections between the mesencephalic reticular formation and the lateral region of the hypothalamus, and also the fact that a duplicate pacemaker of food motivated excitation can be produced in the reticular formation indicate that this structure is directly implicated in the functional system of the food-getting act [1, 4].

Several investigations have shown [4, 10-12] that food-motivated excitation involves neurons of cortical and deep brain structures in the performance of a behavioral act with the aid of neurotransmitters of both adrenergic and cholinergic nature. For instance, the formation of food motivation at the level of hypothalamic structures takes place on account of adrenergic mechanisms [4, 10]. However, no investigation has yet been undertaken of the neurochemical reactions of single neurons of the reticular formation in the presence of food motivation.

It was accordingly decided to study the neurochemical properties of the neurons of this structure in food-motivated rabbits.

## EXPERIMENTAL METHOD

Experiments were carried out on 12 hungry rabbits (deprivation for 48 h) weighing from


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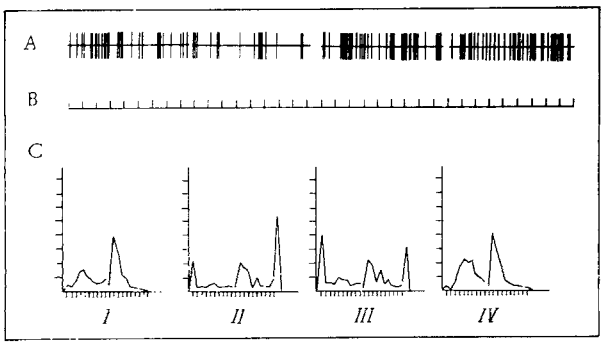


Fig. 1. Time course of unit activity in mesencephalic reticular formation of hungry rabbit during microiontophoretic application of noradrenalin, serotonin, and acetylcholine. A) Unit activity, B) time marker (1 sec), C) interval histogram. I) Spontaneous unit activity, II) microiontophoresis of noradrenalin, III) of acetylcholine, IV) of serotonin.

3 to 3.5 kg. Activity of mesencephalic reticular neurons (P = 8; $\bar{l}$ = 2.5; h = 11-12, atlas of Sawyer, 1954) was recorded by glass five-barreled microelectrodes, extracellularly in an unanesthetized waking, unrestrained animal. The barrel for the recording electrode was filled with 3 M KCl solution. The other barrels of the microelectrode were filled with 1 M solution of L-noradrenalin bitartrate (pH 3.5) and 1 M acetylcholine chloride solution (pH 5.0); isotonic NaCl solution was used as the control.

In the course of the experiments spontaneous activity of a neuron of the mesencephalic reticular formation was recorded for 5 min, the activity of the same neuron was recorded after application of noradrenalin to it by microiontophoresis with a current of 30 nA acting for 3 sec, the activity of the neuron after microiontophoretic application of serotonin to it by a current of the same strength for 3 sec, activity of the neuron after microiontophoretic application of acetylcholine to it by a current of the same strength for 3 sec, and activity of a reticular neuron after microiontophoretic application of the control isotonic NaCl solution were recorded.

The interval between applications of the neurotransmitters and the control NaCl solution varied from 2 to 5 min. For statistical analysis 500 interspike intervals of spontaneous activity of the neuron were always measured before application of the neurotransmitter, and 100-500 intervals after the beginning of action of the substance, depending on its effect. Unit activity was recorded on magnetic tape. The data were analyzed by an HTA-1024 analyzer (Hungary) and M-4030 computer. The mean discharge frequency in spikes/sec was used for statistical analysis of unit activity, by construction of percentile interval histograms. The location of the microelectrodes was verified in laminar brain sections stained by a rapid photographic method.

## EXPERIMENTAL RESULTS

Activity was recorded from 40 neurons of the rabbit's mesencephalic reticular formation. Statistical analysis of spontaneous unit activity showed that the overall mean discharge frequency of these neurons was 21.8 spikes/sec. Twenty of the 40 neurons had a specific pattern of activity on their interval histogram, characterized by dominance of interspike intervals on the histogram in the 2-10 and 100-200 msec regions, i.e., there was a bimodal distribution of intervals which is characteristic of the natural motivated state of hunger in animals [7-9]. The remaining 20 neurons had the following interval characteristics: nine neurons had a bimodal distribution, but with peaks on the histogram in the 20-30 and 100-200 msec regions (six neurons) and the 40-50 and 100-200 msec regions (three neurons); 11 neurons had a monomodal distribution of intervals — intervals in five neurons were chiefly in the 30-60 msec region and in six neurons in 100-200 msec region.

