Effects of Electrical Stimulation of the Hunger Center in the Lateral Hypothalamus and Food Reinforcement on Impulse Activity of the Mylohyoid Muscle in Rabbits under Conditions of Hunger and Satiety

Ju. P. Ignatova and A. A. Kromin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 11, pp. 487-493, November, 2010
Original article submitted October 10, 2009

Effects of electrical stimulation of the hunger center in the lateral hypothalamus and food reinforcement on impulse activity of mylohyoid muscle were studied in chronic experiments under conditions of hunger and satiety. Threshold stimulation of the lateral hypothalamus in starving and satiated rabbits in the absence of food induced searching behavior associated with burst-like impulse activity with a bimodal distribution of interpulse intervals. Regular spike burst in the mylohyoid muscle during stimulation of the lateral hypothalamus in the absence of food serves as an example of the anticipatory type reaction. Increased food motivation during threshold stimulation of the lateral hypothalamus in starving and satiated rabbits with food offered led to successful food-procuring behavior, during which the frequency of spike bursts in the mylohyoid muscle became comparable with that under conditions of natural foraging behavior stimulated by the need in nutrients. Our results suggest that temporal structure of mylohyoid muscle impulse activity reflects convergent interactions of food-motivation excitation with reinforcement excitation on neurons of the masticatory and deglutitive centers.

Key Words: *lateral hypothalamus hunger center; electrical stimulation; mylohyoid muscle; impulse activity; satiety*

According to pacemaker theory of motivation [4], the hunger center in the lateral hypothalamus (LH), an initiative motivation-generating center, activates subcortical structures and cortex, thus forming a system of alimentary motivational brain excitement.

Along with ascending activating influences, the hunger center in the LH exerts descending stimulating influences on neurons of the masticatory center in the medulla oblongata [7]. The peculiarities of the functional organization of the masticatory center are determined, on the one hand, by the ability of the neurons in central mastication pattern generator to maintain rhythmic activity in the absence of sensory feedback in accordance with central mastication program formed by neural circuits of the medulla oblongata, and on another hand, by ability of feedback afferentation appeared in the process of food reinforcement to transform standard program of central mastication pattern generator [6].

Convergent interaction between alimentary motivational excitation and reinforcement excitation are proved to appear on individual neurons of various brain areas [4]. One may assume that alimentary motivational excitation and reinforcement excitation are addressed to the same neurons of the masticatory center in the medulla oblongata. In light of this, it was interesting to study reflection of convergent interactions of alimentary motivational excitation and reinforce-

Department of Physiology, Tver' State Medical Academy, Federal Agency for Health Care and Social Development, Russia. ***Address for correspondence:*** krominaa@mail.ru. A. A. Kromin

0007-4888/11/1505-0559

ment excitation on neurons of the central masticatory pattern generator in impulse activity of the mylohyoid muscle (MM)

The objective of this study was to compare the patterns of impulse activity of MM during electrical stimulation of LH in starving and satiated rabbits in the presence and absence of food.

MATERIALS AND METHODS

Impulse activity of MM in Chinchilla rabbits preliminary subjected to 24-h food deprivation or fed before the experiment was recorded via chronically implanted electrodes [1-3]. The effects of electrical stimulation of LH on impulse activity of MM in the presence and absence of food in the experimental chamber were evaluated under conditions of free behavior. In parallel, behavioral activity of animals was recorded with a web-camera. Electrical stimulation of LH was performed using bipolar nickel-chromium electrodes implanted according to the approach of "travelling electrode". An oval incision on the dorsal surface of the head was made under local anesthesia with 0.5% novocain. Skin and soft tissues were dissected and a wide scalping-type wound was formed. The temporal muscles and the periosteum were removed from the lateral skull surface, hemorrhage was stopped, and wound surface was treated. To prevent infection, the rabbit was intramuscularly injected with 200,000-250,000 U penicillin. The electrodes were implanted on day 5-4 after the intervention using stereotaxic atlas [9] and taking into account Olds data concerning positive, negative, and neutral hypothalamic areas. Before electrode implantation, several holes (0.6-0.8 mm in diameter) were drilled in the skull and guiding cannulas were placed. These cannulas allowed searching for needed brain area in awake animal using "travelling electrode" approach lied in providing test stimulation every 0.5 mm during travelling depthward the brain through the cannula with stimulation electrode (bipolar nichrome electrode in 0.5 mm steel needle with interelectrode distance 1-1.2 mm). After determining the type of behavioral response, the bipolar nichrome electrode was fixed on the skull with stirakryl. Brain stimulation was performed using sinusoidal current with 60 Hz frequency, 2.5-3.5 V, pulse train duration 0.5-1.4 sec.

