

## FEATURES OF BIOELECTRIC ACTIVITY OF THE RETROSPLENIAL CORTEX

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Human brain is one of the most difficult organs to study. The possibility of developing the technologies that have sufficient scientific accuracy and economic accessibility and never violate the moral and ethical standards of human society is of great interest. The study was aimed to study the possibility of assessing the retrosplenial cortex (RSC) structures' activity based on the EEG analysis of brain activity in the alpha frequency range in 36 healthy volunteers with an average age of 29.1 years, no acute central nervous system disorders or exacerbation of chronic central nervous system disorders, severe traumatic brain injuries, mental disorders or epilepsy. Significant source localizations were obtained by solving the EEG inverse problem that could be used for identification of the cerebral retrosplenial cortex structures' bioelectric activity. The use of such technology will allow us to expand the scope of the research focused on assessing the brain functional activity in both research and clinical centers, thereby paving the way for understanding the features of the brain structures' activity in physiologically normal conditions and in individuals with mental disorders caused by various functional alterations in the brain.

**Keywords:** electroencephalography, mathematical methods, retrosplenial cortex, bioelectrical activity of the brain

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**Compliance with ethical standards:** the study was approved by the Ethics Committee of the National Research Nuclear University MEPhI (protocol № 05/23 of 25 May 2023) and conducted in accordance with the principles of biomedical ethics set out in the Declaration of Helsinki (1964) and its subsequent updates.

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## ОСОБЕННОСТИ БИОЭЛЕКТРИЧЕСКОЙ АКТИВНОСТИ РЕТРОСПЛЕНИЯЛЬНОЙ КОРЫ ГОЛОВНОГО МОЗГА

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Головной мозг человека представляет собой один из самых сложных для исследования органов. Огромный интерес представляет возможность разработки технологий, обладающих достаточной научной точностью и экономической доступностью при полном соблюдении морально-этических норм человеческого сообщества. Целью работы было изучить возможность исследования активности структур ретросплениальной коры (RSC) на основе ЭЭГ-анализа биоэлектрической активности головного мозга в альфа-диапазоне частот у 36 здоровых добровольцев возрастом в среднем 29,1 года, не имевших острых и хронических заболеваний центральной нервной системы в стадии обострения, тяжелых черепно-мозговых травм, психических заболеваний и эпилепсии. Получены статистически достоверные локализации источников с помощью решения обратной ЭЭГ-задачи, позволяющие использовать их для идентификации биоэлектрической активности структур ретросплениальной коры головного мозга. Применение данной технологии позволит расширить объем исследований функциональной активности головного мозга как в научных, так и клинических учреждениях, создав условия для понимания особенностей работы мозговых структур в условиях физиологической нормы и при наличии психических заболеваний, основу которых составляют различные функциональные изменения головного мозга.

**Ключевые слова:** электроэнцефалография, математические методы, ретросплениальная кора, биоэлектрическая активность головного мозга

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Human brain is one of the most difficult organs to study, which is due to the features of human anatomy, the need to comply with ethical standards, and the economic component of the use of advanced functional visualization methods: computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI). That is why the possibility of developing the research technologies that have sufficient scientific accuracy and economic accessibility and do not violate moral and ethical standards of modern society is of great interest.

Electroencephalography (EEG), developed in the beginning of the previous century, but given a new impulse with the

development of mathematical data processing systems, has become one such technology [1–3].

In terms of EEG, brain activity is a combination of rhythmic phenomena reflecting the changes in the summed total of postsynaptic potentials. Alpha activity that is currently considered to be associated with the visual analyzer or visual cortex (VC) (Brodmann's areas 17, 18, 19) activity represents the most prominent and commonly recorded EEG phenomenon [4]. Occupying almost the entire occipital lobe, it produces strong occipital rhythmic activity with the frequency of 8–14 Hz that vanishes with eye opening; its association with the visual cortex shows conclusively that there are rhythmic phenomena

associated with the activity of other neural analyzers, the mu and kappa rhythms that have specific stimuli and do not respond to eye opening [5–9]. However, a number of studies [10, 11] have revealed alpha-activity heterogeneity in individuals with borderline disorders and mental deviations manifested in the form of alpha rhythm multimodality. This raises the question of possible perceptual alterations in such individuals. At the same time it has been found [12] that not the visual cortex can be the source of such heterogeneity, but rhythmic activity in the posterior areas of the cingulate cortex, the structure belonging to the retrosplenial cortex (RSC).

