Reactions and Functional Relationships between Nonrespiratory Neurons in the Medulla Oblongata after Central Application of Penicillin

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Changes in impulse activity of nonrespiratory neurons in the medulla oblongata produced by central administration of penicillin were studied in acute experiments on narcotized immobilized rats. The mean firing frequency increased in most neurons. The peaks on histograms for the distribution of interspike intervals were shifted toward shorter intervals and their amplitude increased; the type of distribution was also changed. Tonic activity of neurons was transformed into burst activity. Study of auto- and cross-correlation histograms for neuronal pairs showed that hyperactivation of structures was accompanied by an increase in the degree of synchronization. These changes reflect the appearance of new functional relationships between neurons in the respiratory center. We found that nonrespiratory reticular neurons are involved in the mechanisms of normal and pathological respiratory rhythm generation and serve as a functionally labile component of the neuronal respiratory network.

Key Words: *medulla oblongata; nonrespiratory neurons; impulse activity; cross-correlation; penicillin*

Previous electrophysiological studies showed that disturbances in respiratory rhythm induced by microinjection of penicillin (nonspecific blocker of synaptic transmission) into the respiratory center of the medulla oblongata are related to enhancement of the total activity of medullary neurons, increase in the ratio of neurons with burst firing activity, hyperactivation of inspiratory neurons [3], and changes in spatial configuration of active neurons [2]. The respiratory center also includes reticular neurons not exhibiting specific respiratory pattern and involved in the generation and regulation of the respiratory rhythm [5], reception of afferent impulses with different modality, integration, and realization of efferent respiratory reactions [4,9].

In the present work changes in impulse activity of nonrespiratory neurons in the respiratory center of the medulla oblongata and synchronization of neuronal activity were studied during blockade of inhibitory synaptic transmission by central application of penicillin, which causes pathological respiratory rhythm in spontaneously breathing rats.

MATERIALS AND METHODS

Experiments were performed on 66 male Wistar rats (250-300 g) narcotized with 30 mg/kg nembutal, immobilized with myorelaxin (5 mg/kg intraperitoneally, VEB Arzneimittelwerk), and artificially ventilated. Access to the bottom of the rhomboid fossa was performed under local Novocain anesthesia [3].

Neuronal activity was recorded extracellularly by the standard physiological method using glass microelectrodes (tip diameter 1-5 μ, resistance 5-10 MΩ) filled with 2.5 M NaCl. Impulse activity of each neuron was recorded before, during, and 30 min after microinjection of penicillin.

Impulse activity of neurons was recorded continuously during application of penicillin. A curved

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glass cannula (tip diameter 50 μ) was glued to a microelectrode with lacquer and Mendeleyev putty. The distance between the tips was 100-200 μ. Activity of neuronal pairs was recorded with two glued microelectrodes (distance between tips 150-1000 μ). Electrodes with the cannula were introduced into the dorsal and ventral groups of the respiratory center (solitary tract nucleus, STN; ambiguous nucleus, AN; and reticular gigantocellular nucleus, GN) according to stereotaxic coordinates [11].

Benzylpenicillin sodium salt (50,000 U in 1 ml 0.9% NaCl, 0.5 μl) was administered via an oil pressure microinjector.

Neuronal activity was processed on an ATAK-350 microprocessor (Nihon Kohden). We studied the values and histograms of the mean current firing rate and distribution of interspike intervals. Histograms of cross-intervals and autocorrelation functions were analyzed in studying pairs of neurons. Peaks were considered to be significant when they exceeded the mean interval in the bin (n) for each histogram by more then 3σ. Further studies involved histograms with short asymmetric peaks. The significance of differences was estimated by Student's t test for related samples.

