# Amygdalofugal Modulation of Hering—Breuer Inspiration-Inhibiting Reflex

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Acute experiments on rats showed that the central nucleus of the amygdaloid complex modulates realization of the Hering-Breuer inspiration-inhibiting reflex. It is hypothesized that amygdalobulbar interrelations are realized via modulation of respiratory reflexes by the amygdala. This interaction is mediated by the GABAergic system of the amygdala.

**Key Words:** *central nucleus of the amygdaloid complex; Hering-Breuer inspiration-inhibiting reflex*

Stimulation of the central nucleus of the amygdaloid complex (AC) can produce both stimulatory and inhibitory effects on respiration, which attests to nonspecificity of this reaction [1-3]. According to some reports, AC and the nucleus of the solitary tract, a component of the respiratory center, are connected via direct amygdalofugal projections, in particular, via GABAergic projections [1,6,7,11,13,14]. We hypothesized that AC regulates the respiratory function by modulating respiratory reflexes realized via neurons of the nucleus of the solitary tract. The Hering—Breuer inspiration-inhibiting reflex (HBR) reproduced by stimulation of the vagus central segment in vagotomized animals was used as the experimental model.

## MATERIALS AND METHODS

Experiments were carried out on albino male and female rats ($n$=20) weighing 250-300 g. The rats were anesthetized with urethane (1.5 g/kg) or nembutal (50 mg/kg). Both vagus nerves were transected at the level of the lower third of the trachea. HBR was triggered by unilateral stimulation of the central end of the vagus nerve with bipolar stainless steel electrodes (50 Hz, 60-400 μA). The threshold current was determined and amplified by 1.5, 2, 2.5, 3, and 3.5 times until respiratory arrest for 15 sec from the start of stimulation. The results were standardized by calculating the ratio of acting to threshold current strengths. The nerve was stimulated before and during AC stimulation and after GABA microinjections into AC. The interval between stimulation series was not less than 3-5 min.

Electric current (50 Hz, 150-250 μA) was applied to AC via a gold electrode (20 μ tip diameter). GABA (0.2 μl) was injected with an MS-1 microsyringe via a glass cannula (20-30 μ tip diameter). GABA was *ex tempore* dissolved in cerebrospinal fluid (CSF) to a concentration of $10^{-6}$ M. Control animals received the same volume of CSF. The microelectrode and cannula were stereotactically inserted through trepanation openings [15].

The duration of expiration before vagus stimulation ($T_{Einit}$) and the time from the start of stimulation to the first inspiration ($T_{Einfl}$) were determined by spirography. The standardized expiration time ($T_{Enorm}$) was calculated as the ratio of $T_{Einfl}$ to $T_{Einit}$ [5,9].

The data were processed statistically using Microsoft Excel 7.0 and Statistica 5.5A programs. The means and standard deviations were calculated, significance of differences was estimated by Student's $t$ test for dependent and independent variables, as well as by the correlation test. Differences were considered significant at $p \leq 0.05$.

## RESULTS

Stimulation of the vagus nerve triggered HBR, which manifested in the appearance of an inspiratory pause

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after delivery of the stimulating pulse ($T_{Einfl}$). Threshold values of stimulating current varied from 60 to 100 μA. Increasing current strength by 1.5, 2, 2.5, and 3 times lengthened $T_{Einfl}$ and, correspondingly, $T_{Enorm}$; the dependence of $T_{Enorm}$ on the current parameters was almost linear. Increasing current strength by more than 3 times sharply lengthened $T_{Enorm}$ (Fig. 1).

HBR triggered against the background of AC stimulation decreased the time from the start of stimulation to the first inspiration and, consequently, lengthened $T_{Enorm}$. The effect of AC stimulation on HBR directly depended on the strength of stimulating current (Fig. 1). For instance, when the stimulus applied to the vagus nerve was increased 2-fold (compared to the threshold value) stimulation of AC reduced $T_{Enorm}$ more than 2-fold compared to series without AC stimulation.

We previously demonstrated that activation of the GABAergic system of the central AC nucleus affected the formation of the respiratory pattern, which manifested in increased in respiration depth and frequency, the maximum increase was observed by the 10th minute [6]. Since GABA is the main transmitter in AC [8,14], we examined HBR 10 min after GABA injection into AC. Stimulation of the vagus nerve against the background of activation of the GABAergic system reduced the time between stimulation and the first inspiration and decreased $T_{Enorm}$ for stimuli at least 2.5-fold surpassing the threshold stimulus (Fig. 2).

