# Peculiarities of External Respiration as a Marker of Activity of Cell Respiratory Enzymes in Rat Brain

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Rats with catatonic freezing are characterized by low motor and explorative activity in the open field test and specific pattern of external respiration, so-called strenuous respiration, which is accompanied by a higher activity of the respiratory enzymes succinate dehydrogenase and NADH dehydrogenase in the hippocampus and motor cortex. No dependencies on the brain structure and animal age are noted.

**Key Words:** *succinate dehydrogenase and NADH dehydrogenase activity; external respiration; behavior; freezing (catatonic states)*

Animal behavior is usually characterized by a number of specific actions in response to external stimuli. Frequent repetition of experimental situation leads to habituation and animal's reactions become automatic. However, under conditions of free behavior animals do not necessary respond in the same way to occasionally repeated situation; they either escape or freeze. We have no information explaining the choice of certain behavioral strategy under the same (from experimentator's point of view) experimental conditions. In the present study we compared the energetic characteristics of metabolism in the brain tissue against the background of a specific pattern of external respiration and behavioral parameters in the open field test in conscious rats at rest and in catatonic freezing.

## MATERIALS AND METHODS

Experiments were carried out on two groups of male Wistar rats of different age. The rats were kept under vivarium conditions in standard cages (5 animals per cage). The animals were fed briquetted chow. Group 1 ($n$=8) consisted of 14-15-month-old rats weighing 350-460 g, while group 2 comprised 18 body weight-matched 4-5-month-old rats. Each animal was tested in an open field with a diameter of 1.3 m divided into 32 squares, 4 central squares were designated as the center. A rat was placed to the center and the latency of leaving the central zone was determined. The ambulation (horizontal motor activity), rearing (explorative activity), and the number of boluses and urination (the state of autonomic nervous activity) were evaluated.

External respiration was monitored using a carbon chest-band transducer and an N-327 recorder. A rat was placed into a small chamber with an open cover so that it could look out or lean out of the chamber, but could not climb out of it. The rats were left in the chamber 5 min before and 5 min during recording. The parameters of external respiration in group 1 rats were recorded over 15 days at 3-5-day intervals, while in group 2 the observation period was extended to 45 days. Previous experiments [4] revealed a specific pattern of respiration, strenuous respiration (SR), in the majority of rats (more than 50%). Such respiration was associated with freezing [2], which probably indicated the catatonic state. Strenuous respiration occurred either spontaneously or was induced by pressing rat's neck with a blunt object. In some animals SR could not be induced

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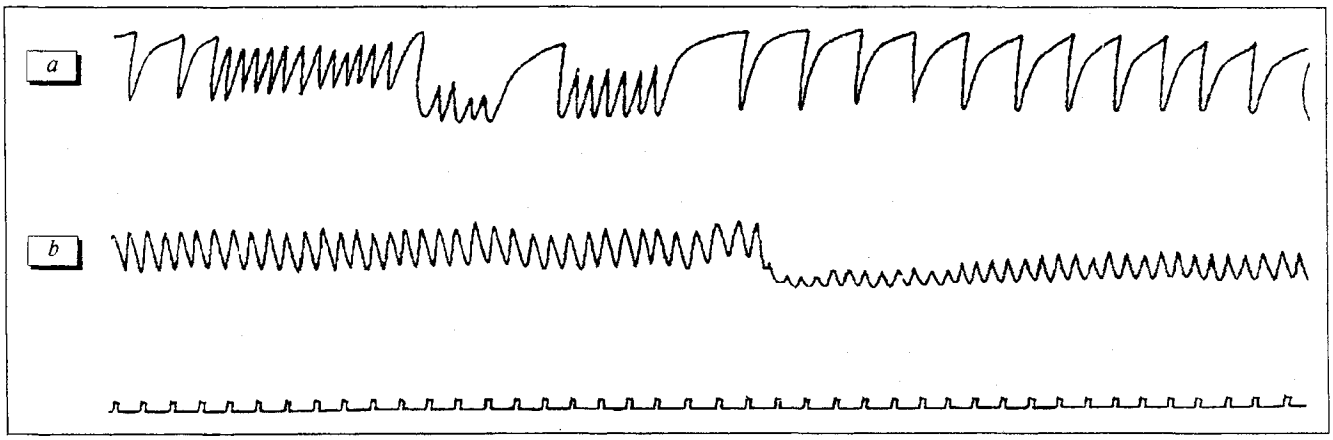


**Fig. 1.** Variants of external respiration in rats. Strenuous respiration during freezing (a), respiration of conscious rats at rest (*b*). Shift of the line down corresponds to inspiration. Time mark: 1 sec.

by this way. The rats selected on the basis of preliminary testing were driven to SR and decapitated after a 5-min period of SR. In group 1, succinate dehydrogenase (SDH) and NADH-dehydrogenase (NADH-DH) activities were measured in 4 animals with SR and in 4 rats without SR, and in group 2 in 6 and 8 animals, respectively.