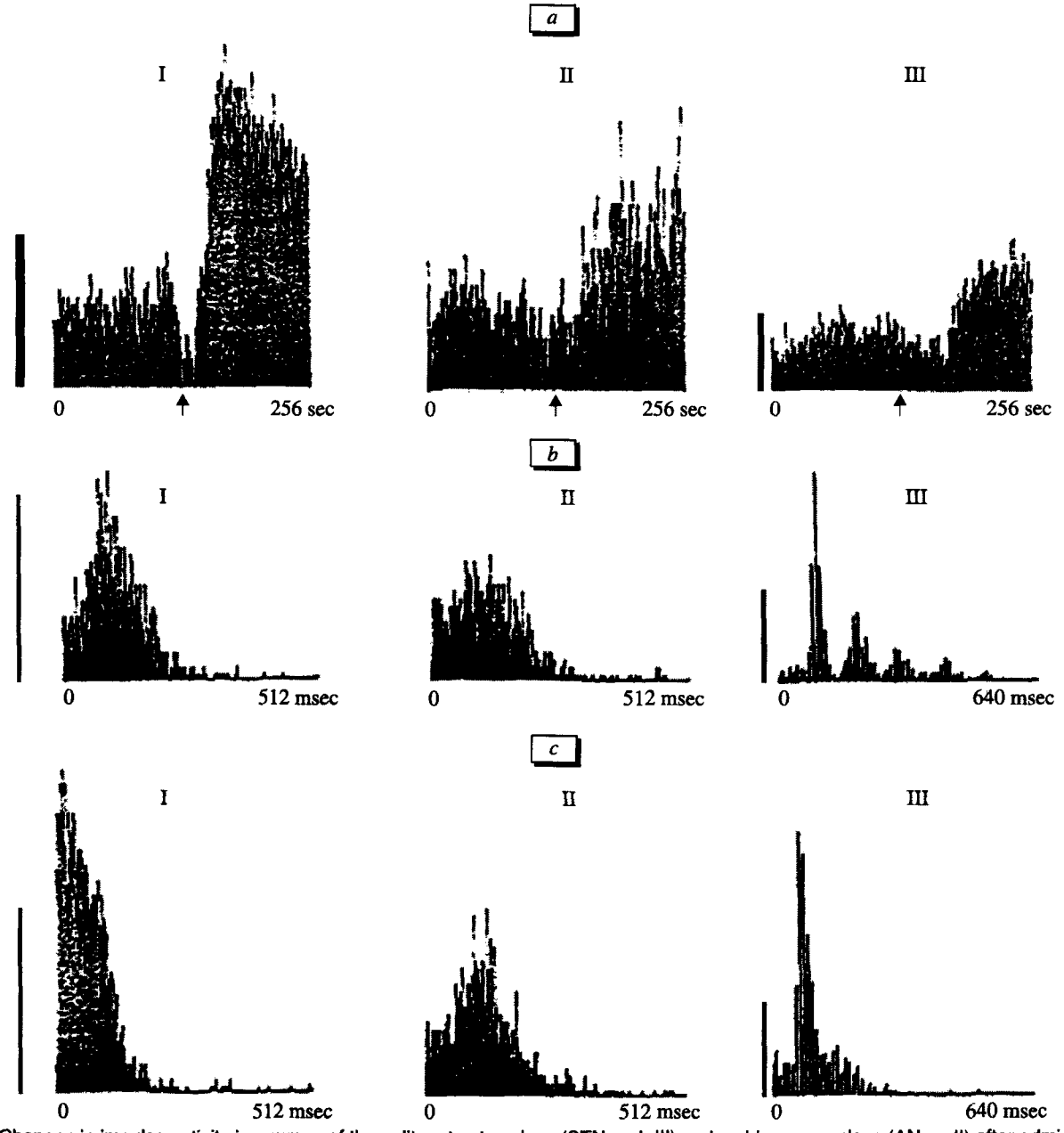

Fig. 1. Changes in impulse activity in neurons of the solitary tract nucleus (STN, *a*, I, III) and ambiguous nucleus (AN, *a*, II) after administration of penicillin. Histogram of the mean current rate (*a*). Arrow: application of penicillin. Calibration: abscissa, 5 sec; ordinate, 20 Hz. Histograms for the distribution of interspike intervals in neurons of STN and AN presented in Fig. 1, *a*: before (*b*) and after administration of penicillin (*c*). Calibration: abscissa, length of interval; ordinate, 100 intervals.

RESULTS

Of 60 examined nonrespiratory neurons the firing rate in response to microinjection of penicillin increased in neurons 45 neurons, decreased in 9 neurons, and remained unchanged in 6 neurons.

The firing rate of 33 neurons in the dorsal (STN) and ventral (AN) groups of the respiratory center increased from 13.42±1.13 to 17.55±1.61 Hz ($p<0.01$) after application of penicillin. The study of histograms for the mean current rate showed that firing rate increased in 24 neurons (Fig. 1, *a*), remained unchanged in 4 neurons, and decreased in 5 neurons (in 2 of these neurons this effect was short-lasting). Impulse activity of a nonrespiratory neuron (STN) progressively decreased and gained a burst pattern (Fig. 2, *a*). Application of physiological saline into STN and AN ($n=10$) had no effect on the firing rate of neurons (13.30±1.18 and 14.20±1.39 Hz, Fig. 2, *b*).