Automatic analysis of temporal parameters of MM pulse activity (in msec) was performed using MP-100 microprocessor and AcqKnowledge software. Temporal parameters of impulse activity of PMM motor units

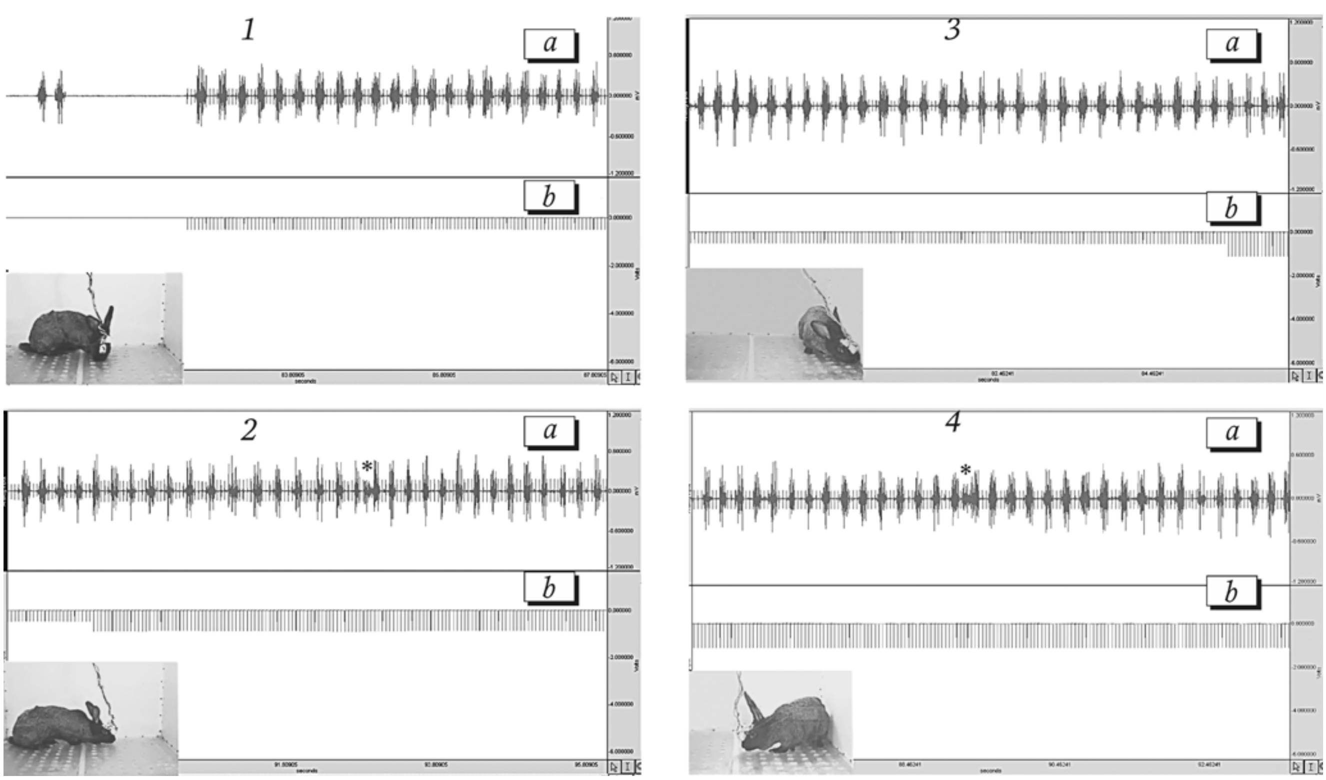
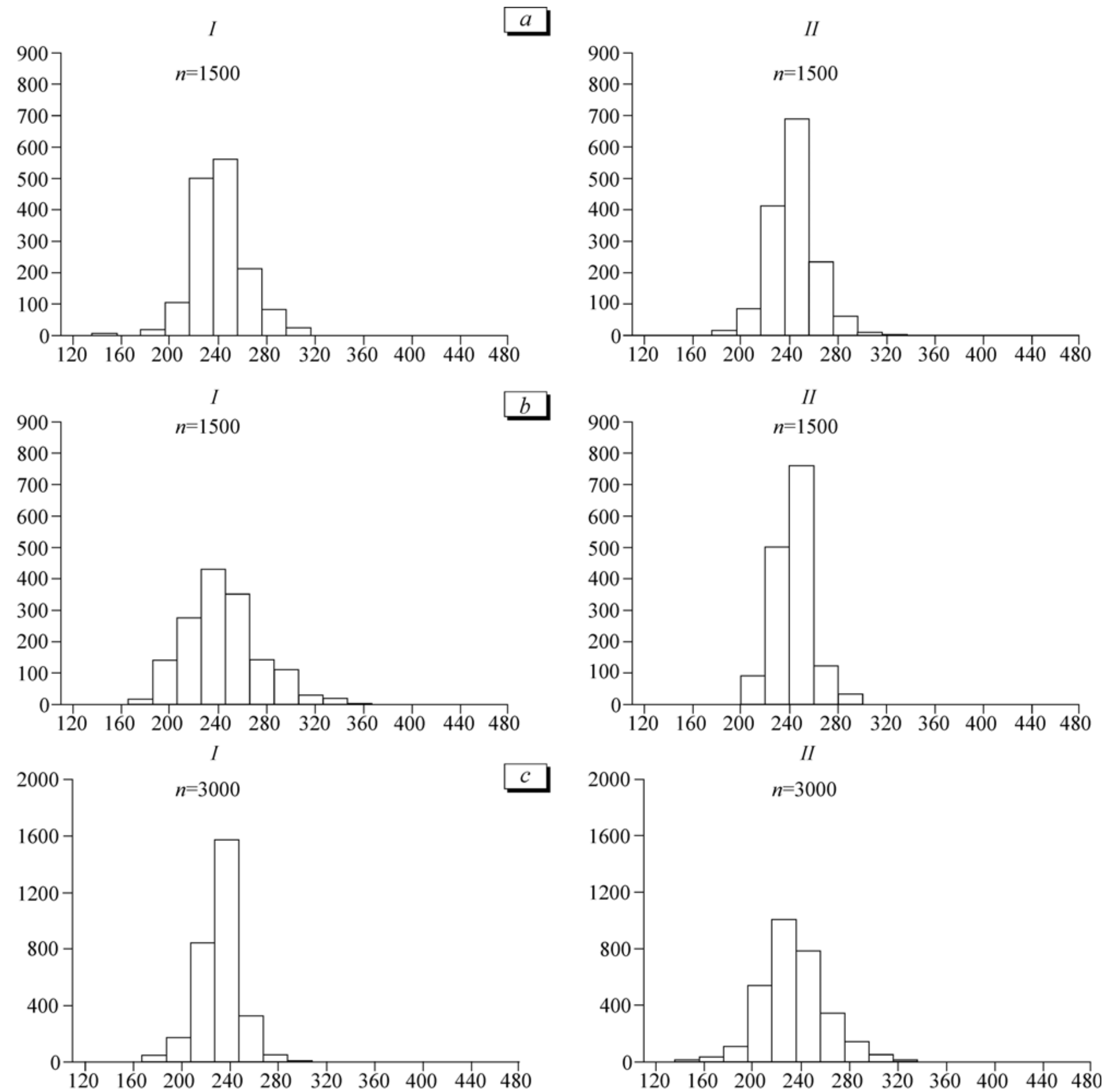


Fig. 1. MM impulse activity in rabbit (*a*) exposed to 24-h food deprivation (*1*, *2*) and satiated (*3*, *4*) during threshold (*1*, *3*) and submaximal (*2*, *4*) LH electrical stimulation in the absence of the food. Here and on Fig. 3: *a*: Low-amplitude artifacts from LH stimulation can be seen. They correspond to frequency of stimulating square electric pulses (*b*). Asterisks: spike bursts in deglutition pattern. On the bottom: time mark 2000 msec; on the right: calibrating signals: mV (*a*), V (*b*); photographs in the lower left rectangle: animal behavior.



