Multiparameter Analysis of EEG in Old Wistar Rats after Bilateral Carotid Artery Ligation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 5, pp. 502-506, May, 2009
Original article submitted September 25, 2008

We showed that 302 of 840 EEG parameters in old Wistar rats significantly change 3 h after bilateral carotid artery ligation. All animals (n=7) died 250±47 min after ligation. EEG power spectra had signs of suppression (increased δ-activity, decreased τ-activity, and increased low-frequency α-activity) or activation of CNS (decreased high-frequency α-activity and paroxysmal increase in β-activity). The coherence, synchrony, and entropy of potentials were reduced in the majority of brain regions. This method and scheme of experiments are suitable for the search and study of anti-stroke drugs.

Key Words: *electroencephalogram; brain; ischemia; carotid arteries; Wistar rats*

Ischemic stroke is one of the major causes for disability and death of elderly and senescent people. There are no efficient approaches to the therapy of this disorder. The search for anti-insult drugs requires the development of new experimental methods and reliable criteria of cerebral ischemia.

Ischemic stroke is related to atherosclerotic occlusion of both carotid arteries or occlusion of one of the carotid arteries in combination with stenosis of another artery. Ligation of both carotid arteries for 6-20 min in combination with bloodletting is a widely used model of ischemic stroke in rats [7]. Some investigators in addition to these measures perform vertebral artery occlusion and use hypertensive animals. Thus, most methods suggest complete or partial arrest of cerebral circulation, which is rarely observed in clinical practice. Experiments with severe (total) ischemia of the brain are performed to evaluate the consequences of this disorder from hemodynamic, morphological, and biochemical parameters. Changes in these parameters are observed only during severe ischemia.

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Little is known about electrophysiological markers of cerebral ischemia in animals. The majority of studies were performed at low methodological level. They are devoted to EEG-monitoring in patients during the post-stroke period or after arteriotomy. We hypothesized that multiparameter analysis of EEG from various regions of the brain cortex will provide a sufficient number of reliable criteria for cerebral ischemia caused by bilateral ligation of the carotid arteries in Wistar rats. To simulate the pathological state in humans, experiments were performed on old rats (similarly to the biological age of elderly and old people).

MATERIALS AND METHODS

Experiments were performed on 12 male Wistar rats (Svetlye Gory) weighing more than 300 g (older than 40 weeks). Freely moving animals were maintained in a light-tight and soundproof chamber. Steel needle electrodes were fixed to the skull bones with a plastic. EEG was recorded unipolarly from 24 points of the dorsal brain surface. After Fourier transformation, electric potentials were processed on a computer. The sampling rate was 128 Hz. The analysis epoch was 4 sec. Each sample consisted of 50 epochs. The frequency range (0.03-

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30 Hz) was divided into 22 frequency sub-ranges (Table 1). The spectral power, cross-correlations (synchrony), coherence, spatial randomness (entropy) of biological potentials, and various types of asymmetry were evaluated in each sub-range. Eight hundreds and forty parameters were recorded before and 3 h after bilateral ligation of the common carotid arteries. The arteries were ligated under ether anesthesia. The adjacent nerve trunks and plexus remained intact. Sham-operated rats (n=5) were subjected to all manipulations except for carotid artery ligation. The significance of changes in EEG parameters was estimated by Student's t test.

TABLE 1. EEG Frequency Bands (0.03-30.00 Hz) and Their Correspondence to Standard Frequency Ranges of EEG (Fig. 1)

No.	Hz	Range
1	0.03-1.7	
2	1.95-3.00	δ
3	3.25-4.50	
4	4.75-5.75	
5	6.00-7.25	τ
6	7.50-8.50	
7	8.75-9.75	
8	10.00-11.00	
9	11.25-12.50	α
10	12.75-13.50	
11	13.75-14.75	
12	15.00-16.00	
13	16.25-17.25	
14	17.50-18.50	
15	18.75-20.00	β_1
16	20.25-21.25	
17	21.50-22.50	
18	22.75-23.75	
19	24.00-25.00	
20	25.25-26.50	
21	26.75-28.00	
22	28.25-30.00	β_2

RESULTS

Seven rats died at various periods after bilateral ligation of the common carotid arteries. One rat died 10 min after ligation. EEG was recorded in 6 animals, which survived 3 h after surgery. The lifespan of animals appeared as follows (in increasing order): 3 h 30 min, 3 h 55 min, 4 h 58 min, 5 h 18 min, 5 h 20 min, and 6 h 01 min. The average lifespan was 250±45 min.