Changes were observed in the distribution of interspike intervals. The mode of the histogram for a STN neuron was shifted towards short interspike intervals. The unimodal distribution was transformed into the exponential distribution (Fig. 1, *b*, I; *c*, I). The amplitude of the histogram peak increased in STN and AN neurons (Fig. 1, *b*, II; *c*, II). The multimodal distribution of interspike intervals for another STN neuron was transformed into unimodal distribution (Fig. 1, *b*, III; *c*, III).

Penicillin application increased the firing rate of 27 neurons in GN from 9.63±1.24 to 12.11±1.59 Hz ($p<0.05$).

The study of histograms for the mean current rate revealed an increase in the firing rate of most neurons ($n=21$) after application of the convulsant (Fig. 3, *a*, I, II; *c*, I, II; *e*, I). The firing rate of several neurons increased over the first minute after application of penicillin (Fig. 3, *e*, I). Five neurons in the lateral reticular formation exhibited high-frequency firing activity (Fig. 3, *a*, II). Published data show [13] that this region includes functionally labile reticular cells capable of transforming tonic activity into burst pattern or modulated activity during induction of protective respiratory mechanisms [1] or electrical stimulation [9]. The firing rate decreased in 4 neurons (Fig. 3, *e*, II; *f*, I, II), but remained unchanged in 2 neurons. Application of physiological saline (control, $n=10$) had no effect on the mean firing rate of GN neurons (9.60±2.03 and 9.80±1.98 Hz, Fig. 3, *g*).

We observed changes in the shape of histograms for the distribution of interspike intervals in GC neu-

TABLE 1. Impulse Activity of Neuronal Pairs under Normal Conditions and after Microinjection of Penicillin

Experimental conditions, No.		Localization	Mean current rate		Peak maximum	Maximum function	Relationship
			neuron 1	neuron 2			
Normal	5	STN, STN	6.43	1.16	-2350	1.53	Common excitatory source
	6	GN, AN	16.07	5.24	400	1.18	Insignificant CC
	8	STN, AN	3.4	0.66	130	2.88	1-2 excitatory
	9	AN, STN	5.19	3.45	-280	1.73	2-1 excitatory
	12	STN, GN	1.68	0.32	-2450	2.23	No CC were found
	13	GN, AN	6.28	1.43	-800	2.77	Insignificant CC
	15	AN, AN	8.02	1.83	10	1.65	No CC were found
	19	GN, GN	1.38	7.74	460	2.01	No CC were found
	20	GN, STN	0.4	1.26	-800	2.8	Insignificant CC
After microinjection	1	STN, AN	11.38	2.41	230	1.47	1-2 excitatory
	2	AN, GN	12.28	1.78	280	1.46	1-2 excitatory
	3	STN, STN	26.04	1.78	-30	2	CC was related to AC 1
	4	ST N, STN	6.34	1.16	490	2.55	Common excitatory source
	7	GN, GN	9.3	1.09	98	2.81	Insignificant CC
	10	GN, STN	2.93	0.32	2100	2.53	Common excitatory source
	11	AN, STN	2.93	1.88	1500	1.84	1-2 excitatory
	14	STN, GN	7.67	0.32	-250	2.05	Common excitatory source
	16	AN, GN	5.02	1.83	-110	2.17	2-1 excitatory
	17	AN, ДЯ	8.92	5.62	10	1.98	1-2 excitatory
	18	STN, STN	1.95	5.5	-240	1.89	No CC were found

Note. CC, cross-correlation; AC, autocorrelation. *1-2*: influence of neuron 1 on neuron 2. *2-1*: influence of neuron 2 on neuron 1.

