

---

## METHODS

---

# Comparison of the Rate of Hydrogen Release from Brain Tissue with Parameters of Rat External Respiration

I. P. Levshina and N. N. Shuikin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 1, pp. 118-120, January, 1996  
Original article submitted December 23, 1994

---

Comparison of the rate of hydrogen release from the brain tissue with the parameters of external respiration is performed in rats with free behavior. Regression of the hydrogen release rate is found at the parameter of external respiration. The scatter of results on the recorded hydrogen release rate obtained by measuring the local brain blood flow may be cut in half by monitoring the external respiration characteristics.

---

**Key Words:** *hydrogen clearance; parameters of external respiration*

---

For many years the local blood flow has been monitored from the hydrogen clearance in studies of the blood supply of different tissues in laboratory animals [8]. It has been repeatedly noted that the time of hydrogen release varies markedly in the same brain structures in different rats and in various functional states, especially in narcotic and natural sleep. The background rate of the local brain blood flow (rLBF) determined by hydrogen clearance may differ in three or more times during wakefulness [3,4,6]. As a rule, such a variability of the background values of rLBF is not discussed. However, according to the same scientists, the variation of rLBF in the case of sensory stimulations or electrostimulation of brain structures is markedly lesser, constituting tens of percentage points of the background values [4,7].

Since the method of hydrogen clearance is widely used to measure the volume rate of LBF, it is

necessary to know how heavily this method depends on the changes of other physiological factors, which may account for such large fluctuations of the rLBF background values.

External respiration is a physiological process which reflects the functional state of the nervous system and is important in gas exchange. Some authorities note that the brain blood flow may be increased both in hypoventilation, which results in carbon dioxide accretion in the blood, and in hyperventilation induced by hypoxic hypoxia [1,9]. The present investigation demonstrates the expediency of monitoring external respiration when measuring brain circulation using the method of hydrogen clearance.

## MATERIALS AND METHODS

The experiment was carried out on 12 rats with electrodes chronically implanted into the hippocampus and motor cortex. The active electrode was made of platinum wire 0.3 mm in diameter insulated with Viniflex lacquer along its entire length except for the tip. The indifferent electrode was a plate of chlorinated

---

Group of Experimental Pathology and Therapy of Higher Nervous Activity, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow (Presented by the late S. E. Severin, Member of the Russian Academy of Medical Sciences)

silver with an interlayer containing NaCl isotonic solution mounted on the animal's hind paw.

The time  $T$  during which the gas concentration was lowered by 50% was determined according to the dynamics of hydrogen release recorded polarographically. Hydrogen inhalation was performed in a small closed chamber with a ventilation installation which quickly removed the hydrogen-air mixture from the chamber.

Respiratory movements of the rat were recorded with an H-337 automatic recorder using a carbon sensor for 6 min. Periods of total rest of the animal were analyzed. The minimal and maximal levels of the sensor's tension were noted and the midline was drawn between these points (Fig. 1). The portion of time ( $\Sigma \tau_i/t$ ) during which the expansion of the thorax exceeded the noted mean level was calculated according to the midline. The mean ( $\Sigma a_i t_i/t$ ) and maximal ( $A$ ) signal amplitude was determined according to the same record. The external respiration was characterized by the value  $K=(\Sigma \tau_i/t) \times (\Sigma a_i t_i/At)$ .

## RESULTS

Records of respiratory movements of rats in different functional states are shown in Fig. 2, a downward line corresponding to an inspiration. The tracing on Fig. 2, a, shows a deep, uniform respiration with an equal duration of inspiration and expiration and, accordingly, with  $K=0.5$ . Figure 2, b gives an example of superficial respiration with the thorax collapsed with the occasional inspiration. The calculated  $K$  value in this case approximates zero. Active wakefulness (Fig. 2, c) is accompanied by deep respiration with a short phase of expanded thorax ( $K=0.27$ ). And, finally, shallow respiration against the background of an expanded thorax is noted at rest ( $K=0.39$ ) (Fig. 2, d). Expansion of the thorax signifies an intensified pulmonary blood flow and increased diffusion capacity of the lungs [2,5]. Therefore, an acceleration of the gas release rate may