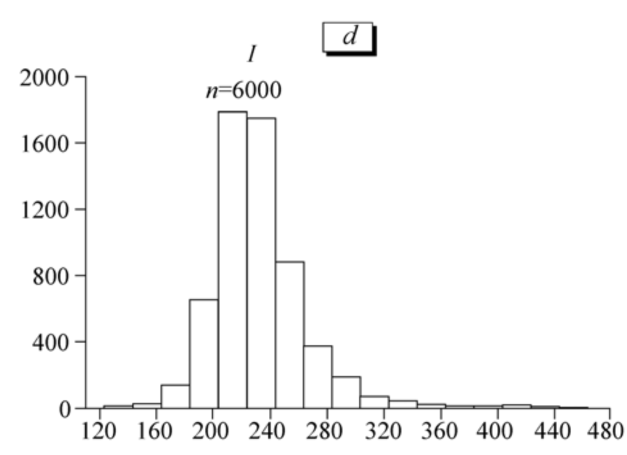


Fig. 2. Distribution histograms of MM burst-like activity periods in rabbits exposed to 24-h food deprivation (*I*) and in satiated rabbits (*II*): during threshold (*a*) and submaximal (*b*) LH electrical stimulation in the absence of food; during threshold LH electrical stimulation and subsequent effective foraging behavior (*c*); during natural foraging behavior (*d*). Abscissa: values of burst-like activity periods, msec; ordinate: absolute frequency of burst-like activity periods.

TABLE 1. Statistical Parameters of MM Impulse Activity (msec) in Rabbits Exposed to 24-h Food Deprivation and Satiated Rabbits during Threshold and Submaximal LH Stimulation in the Absence of Food

Temporal parameters	Threshold stimulation					Submaximal stimulation				
	interpulse intervals	interpulse intervals in spike bursts	burst duration	interburst intervals	periods of burst-like activity	interpulse intervals	interpulse intervals in spike bursts	burst duration	interburst intervals	periods of burst-like activity
Rabbits exposed to 24-h food deprivation										
n	6414	4914	1500	1500	1500	5620	4120	1500	1500	1500
M	56.46	13.06	42.77	198.68	241.44	64.85	14.43	39.63	203.33	242.96
Me	13.00	10.50	42.00	198.50	238.75	16.00	12.50	40.50	203.50	244.00
s	79.55	7.38	16.38	21.93	21.46	85.11	7.46	13.06	28.66	29.21
m	0.99	0.11	0.42	0.57	0.55	1.14	0.12	0.34	0.74	0.75
As	1.30	1.78	0.61	0.01	0.16	1.14	1.48	0.93	0.37	0.52
s_{As}	0.03	0.03	0.06	0.06	0.06	0.03	0.04	0.06	0.06	0.06
Ex	-0.19	3.79	0.85	0.78	1.53	-0.51	2.70	2.37	0.07	0.56
s_{Ex}	0.06	0.07	0.13	0.13	0.13	0.07	0.08	0.13	0.13	0.13
q. 25%	8.50	8.00	31.50	184.50	228.00	10.00	8.50	30.00	181.50	222.00
q. 75%	33.00	15.50	51.00	210.50	252.00	160.50	18.00	47.00	222.00	255.50
Satiated rabbits										
n	6384	4884	1500	1500	1500	6850	5350	1500	1500	1500
M	57.03	15.47	50.36	192.38	242.74	53.11	13.41	47.83	194.71	242.55
Me	17.00	13.50	50.00	190.50	243.50	14.00	11.50	47.50	192.75	244.75
s	75.89	7.68	16.06	19.33	18.47	75.50	6.32	11.85	14.62	15.49
m	0.95	0.11	0.41	0.50	0.48	0.91	0.09	0.31	0.38	0.40
As	1.28	1.13	0.34	0.35	0.15	1.37	1.32	0.07	0.33	0.21
s_{As}	0.03	0.04	0.06	0.06	0.06	0.03	0.03	0.06	0.06	0.06
Ex	-0.24	1.18	0.66	0.20	0.86	-0.04	1.79	0.55	0.30	1.01
s_{Ex}	0.06	0.07	0.13	0.13	0.13	0.06	0.07	0.13	0.13	0.13
q. 25%	11.00	9.50	39.50	178.50	231.00	9.00	8.50	40.50	185.50	232.00
q. 75%	36.00	19.50	62.00	204.25	253.00	27.00	17.00	56.00	204.00	250.50