The activities of respiratory enzymes in the hippocampus and motor cortex were assessed histochemically as described elsewhere [5] using nitro blue tetrazolium. Enzyme activity was measured as the amount of formazan (in mol) formed by 1 mol protein nitrogen for 1 min at 37°C and expressed in mU formazan per min.

Experiments with group 1 animals were completed in the beginning of December, with group 2 animals in the end of February. The significance of differences was evaluated by Student's *t* test.

## RESULTS

Figure 1 shows a typical SR curve during freezing (*a*) and a record of external respiration of conscious rats at rest (*b*). In the animals predisposed to spontaneous SR, this respiration pattern is usually observed in all records or it can be readily induced by pressing rat's neck with a blunt object. However, in some animals with SR observed in preliminary experiments, this respiration pattern could not be induced immediately before decapitation. Thus, the activity of respiratory enzymes was studied in animals with different patterns of external respiration both before decapitation and during preliminary tests, which allowed the identification of predominant pattern in each animal.

Activity of SDH and NADH-DH in the motor cortex of rats from both groups is presented in Table 1. Both SDH and NADH-DH activities depend on the type of external respiration immediately before decapitation. It should be noted that the subgroups with SR included rats who exhibited SR in their last record. In subgroups without SR, the respiration pattern corresponded to that of conscious rats at rest. The activity of respiratory enzymes in rats predisposed to SR but not exhibiting such respiration before decapitation was close to the upper limit of this parameter in rats without SR (Table 1). In rats who exhibited no spontaneous SR during preliminary tests but had SR in the last record, SDH and NADH-DH activities were close to the lower limit of this parameter in the rats with SR. Thus, in the animals with atypical pattern of external respiration (the presence

**TABLE 1**. Activity of Respiratory Enzymes in Motor Cortex of Rats ($X \pm S$)

| Group (age, months) | Respiration pattern | Number of animals | Enzyme activity, arb. units | |
|---|---|---|---|---|
| | | | SDH | NADH-DH |
| 1st (14-15) | With SR | 4 | 126.5±10.2 (118.3—139.5) | 4.83±1.19 (3.53—5.67) |
| | Without SR | 4 | 99.7±6.6 (98.3—108.6)* | 3.02±0.17 (2.80—3.20)*** |
| 2nd (4-5) | With SR | 6 | 125.4±4.24 (120.2—130.8) | 4.52±0.57 (3.73—5.13) |
| | Without SR | 8 | 114.2±5.6 (106.2—120.0)** | 2.79±0.19 (2.40—2.97)** |

**Note.** Here and Table 2: minimal and maximum values are shown in parentheses; *$p<0.01$, **$p<0.001$, ***$p<0.03$ compared with rats with SR.

**TABLE 2.** Motor (Ambulation) and Explorative (Rearing) Activity in the Open Field Test ($X \pm S$)

| Respiration pattern | Behavioral parameters | |
|---|---|---|
| | ambulation | rearing |
| With SR ($n$=9) | 13.8±8.6 (4—27) | 3.1±1.8 (0—13) |
| Without SR ($n$=17) | 67.2±61.5 (8—243)** | 12.2±3.5 (0—56)*** |

or absence of SR), brain activities of the studied respiratory enzymes were shifted toward the opposite subgroup, but their ranges did not overlap. The maximum SDH and NADH-DH activities were noted in rats with readily inducible (without visible causes) and long-term periods of SR. We present no data on the activity of the studied respiratory enzymes in the hippocampus, because it does not differ significantly from that in the motor cortex and retains the same relationships.

Considerable differences in SDH activity were found between rats with the same respiration pattern (without SR) but from different groups: in conformity with our previous data, younger rats exhibited higher activity of SDH [3].

Behavioral characteristics (open field test) were the same in young and mature rats, therefore, Table 2 summarizes the data on these two groups. The rats were assigned to different groups (with or without SR) on the basis of their respiration records made on the day of the open field test. Thus, these groups differ in the number of animals from those formed before decapitation due to changes in the respiration pattern. Ambulation and rearing differed significantly in animals with and without SR (Table 2). No differences in the number of boluses and urinations, groomings, and visits to the central zone, as well as in the duration of the initial stay in the central zone were noted.

A correlation between such apparently dissimilar parameters as activity in the open field test, pattern of respiration and cell respiratory enzyme activity confirm the existence of a complex multilevel hierarchy of processes responsible for the strategy of animal's behavior in a given situation. It has been previously found that hydrogen clearance (and, consequently, the rate of local circulation) in the hippocampus and motor cortex of rats with SR is higher than in conscious rats at rest. This probably reflects the existence of a tension in the organism, so-called energetic mobilization, including both the emotional component and readiness to act [1]. The presence of SR fragments in the records of external respiration in rats can serve as a marker of predisposition to catatonic freezing. This state is characterized by activation of cell respiratory enzymes, SDH and NADH-DH, in the brain of rats with low motor and explorative activity in the open field test. Thus, the reaction of freezing, i.e., inactivity, is accompanied by strengthening of the physiological (respiration) and biochemical (SDH and NADH-DH activity) systems.

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