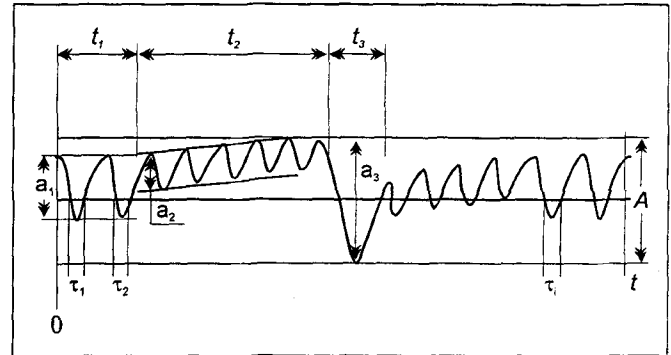


Fig. 1. Analysis of a respiration record. Downward lines correspond to inhalations.  $\tau_i$  ( $i=1,2,3, \dots$ ) shows the regions where the expansion of the thorax exceeds the mean level;  $t_i$  indicates the regions with amplitude  $a_i$  ( $j=1,2,3, \dots$ ).  $A$  is the maximum amplitude of the respiratory movement;  $t$  is the duration of the record.

be expected in the phase of expanded thorax. The first cofactor in the formula for  $K$  assesses the total duration of respiration with expanded thorax. The second cofactor corrects for the mean depth of respiration.

A reliable regression for the motor cortex ( $p<0.001$ ) and for the hippocampus ( $p<0.01$ ) (Fig. 3, a, b) was detected by comparing the calculated  $K$  values with the corresponding measured rates of hydrogen release from the brain tissue ( $1/T$ ). Depending on the respiratory pattern of a rat, the points are situated nearer to or farther from the origin of the coordinates, i.e., the regression is noted both for the individual animal and for the whole sampling. No reliable regression relationship was found between the hydrogen release rate and either of the most frequently measured parameters (frequency and amplitude) of external respiration or for the combination of them. A tendency toward a stronger regression of the selected indexes is found for the motor cortex as compared to the hippocampus (Fig. 3), but the difference between the regression coefficients is insignificant. The scatter of the values of the hydrogen release rate clearly diminishes when the regression is taken into account. Calculation shows a 2-fold decrease of the variance.

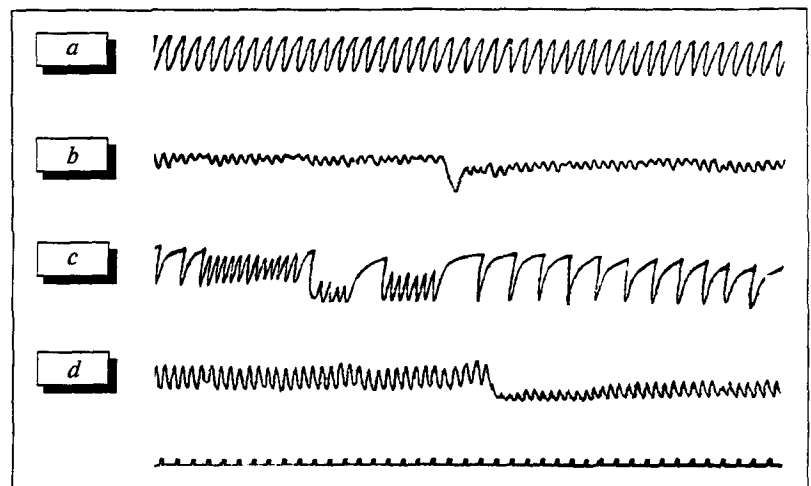


Fig. 2. Tracings of respiratory movements of rats in different functional states, namely, deep narcosis (a), awakening (b), active alertness (c), and alertness (d). Time mark 1 sec.