Note. Here and in Table 2: *n*: number of time intervals of MM impulse activity; *M*: mean, *m*: error of the mean, *Me*: median, s: standard deviation, quartile 25% and 75% (msec); As: asymmetry coefficient, Ex: excess coefficient, s_{As} and s_{Ex}: errors of asymmetry coefficient and excess coefficient.

TABLE 2. Statistical Parameters of MM Impulse Activity in Rabbits Exposed to 24-h Food Deprivation and Satiated Rabbits during Effective Foraging Behavior against the Background of Threshold LH Stimulation and during Natural Foraging Behavior (after 24-h Food Deprivation)

Temporal parameters	Foraging behavior during LH stimulation (hunger)					Foraging behavior during LH stimulation (satiety)					Natural foraging behavior (without LH stimulation)				
	interpulse intervals	interpulse intervals in spike bursts	burst duration	interburst intervals	periods of burst-like activity	interpulse intervals	interpulse intervals in spike bursts	burst duration	interburst intervals	periods of burst-like activity	interpulse intervals	interpulse intervals in spike bursts	burst duration	interburst intervals	periods of burst-like activity
n	14,406	11,406	3000	3000	3000	16,091	13,091	3000	3000	3000	29,566	23,566	6000	6000	6000
M	48.43	13.71	52.12	180.45	232.58	43.63	14.29	62.37	171.63	234.00	47.12	13.16	51.57	180.51	232.20
Me	14.00	12.50	50.50	181.50	233.00	14.00	11.00	60.50	171.00	232.50	11.50	10.00	48.50	179.00	227.00
s	68.38	5.95	14.89	17.43	16.35	62.99	8.41	22.30	28.91	25.87	69.63	8.87	23.32	35.48	34.28
m	0.57	0.06	0.27	0.32	0.30	0.50	0.07	0.41	0.53	0.47	0.40	0.06	0.30	0.46	0.44
As	1.46	1.05	0.48	-0.22	-0.32	1.71	1.59	0.31	0.18	0.26	1.66	2.28	0.83	1.48	1.86
s_{As}	0.02	0.02	0.04	0.04	0.04	0.02	0.02	0.04	0.04	0.04	0.01	0.02	0.03	0.03	0.03
Ex	0.25	0.98	0.03	0.05	1.63	1.26	2.71	-0.20	-0.01	0.68	1.39	5.59	0.44	6.66	7.80
s_{Ex}	0.04	0.05	0.09	0.09	0.09	0.04	0.04	0.09	0.09	0.09	0.03	0.03	0.06	0.06	0.06
q. 25%	10.00	9.00	41.50	169.50	225.00	8.50	8.00	46.50	151.50	217.50	8.00	7.50	34.50	160.50	121.50
q. 75%	25.00	17.00	62.00	192.00	241.50	28.00	18.50	76.50	190.00	249.00	31.00	14.50	63.50	197.00	245.50

(MU) were statistically processed [1]. Significance of differences was assessed using Mann–Whitney *U* test, $p<0.05$.

RESULTS

Threshold stimulation of the hunger center in LH of satiated and food-deprived rabbits in the absence of food led to the appearance of searching, orientation, and exploratory activities associated with uniform reorganization of MM pulse activity manifesting in a shift of irregular phasic impulse activity consisting of 2-3 spike bursts to regular spike burst generation (Fig. 1). We previously showed that such reorganization of MM pulse activity appears during natural successful foraging and that it is not characteristic for exploratory behavior formed on the basis of organism requirements in nutrients [1,2]. Therefore, regular generation of spike bursts in MM during threshold stimulation of LH in the absence of the food can be regarded according to functional system theory as an advanced-type reaction [4].