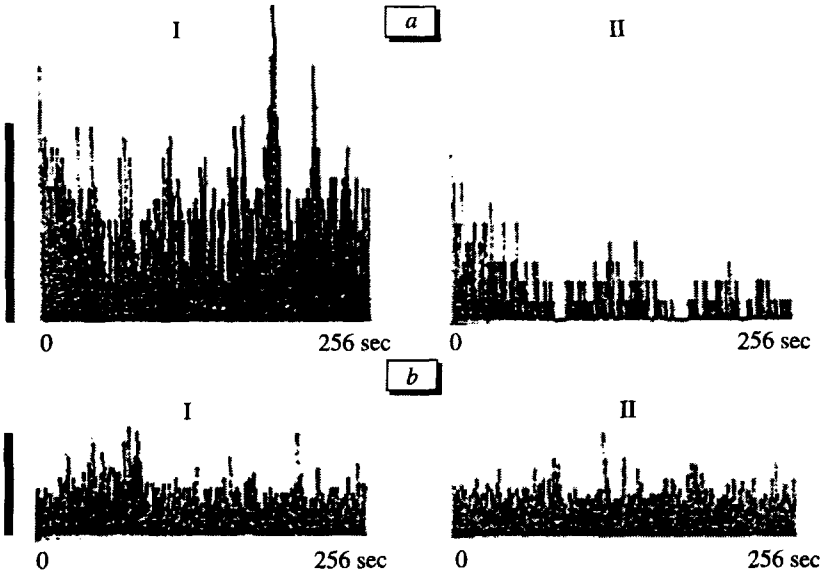

Fig. 2. Histograms for the current mean rate of neurons in STN (*a*) and AN (*b*). Before treatment (*a*, I; *b*, I); after application of penicillin (*a*, II); after administration of physiological saline (*b*, II). Calibration: abscissa, 5 sec; ordinate, 20 Hz.

rons. Histogram peaks became sharper with the increasing firing rate. The mode of histograms was shifted toward short intervals (Fig. 3, *b*, I-II). The unimodal distribution of interspike intervals was transformed into the exponential distribution (Fig. 3, *d*, I, II). The distribution of interspike intervals in several neurons remained unchanged after microinjection of penicillin.

Impulse activity of neuronal pairs was recorded with two microelectrodes under normal conditions (n=9) and after microinjection of penicillin (n=11). A strict cross-correlation was revealed between firing activity of two neuronal pairs under normal conditions, which reflected the excitatory effect of one of these neurons (Table 1). One neuronal pair had a common excitatory source. In three cases no correlations were found, other pairs were characterized by insignificant cross-correlations. The inhibitory effect was studied by typical dips in the histogram. It did not exclude the possibility of a common excitatory source. Hyperactivation of structures in the medulla oblongata after penicillin administration was accompanied by an increase in the degree of synchronization between neurons (Table 1). A significant cross-correlation was revealed in 6 pairs, which reflected the excitatory relationship between neurons. Cross-correlation in 1 neuronal pair was related to autocorrelation of 1 of the neurons. Nonrespiratory neurons modulated the activity of respiratory neurons (experiments 1 and 16). Our results are consistent with published data that respiratory neurons gain tonic firing activity with changes in the pattern of nonrespiratory reticular neurons during severe respiratory arrhythmias in cats and rabbits [12]. A common excitatory source was revealed in 3 neuronal pairs. We observed common inhibition and reciprocal or common excitation. In other experiments, cross-correlations were statistically insignificant (1 pair) or absent (1 pair).

Activity of nonrespiratory neurons in STN, AN, and GN changed over the first minutes after the impairment of inhibitory synaptic mechanisms. Our results are consistent with published data that nonrespiratory neurons exhibit more rapid and significant changes in firing activity during electrical stimulation compared to respiratory neurons [4,5]. Moreover, nonrespiratory neurons play a more important role in the expiration reflex compared to respiratory neurons [1]. Nonrespiratory neurons in the respiratory center more readily react to treatment with agonists of inhibitory synaptic transmission than respiratory neurons [6]. We revealed an increase in the firing rate of most neurons. Several neurons whose firing rate was reduced after application of penicillin probably acted as inhibitory interneurons.

The peak and mode of histograms for the distribution of interspike intervals were shifted toward shorter intervals. The type of distribution sometimes underwent changes (multimodal-unimodal, unimodal-exponential). Published data show that reticular neurons can transform their tonic activity into modulated respiratory activity under the influence of various factors on medullary structures [5,9]. Long-term tonic transformations confirm the notions that reticular neurons are complex and labile components of the respiratory center [5]. These data reflect the possibility of plastic reconstructions.

Hyperactivation of structures was accompanied by an increase in the degree of synchronization between firing activity of reticular neurons. Most relationships had mono- and polysynaptic excitatory nature and reflected the appearance of new functional relationships between neurons of the respiratory net-