The study of the RSC is of interest due to its direct involvement in complex cognitive processes, such as spatial cognition, analysis and error correction for current sensory states with internal representations of the environment [13]. Occupying the posterior part of the cingulate cortex (Brodmann's areas 26, 29, 30, 23, 31) [14], this area is linked to the anterior thalamic nuclei, entorhinal and parietal cortex, subiculum and hippocampus [15, 16, 17], which determines its key role in the processes underlying spatiotemporal orientation (human self-determination and navigating in the surrounding space). Changes in the RSC activity may be the earliest signs of dementia [18], which has been confirmed by clinical trials [19–21]. Furthermore, the RSC is associated with memory and attention [22–24], as well as with knowledge of the world [24, 25]. The RSC is closely related to the visual stimuli encoding by the visual cortex [26] and to formation of personal orientation towards a specific goal [27–29]. This view is confirmed by the presence of structural links between the RSC and the prefrontal cortex, parahippocampal areas, hippocampus, anterior thalamic nuclei, and parietal cortex [30, 31]. The functional neuroimaging studies have shown that the RSC structures respond more strongly during virtual or imaginary navigating compared to other tasks [32–34]. Thus, according to current research, the RSC is a major area of the cerebral cortex that is associated with cognitive and mental functions. The RSC extensive study will make it possible to acquire new data on the features of the brain functional activity at the earliest stages of the disease development.

The study was aimed to demonstrate the possibility of assessing the RSC activity based on the analysis of the EEG alpha waves in order to determine the features associated with various relaxed wakefulness states.

## METHODS

### Study design

According to modern literature, the RSC is primarily an area responsible for spatial positional orientation (and possibly temporal orientation). That is why its EEG identification becomes possible during the periods when the RSC structures produce rhythmic activity in the conditions of afferent stimuli disconnected from the systems controlling body position in space, the main of which is proprioceptive system. Therefore, a matched study involving EEG recording of alpha waves became the main functional test. During the test the subject, who was in the relaxed wakefulness state, was seated in a chair (active proprioceptive system) or was lying in bed before falling asleep (a fragment of EEG recording with the same length as the first recording was extracted that showed strong occipital alpha activity before fragmentation), when his/her proprioceptive system was minimally involved. This functional test was selected based on the clinical phenomenon “falling sensation when falling asleep”. Primary data were recorded with

the digital electroencephalography system (Medical Computer Systems; Zelenograd, Russia). The analog-to-digital converter sampling rate was 500 Hz, and the signal filtering input parameters were 0.03–70 Hz. Electrodes were placed on the scalp according to the international 10–20 settlement system. The electrode positions were refined by performing linear measurements with subsequent adjustment of the electrode spatial arrangement standard tables. This electrode settlement system was selected due to the fact of the increasing number of the recording artifacts associated with exposure to physical and technological environmental factors in the multichannel system [35].

During the first phase of processing physical artifacts were minimized. For that the by-standing electrical devices that generated parasitic electromagnetic fields were switched off, and the interface impedance was controlled. The temperature in the room was also adjusted, and parasitic muscle movements were minimized whenever possible, which reduced the biological artifacts' intensity.

During the second phase the data pool obtained was through standardization of basic assembly to create a common electrode space, as well as to artifact removal via extraction of independent signal components. This made it possible to purify the native signal of various physiological artifacts that had not been eliminated by filtration.

During the third phase the EEG signal segmentation was performed to extract individual EEG microstates by using the procedure implemented in the sLORETA software package (v. 20210701 University of Zurich; Switzerland) involving allocating eight classes of individual microstates (conventional I–IV [36] and four extra ones (V and VIII) taking into account their variability). The final phase of the study involved solving the EEG inverse problem for each of the allocated EEG microstate classes using the EEG inverse problem solution algorithm implemented in the sLORETA software package. The results provided information about eight variants of sources of individual EEG microstates in accordance with the Brodmann area atlas (based on the atlas by the Montreal Neurological Institute (MNI)).

### Patients

A total of 36 healthy volunteers of different ages, who submitted informed consent, were assessed. Among them 19 individuals were under the age of 30 years, while 17 were over the age of 30 years. The average age of the subjects was 29.1 years, (Mo — 10 years, Me — 26 years, 1<sup>st</sup> quartile — 18 years, 1<sup>st</sup> quartile — 33 years). The average age of the subjects under the age of 30 years was 17.4 years (standard deviation — 1.7 years, Mo — 10 years, ME — 18 years, 1<sup>st</sup> quartile — 12.3 years, 3<sup>rd</sup> quartile — 23.3 years). The average age of the subjects over the age of 30 years was 43.3 years (Mo — 31 years, Me — 34.5 years, 1<sup>st</sup> quartile — 31 years, 1<sup>st</sup> quartile — 55.8 years).