Activating influences of threshold stimulation of LH hunger center on motor neurons of the masticatory center in the medulla oblongata in starving and satiated animals in the absence of food were uniformly reflected in the structure of temporal organization of MM pulse activity in the form of bimodal distribution of interpulse intervals with peaks at 5-20 and 170-230 msec (starving) and 5-25 and 160-220 msec (satiety), respectively. Testing the values of successive interpulse intervals for normal distribution using Shapiro–Wilk *W* test showed deviation of the experimental data from normal distribution model ($W=0.616355$; $W=0.635896$, $p<0.001$). The same was shown using parameters of descriptive statistics (Table 1). Duration of burst-like activity periods was characterized by low variability, which was demonstrated by unimodal distribution of the time intervals (Fig. 2). The periods of burst-like activity generated by MM in starving and satiated animals lasted for about 241.44 msec with standard deviation 21.46 msec, which corresponded to the frequency of 4.14 Hz or 248.50 cycles per min, and 242.74 msec with standard deviation 18.47 msec or 4.12 Hz or 247.18 cycles per min, respectively, *i.e.* there were no statistically significant differences ($p>0.05$ according to Mann–Whitney *U* test). Our results showed that efferent influences induced by threshold stimulation of LH hunger center in rabbits in the absence of food enhance neuron excitability of the central generator of mastication pattern in the medulla oblongata, tune them on reinforcement, and activate central mastication program. Hence, our data confirms modern conceptions of organization of the medulla oblongata mastication center [5].