Microiontophoretic application of noradrenalin to reticular formation neurons increased the overall mean discharge frequency to 24.8 spikes/sec, evoking an activation response in 19 neurons and inhibition in 13; eight neurons were areactive. Meanwhile, of 20 neurons characterized by a bimodal distribution of intervals with maxima in the 2-10 and 100-200 msec regions, 15 preserved the same interval characteristics on their histogram after microiontophoretic application of adrenalin, which increased the mean discharge frequency of 12

of these neurons to 26.2 spikes/sec but did not change the frequency characteristics of the other three neurons (22.1 spikes/sec); the remaining five neurons switched to a different but regular type of activity. Their overall mean discharge frequency was 24.5 spikes/sec (Fig. 1). Of the 20 other neurons with widely different interval characteristics in the background, untypical of an animal in a food-motivated state, application of noradrenalin not only caused changes in the frequency characteristics of 10 neurons (overall mean frequency 19.5 spikes/sec), but also altered their firing pattern, as shown by predominance of intervals in the 2-10 and 100-200 msec regions on the histogram. Of the remaining 10 neurons, five did not respond to the drug and five neurons had a different distribution of intervals on their histogram.

Microiontophoretic application of acetylcholine also increased the overall mean discharge frequency of neurons in this region to 28.8 spikes/sec. Acetylcholine evoked activation of the discharges of 21 neurons, inhibited the activity of eight neurons, and 11 neurons were areactive. Of 40 neurons only 10 were characterized by dominance of interspike intervals in regions under 10 and between 100 and 200 msec. Two of the 10 neurons, which initially had a regular type of activity, gave a bimodal distribution on their interval histogram after application of acetylcholine, whereas eight neurons, with initially a bimodal distribution of intervals, preserved this same type of activity after application of the drug and responded by increased dominance of intervals in regions between 2 and 10 msec.

If the ratio between peaks at 5-10 and 200 msec on the interval histogram after application of noradrenalin, namely 2:1, is compared with the same ratio for application of acetylcholine, namely 5:1, the clear increase in the ratio in the latter case will be evident.

Similar application of serotonin to reticular neurons reduced the overall mean frequency to 17.8 spikes/sec. The microiontophoretic effect of serotonin was expressed as activation of the discharge in 13 neurons and inhibition in 10; the activity of 17 neurons was unchanged. Of 20 neurons with a bimodal distribution of intervals on the histogram of their spontaneous activity, with maxima in the 2-10 and 100-200 msec regions, six neurons continued to have such a distribution after application of serotonin (only their frequency characteristics were changed); six neurons showed no change in either the frequency or the interval characteristics of their discharge; eight neurons switched to a different, regular type of activity, characterized by dominance of intervals in the 50-60 or 100-200 msec regions on their interval histogram.

Analysis of interval histograms of reticular neurons of hungry rabbits thus revealed a specific distribution of intervals in 50% of the neurons, expressed as dominance of interspike intervals in the 2-10 and 100-200 msec regions, as was demonstrated previously in the case of neurons of structures such as the lateral hypothalamus, orbital cortex [7], hippocampus [8], and ventral posteromedial thalamic nucleus [3]. Since the same distribution of intervals in satiated animals was found for neurons of the reticular formation in response to stimulation of the lateral hypothalamus, it can be concluded that food-motivated excitation is reflected at the neuronal level in the mesencephalic reticular formation as a specific pattern of activity, corresponding to a definite distribution of interspike intervals on the histogram.