Behavioral activity of animals was reduced after surgery (end of anesthesia). Pupillary reflex and corneal reflex were present. However, the majority of rats were characterized by unilateral or bilateral ptosis. Random running and circular movements were observed 2-5 h after artery ligation (shortly before death). The attacks of motor agitation were revealed 30-60 min before death. These rats dashed around the cage, rushed to the wall, and fell in convulsions. After 2-4 paroxysmal attacks, the animals were characterized by reduced locomotor activity and died. Death of 2 rats was accompanied by generalized tonic-clonic convulsions of skeletal muscles.

EEG was recorded 3 h after common carotid artery ligation (no motor agitation). The total power

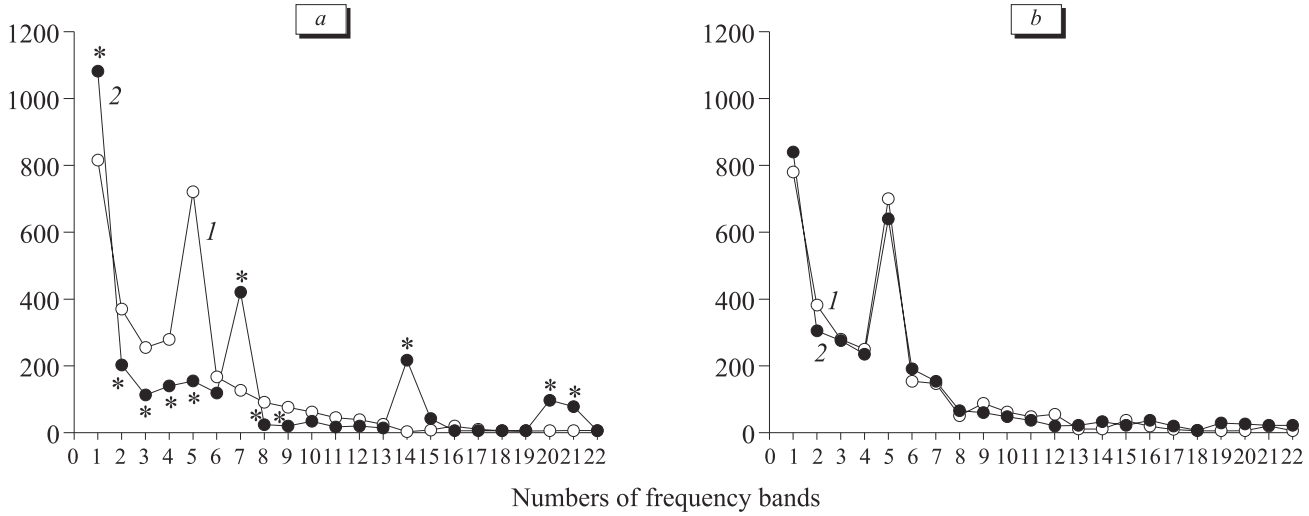

Fig. 1. EEG power spectra in old Wistar rats 3 h after common carotid artery ligation (*a*) and sham operation (*b*). Ordinate, total value of PS in 24 leads (all rats, arbitrary normalized units). Before surgery (control, *1*); 3 h after bilateral carotid artery ligation (*2*). *p<0.01: significant changes in PS compared to the control.

spectrum (PS) of EEG (Fig. 1, *a*) was significantly increased in the range of δ-waves (frequency band No. 1), low-frequency α-waves (No. 7), β_1-waves (No. 14), and β_2-waves (No. 21 and 22). PS of τ-activity (dominant rhythm of EEG in rats) significantly decreased in all sub-ranges (No. 3, 4, and 5). The dominant peak of τ-activity (No. 5) was undetected. PS of frequency ranges did not change in sham-operated rats (Fig. 1, *b*).

Figure 2 shows a typical EEG pattern in one rat, which was recorded bilaterally in the frontal, parietotemporal, and occipital region of the cerebral cortex. A decrease in the amplitude of EEG, reduction of τ-waves with a frequency of less than 7 Hz, and predominance of α- and β-waves were revealed in all leads.

Changes in the coherence of biological potentials of the brain coincided with variations in PS. In all regions of the cortex, the coherence was shown to decrease in the majority of EEG frequency bands. This parameter increased in the bands that were characterized by an increase in PS (No. 7, 14, and 21).