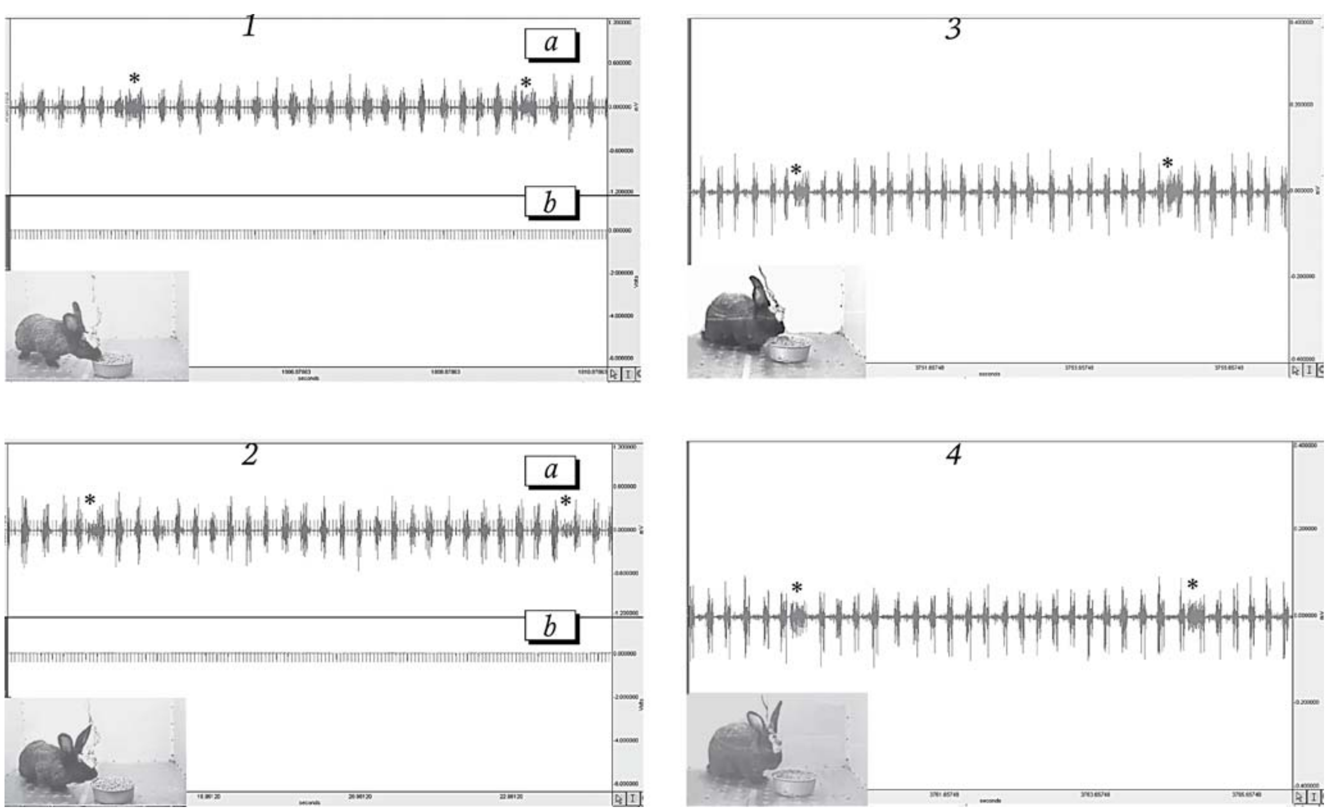


Fig. 3. MM impulse activity in rabbit (*a*) exposed to 24-h food deprivation (*1*) and satiated (*2*) during threshold LH stimulation and associated effective foraging behavior. *3*, *4*: MM impulse activity in a rabbit exposed to 24-h food deprivation during natural effective foraging behavior.

Submaximal stimulation of LH in starving and satiated rabbits in the absence of food did not change MM burst activity during threshold electrical stimulation (*p*>0.05, Fig. 1, Table 1).

Threshold stimulation of LH in hungry and satiated animals with free access to food was associated with successful foraging behavior (Fig. 3) associated with generation of spike bursts in MM in mastication rhythm, which frequency significantly exceeded the corresponding value in the absence of food (*p*<0.05, Fig. 1, 3, Tables 1, 2): 4.30 Hz or 257.97 cycles per minute for starving rabbits and 4.27 Hz or 256.41 cycles per minute for satiated rabbits, which did not significantly differ from the values obtained during natural foraging behavior (*p*>0.05, Table 2).

Threshold stimulation of LH in hungry and satiated animals having free access to food was associated with generation of spike bursts in MM in mastication rhythm and burst activity in deglutition rhythm (Fig. 3). This relationship was previously observed under conditions of natural foraging behavior stimulated by the need in nutrients [1,2,8]. Under conditions of stimulation of the hunger center in the lateral hypothalamus and subsequent effective foraging behavior, the burst frequency in deglutition rhythm in starving animals (0.19 Hz or 11.42 cycles per minute) was higher than in satiated rabbits (0.14 Hz or 8.90 cycles per minute, *p*<0.05).

Thus, our results suggest that MM pulse activity reflects convergent interactions of motivation food excitement and back afferentation from food reinforcement are addressed to the same neurons of the masticatory center in the medulla oblongata. Such possibility was demonstrated for various neurons of the brain [4].

REFERENCES

1. Ju. P. Ignatova, A. A. Kromin, *Zh. Eksp. Klin. Gastroenter.*, No. 1, 45-54 (2009).
2. Ju. P. Ignatova, A. A. Kromin, *Bull. Eksp. Biol. Med.*, **147**, No. 1, 20-24 (2009).
3. A. A. Kromin, Ju. P. Ignatova, O. Yu. Zenina, *Bipolar Silver Ball Electrode for Electrography. Patent № 5256. Bull. Izobr. Polezn. Modeli*, No. 10, Pt. 3, 809 (2006).
4. K. V. Sudakov, *Dominant Motivation* [in Russian], Moscow (2004).
5. T. Athanassiadis, K. A. Olsson, A. Kolta, and K. G. Westberg, *Exp. Brain Res.*, **165**, No. 4, 478-489 (2005).
6. K. D. Foster, A. Woda, and M. A. Peyron, *J. Neurophysiol.*, **95**, No. 6, 3469-3479 (2006)
7. M. Inoue, K. Nozawa-Inoue, Y. Miyaoka, and Y. Yamada, *Neurosci Res.*, **41**, No. 1, 61-65 (2001).
8. Y. Meng, K. Vchida, T. Sato, *et al.*, *Dysphagia*, **14**, No. 2, 78-84 (1999).
9. G. H. Sawyer, J. Everett, J. O. Green, *J. Comp. Neurol.*, **101**, No. 3, 801-924 (1954).