Our findings agree with previous data on the modulatory effect of AC on the reflexes realized via the vagosolitary complex. It was shown that descending projections of AC regulate activity of preganglionic vagus neurons innervating the peripheral regions of

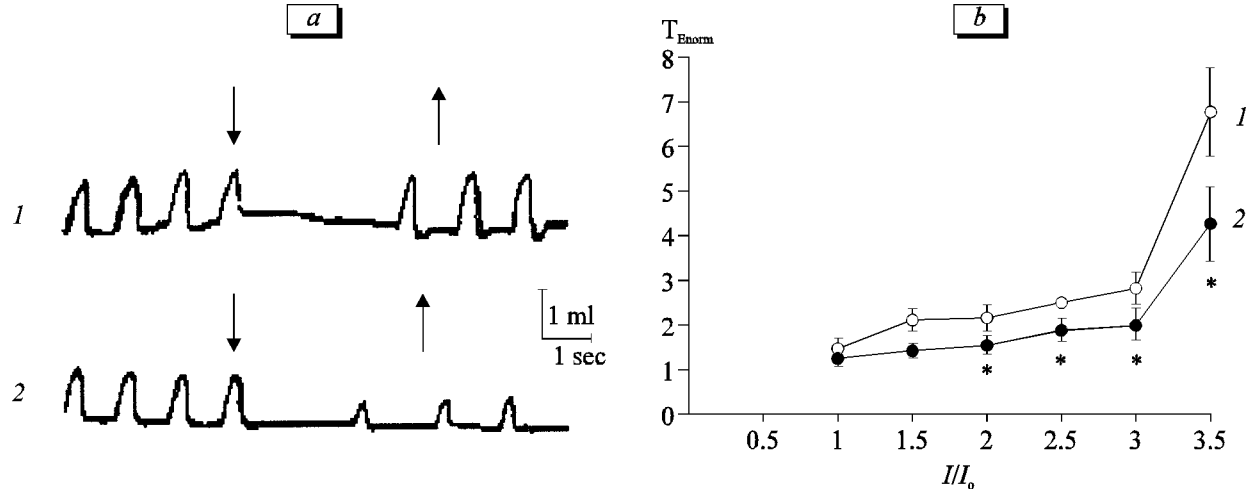


**Fig. 1.** Inspiration-inhibiting Hering—Breuer reflex against the background of stimulation of the central nucleus of the amygdaloid complex (AC): spirograms (*a*) and expiration time ($T_{Enorm}$, *b*) before (*1*) and during AC stimulation (*2*). **p*<0.05 compared to the levels before treatment. Here and in Fig. 2: $I/I_o$ — the ratio of acting to threshold current strengths. Downward and upward arrows show the start and end of stimulation, respectively.

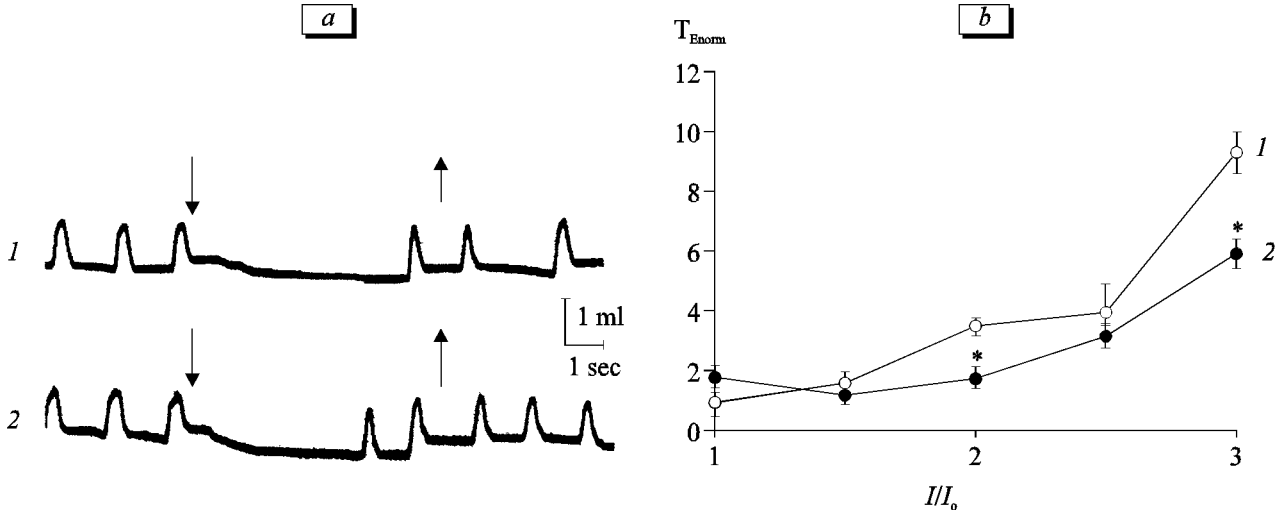


**Fig. 2.** Inspiration-inhibiting Hering—Breuer reflex 10 min after microinjections of artificial cerebrospinal fluid (*1*) and GABA (*2*) into the central nucleus of the amygdaloid complex: spirograms (*a*) and changes in expiration duration ($T_{Enorm}$, *b*). **p*<0.05 compared to cerebrospinal fluid.

the respiratory system [10]. Moreover, destruction of AC alters the pressor response induced by vagal stimulation [12]. Recent studies demonstrated a modulatory effect of AC on the vago-vagal reflex in the stomach [4]. Thus, the observed effect of AC on HBR presents a particular case of modulatory action of AC structures on autonomic reflexes realized via neurons of the vagosolitary complex. A possible mechanisms of HBR modulation include changes in specific neuromediatory projections (including GABAergic) from AC to the nucleus of the solitary tract, which, in turn, reduce the sensitivity of solitary nucleus neurons to afferent signals from stretch receptors in the lungs.

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