The synchrony and entropy of biological potentials significantly decreased in all regions of the cerebral cortex.

Our results indicate that bilateral carotid artery ligation is followed by a combination of EEG signs for the central inhibition and excitation.

The inhibition of CNS was probably related to inactivation of a considerable number of brain neurons. This conclusion was derived from the increase in the δ-rhythm and low-frequency α-rhythm and decrease in the τ-rhythm.

Published data show that an increase in the number of δ-waves is typical of slow-wave sleep in humans and animals. δ-Activity increases in patients with cerebral ischemia [5], hypertensive rats with carotid artery occlusion [10], and rats with local cerebral ischemia [9].

τ-Wave suppression in all frequency ranges (3.25-7.25 Hz) reflects a decrease in activity of septal-hippocampal-cortical pathways. Previous experiments showed that various types of cerebral ischemia in humans and animals are accompanied by hippocampal neuronal damage (major generator of τ-waves) [1,3,11] and decrease in τ-activity [3, 12]. The hippocampus has a key role in higher nervous functions (*e.g.*, orientation-and-exploratory behavior, attention, memory, learning, *etc*.) [1]. Damage or destruction of the hippocampus is followed by cognitive deficit, memory disorders (in humans) [6], and impairment of learning (in rats) [11]. Impairment of conditioned-reflex activity and memory was found in rats with carotid artery occlusion [8,12].

The decrease in PS of high-frequency α-waves in frequency bands No. 8 and 9 (10.0-12.5 Hz) serves as a criterion of cerebral excitation. α-Rhythm suppression reflects activation of CNS, awakening, and wakefulness.

The paradoxical increase in PS in narrow frequency bands of the α- and β-rhythm (8.75-9.75 and 17.50-18.50 Hz; and 25.25-28.00 Hz) prob-

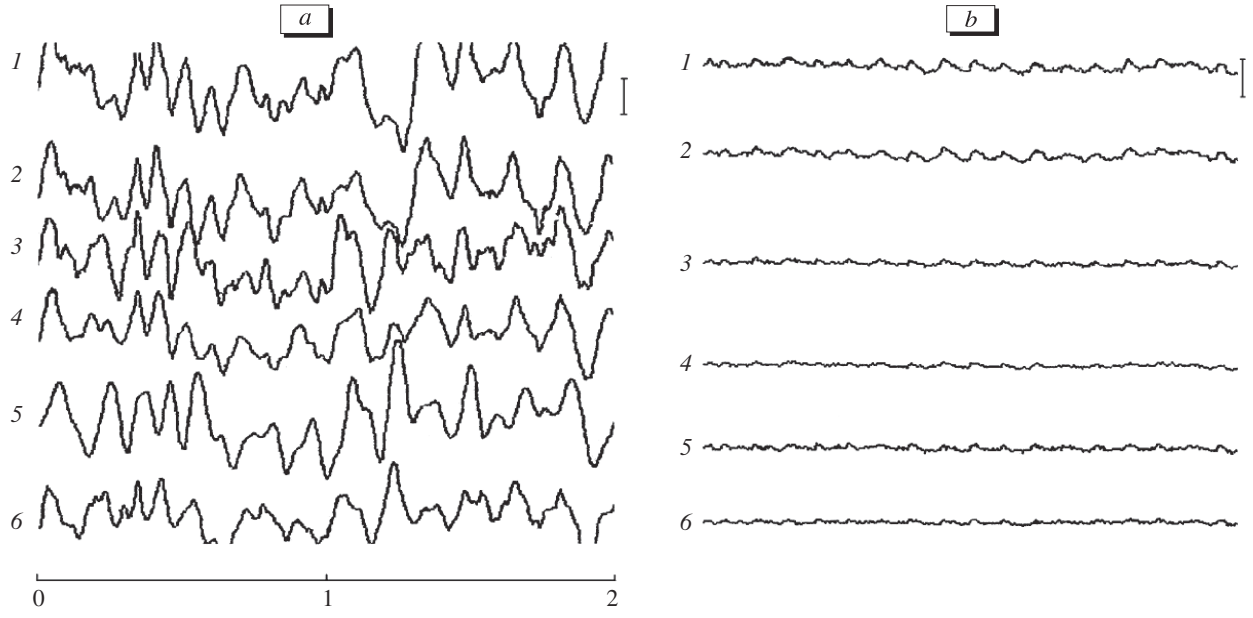

Fig. 2. EEG recording in the rat 3 h after bilateral carotid artery ligation. The rat died 4 h 58 min after surgery. Before surgery (*a*); after carotid artery ligation (*b*). Abscissa: time, sec. Left and right frontal region (*1*, *2*); parietotemporal region (*3*, *4*); occipital region of the brain (*5*, *6*). Calibration 70 μV.