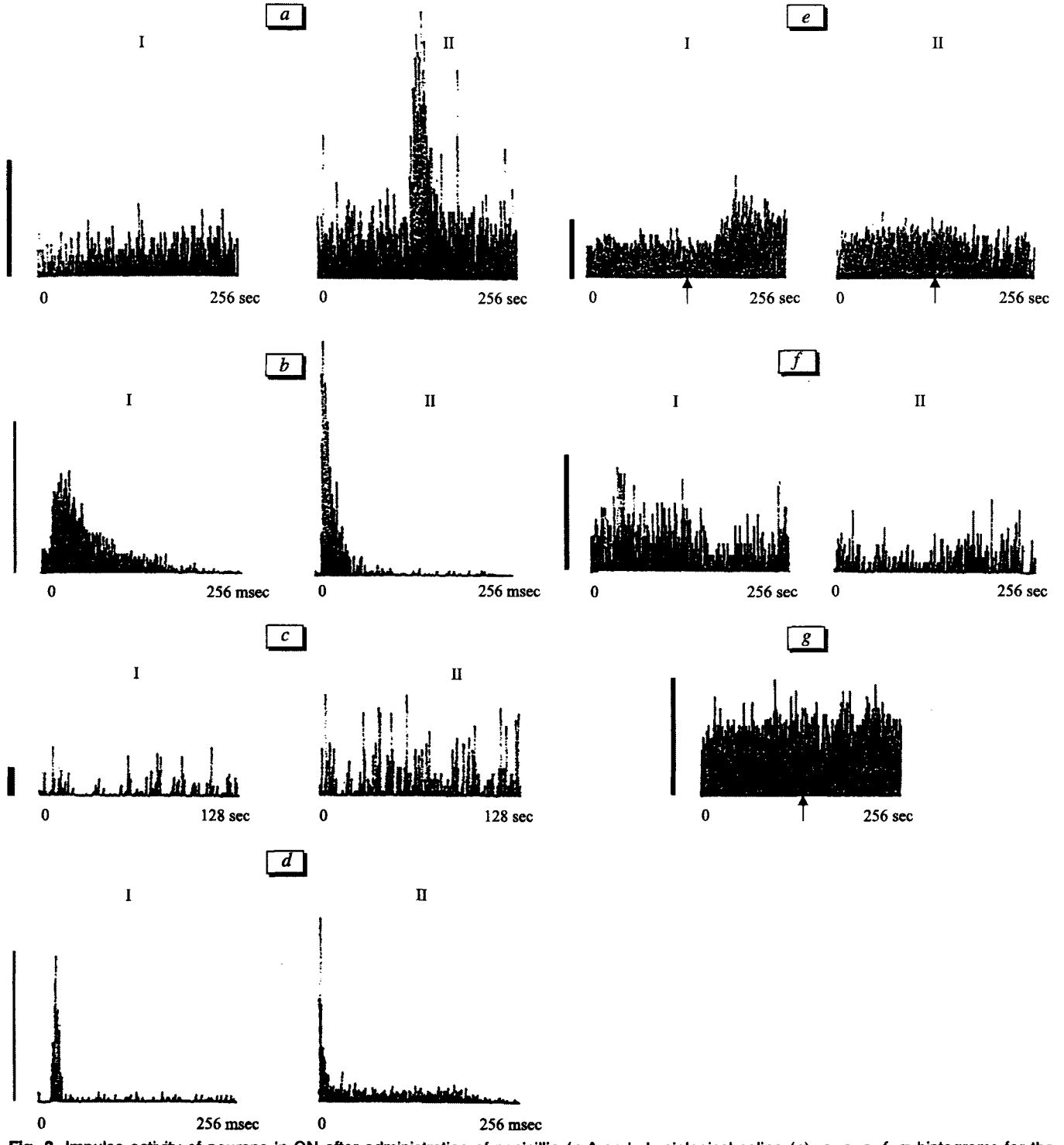

Fig. 3. Impulse activity of neurons in GN after administration of penicillin (*a-f*) and physiological saline (*g*). *a*, *c*, *e*, *f*, *g*: histograms for the current mean rate; on *a*, *c*, and *f*: I - before and II - after convulsant administration; arrows on *e* and *g* show moment of application. Calibration: abscissa, 5 sec; ordinate, 20 Hz. *b*, *d*: histograms for the distribution of interspike intervals in neurons of GN presented in Fig. 3, *a*, *c* before (I) and after administration of penicillin (II). Calibration: abscissa, length of interval; ordinate, 100 intervals.

work. Previous studies revealed interaction between structures of the dorsal respiratory group and nuclei of the reticular formation [14]. The medial reticular formation plays an integrative role [9] and modulates excitability of respiratory motoneurons [7]. The intermedial reticular formation connecting with AN and motor nuclei of the facial and sublingual nerves determines neurogenesis of gasping [8]. These data indicate that reticular neurons of the respiratory center have integrative, coordinating, and synchronizing functions.

Disturbances in the respiratory rhythm [2] induced by blockade of inhibitory synaptic transmission

result from changes in functional activity of the neuronal respiratory network [10], which consists of respiratory and nonrespiratory neurons.

Our results indicate that nonrespiratory reticular neurons constitute the most labile functional pool of the respiratory center. They interact with other neurons and play an important role in the regulation of respiratory rhythmogenesis under normal and pathological conditions.

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