Microiontophoretic application of noradrenalin and acetylcholine significantly increased the overall mean frequency of unit activity in the reticular formation. The action of noradrenalin also leads to an increase in the number of neurons that respond to application by dominance of intervals below 10 and 100-200 msec on the histogram, evidence of facilitation of the realization of food-motivated excitation on reticular neurons. The action of acetylcholine can evoke the same distribution of intervals, although in fewer neurons, but with a more marked difference in the values of the 5-10 and 200 msec peaks, due to enhanced "burst formation," i.e., an increase in the number of spikes in the bursts. These data indicate activation of neurons of the reticular formation into a functional system of food behavior by means of the neurotransmitters noradrenalin and acetylcholine, but the level of their participation and their qualitative contribution to the realization of food-motivated excitation are different.

Microiontophoretic application of serotonin reduces the overall mean discharge frequency of these neurons without causing the changes described above in their firing pattern, and it gives a completely different distribution of intervals on the histogram; this may indicate that serotonin participates in processes unconnected with the initial stages of the functional system of food-getting behavior.

LITERATURE CITED

1. D. Adams, V. I. Badikov, V. M. Smirnov, et al., Byull. Éksp. Biol. Med., No. 11, 515 (1978).
2. P. K. Anokhin, The Biology and Neurophysiology of the Conditioned Reflex [in Russian], Moscow (1969).
3. B. V. Zhuravlev and I. Yu. Orbachevskaya, Byull. Éksp. Biol. Med., No. 2, 137 (1980).
4. V. G. Zilov, in: Problems in the Physiology of the Hypothalamus [in Russian], No. 13, Kiev (1979), p. 47.
5. K. V. Sudakov, Biological Motivations [in Russian], Moscow (1971).
6. K. V. Sudakov, B. V. Zhuravlev, and A. V. Kotov, Zh. Vyssh. Nerv. Deyat., No. 1, 24 (1973).
7. K. V. Sudakov and B. V. Zhuravlev, Zh. Vyssh. Nerv. Deyat., No. 3, 643 (1979).
8. L. V. Timofeeva and B. V. Zhuravlev, Zh. Vyssh. Nerv. Deyat., No. 2, 412 (1981).
9. A. I. Shumilina, B. V. Zhuravlev, and I. Yu. Orbachevskaya, Zh. Vyssh. Nerv. Deyat., No. 6, 1298 (1980).
10. L. J. Herberg and K. B. J. Franklin, Physiol. Behav., 8, 1029 (1972).
11. R. D. Myers and M. L. McCaleb, Neuroscience, 6, 645 (1981).
12. P. Stark, J. A. Turk, and C. W. Totty, Am. J. Physiol., 220, 1516 (1971).

# ROLE OF MEDULLARY CHEMOSENSITIVE STRUCTURES IN BLOOD PRESSURE CONTROL AND THEIR MEMBERSHIP IN THE APUD SYSTEM

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One of the functions of the medullary chemosensitive structures is not merely to organize and modify the respiratory drive from its ventral surface but also to take part in the formation of pressor reactions of the arterial pressure [2]. By central chemoreceptors are implied structures of nerve tissue located directly beneath the pia mater at a depth of under 150-200 μ from the ventral surface of the medulla [7, 8]. It has been suggested that these chemoreceptive structures determine the activity of sympathetic vasomotor neurons [9]. The problem of the nature of these formations is particularly interesting in connection with the extensive study of the apud system: This is a highly specialized system of cells which produce vitally important chemical agents — biogenic amines and peptide hormones [1, 5]. It has recently been shown that substances which also have been identified in the brain are synthesized in cells of the apud system (apud cells): gastrin, vasoactive intestinal peptide, substance P, somatostatin, and enkephalins [6]. These have been called neuropeptides.

Considering that the functional state of structures located within the central chemosensitive areas correlates clearly with the intensity of pressor reactions, it was decided to study whether biologically active substances (biogenic amines and their analogs) are present in these regions and to clarify the role of the chemosensitive input in blood pressure (BP) regulation.

## EXPERIMENTAL METHOD

Acute experiments were carried out on 25 cats anesthetized by intravenous injection of a mixture of chloralose (40 mg/kg) and urethane (200 mg/kg). In a separate series of experiments the lungs were artificially ventilated, for which purpose the animals were relaxed with tubocurarine (0.5-1.0 mg/kg, intravenously for 1 h) and connected to an artificial respiration apparatus (AID). The medulla was exposed through a ventral approach from the

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