ably reflects activation of the brain. These EEG parameters do not serve as a sign of seizure activity, which is often observed in patients during the post-stroke period [2,13] and in animals with experimental cerebral ischemia [5,9]. Seizure activity (*e.g.*, nonconvulsive status) is usually accompanied by the appearance of high-amplitude low-frequency waves in EEG (polymorphic δ-rhythm, peak weaves, *etc*.) [9]. The mechanism for the narrow-band increase in PS of α- and β-ranges remains unclear. These EEG parameters reflect the development of severe cerebral ischemia and predict irreversible brain injury or death of animals. Terminal convulsions are probably related to general metabolic disorders in CNS, including acidosis, cerebral edema, massive neuronal death, and accumulation of free radicals and intracellular or extracellular Ca^{2+} [15].

The decrease in the coherence of biological potentials in various frequency ranges reflects impairment of synaptic relationships between brain structures. It is probably associated with dysfunction and death of a considerable number of neurons in the cerebral cortex and subcortical structures.

The reduction of spatial entropy of biological potentials illustrates suppression of brain functions, decrease in the diversity of synaptic relationships between brain structures, and diminished degree of freedom for nerve cells. The most resistant regions (pacemaker centers) of the brain can survive for a certain period of time during the postischemic period after neuronal damage and death. They provide various autonomic functions that are associated with the maintenance of vital activity (respiratory center, cardiovascular center, *etc*.).

Published data show that the early mortality rate after bilateral carotid artery ligation varies from 0 to 100% for various strains of rats [14]. The mortality rate is low for young Wistar rats (10-20%), but significantly increases with age [4]. It probably results from age-related morphological (atherosclerotic) and hemodynamic changes and decrease in reserve capacities of cerebral circulation.

It is accepted that bilateral carotid artery ligation causes moderate global ischemia of the brain. We believe that this point of view is erroneous (at least, for old rats). Our conclusion is derived from high mortality rate and significant changes in EEG parameters. We showed that 302 of 840 parameters for electrical activity of the brain significantly change 3 h after carotid artery ligation (Fig. 3). Sham-operated animals were characterized by significant changes in 47 secondary parameters (various types of brain asymmetry *etc*.), but not in main parameters of bioelectric activity (PS, coherence, synchrony, and entropy).

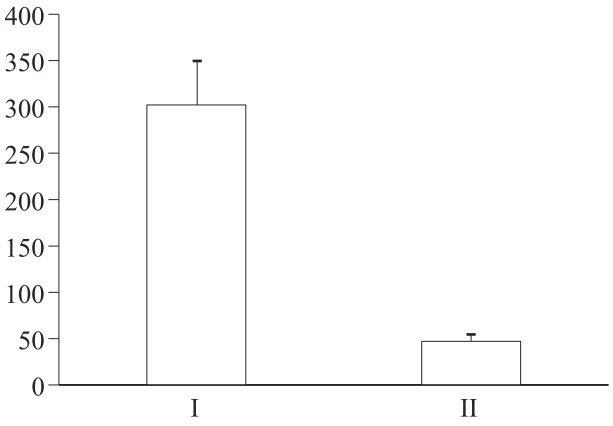

Fig. 3. Number of modified parameters of EEG (840 estimated parameters) 3 h after bilateral ligation of the common carotid arteries (I) and sham operation (II). Averaged data for both groups of Wistar rats.

The calculation of changed parameters for bioelectric activity of the brain may be used in experimental pharmacology. When a chemical substance or other treatment significantly decreases the number of abnormal parameters in EEG after artery ligation (particularly main parameters), it can be concluded that this agent has high antiischemic activity (even without increase in animal survival).

The model of bilateral carotid artery ligation in combination with a multiparameter analysis of EEG is suitable for studying the pathogenesis of cerebral ischemia. This method holds much promise for the search and efficacy evaluation of new antiischemic (anti-stroke) drugs of various types and mechanisms for action.

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