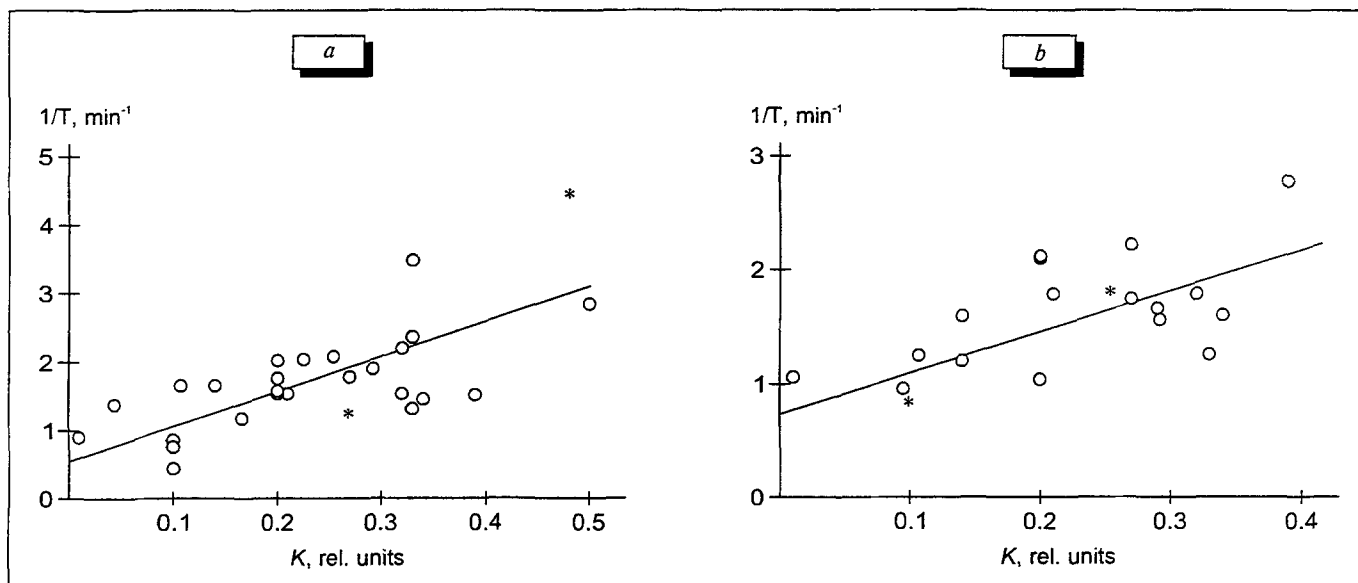


Fig. 3. Comparison of the rate of hydrogen release ( $1/T$ ) from the motor cortex (a) and from the hippocampus (b) of the rat brain with the external respiration coefficient ( $K$ ). Examples of these correlations for an individual rat during wakefulness and in narcotic sleep are indicated by asterisks.

The findings suggest that the scatter of the background values of the rate of hydrogen release from the brain tissue may be due to the specific pattern of respiration. Combined recordings of the brain blood flow by the hydrogen clearance method and of respiration by the carbon sensor permit the investigation of alert, free animals. Assessment of external respiration using the method described allows for a more comprehensive analysis of the results obtained in studies of the local brain circulation.

## REFERENCES

1. E. S. Gabrielyan, *Some Aspects of the Physiology and Pharmacology of the Brain Circulation* [in Russian], Erevan (1976), pp. 20-29.
2. D. P. Dvoretiskii, in: *Physiology of the Circulation. Physiology of the Vascular System* [in Russian], Leningrad (1984), pp. 281-305.
3. M. I. Devdariani, V. G. Meladze, N. P. Mitagvariya, et al., *Fiziol. Zh. SSSR.*, **75**, № 11, 1602-1607 (1989).
4. I. T. Demchenko, *Blood Supply of the Wakeful Brain* [in Russian], Leningrad (1983).
5. E. M. Marshak, *Regulation of Human Respiration* [in Russian], Moscow (1961).
6. Yu. N. Morgalev and I. T. Demchenko, *Fiziol. Zh. SSSR*, **65**, № 7, 985-990 (1979).
7. Yu. E. Moskalenko, T. V. Zhuravin, T. V. Ryasina, et al., *Ibid.*, **78**, № 11, 87-97 (1992).
8. K. Aukland, F. Bruce, M. Bower, et al., *Circ. Res.*, **14**, 164-187 (1964).
9. N. V. Santiago, E. Guerra, J. A. Neubauer, and N. H. Edelman, *J. Clin. Invest.*, **73**, 497-506 (1984).