All the subjects were through EEG test involving assessing the background activity of the brain in the relaxed wakefulness state with the eyes closed performed when the subject was in a sitting position and the same test performed when the subject was lying down (the onset of physiological sleep was controlled), since in healthy people the RSC activity is represented by the development of the phenomenon “falling sensation when falling asleep” observed before falling asleep or when lying in bed with the eyes closed. It is associated with the sense of spatial disorientation described as flying and/or falling down before falling asleep [37]. This makes it possible to use this phenomenon as a physiological test for extraction of the RSC activity during the experiment.

**Table 1.** Pairwise comparison of the alpha activity frequency characteristics (Hz) in occipital and parietal areas in the relaxed wakefulness state in the sitting position (observation № 1) and in the relaxed wakefulness state when lying down before falling asleep (observation № 2) (Student's *t*-test, KS-test norm < 0.01)

Region	O1		O2		P1		P2	
	1	2	1	2	1	2	1	2
Observation	1	2	1	2	1	2	1	2
M	10.3	10.3	10.3	10.3	10.3	10.1	10.3	10.1
$\sigma$	0.7	1	0.7	1	0.8	0.9	0.8	0.9
Mo	10	9.5	10	9	10	9.5	10	9.5
Me	10.2	10.4	10.2	10.3	10.2	9.8	10.2	9.8
<i>p t</i> -Student	1		0.8		0.2		0.1	

Inclusion criteria: no history of acute nervous system disorder; no exacerbation of chronic disorder; no history of severe traumatic brain injury, mental disorder, epilepsy.

Clinical assessment was performed in the La Salute Clinic in accordance with the cooperation agreement between the La Salute Clinic and the National Research Nuclear University MEPhI (№ 09-01/23 of 09 January 2023).

**Statistical analysis**

The results obtained were processed in accordance with the guidelines [38] using PSPP (GNU software ver. 1.6.2-g78a33a) for OC Linux Mate (v. 10.10, GNU-GPL licence). Calculation involved pairwise comparison of the EEG inverse problem solution results obtained for eight EEG microstates using the Kolmogorov-Smirnov test (KS-test) for normality; calculation of Student's *t*-test for samples with normal distribution and Wilcoxon signed-rank test for related samples with non-normal distribution. The same degree of freedom was used, the significance level was set as  $\alpha < 0.05$ .

**RESULTS**

The analysis of occipital and parietal alpha activity performed in the general group revealed the decrease in alpha activity frequency before falling asleep, but there were no significant differences in the values of the general group (Table 1) and individuals under the age of 30 years (Table 2). In contrast, in the group of subjects over the age of 30 years the decrease in alpha activity frequency observed before falling asleep was significant (Table 3).

When studying individual EEG microstates in the alpha range, heterogeneity of the alpha activity sources associated with the changes in the subject's state was revealed in the general group (Table 4). Thus, in the sitting position the rhythmic phenomena were generated mainly by Brodmann's areas 17, 18, and 19, which represented the expected alpha activity produced by the visual cortex structures functioning in

the "idle" mode. Persistence of these indicators when lying down (without the emergence of significant differences) also suggested the visual cortex response to eye closing, however, when lying down, the recorded alpha activity source shifted to the Brodmann's areas 23, 29, 30, and 31 characterizing the RSC structures.

Assessment of the age-related features of this response showed that individuals under the age of 30 years demonstrated significant differences in alpha rhythm production, since when sitting in the relaxed wakefulness state, alpha activity was produced by the VC structures (Brodmann's areas 17, 18, and 19), while prior to falling asleep the RSC became the source of alpha activity (Brodmann's areas 23, 29, 30, and 31). A reliable RSC response was observed in individuals over the age of 30 years, while the VC structures showed no significant differences before falling asleep (Table 5).

**DISCUSSION**

The findings have shown that even the assessment of the brain rhythm frequency characteristics makes it possible to determine that alpha rhythm recorded during conventional EEG tests is not a stable parameter determining the "basic" characteristics of brain activity in humans. Alpha activity represents the group of rhythmic phenomena showing significant differences in individuals over the age of 30 years having fully developed brain structures.

However, the shift of alpha activity source between the visual cortex and retrosplenial cortex (clinically manifesting in the phenomenon "falling sensation when falling asleep") is clear in individuals under the age of 30 years that can be considered as involvement of the larger number of brain structures in implementation of higher nervous functions and the need for integration of their activity.

At the same time, after 30 years the neural centers are likely to acquire marked specialization, especially the brain's parietal and RSC structures. Specialization manifests itself in the changes of parietal alpha rhythm and the recording of rhythmic

**Table 2.** Pairwise comparison of the alpha activity frequency characteristics (Hz) in occipital and parietal areas in the relaxed wakefulness state in the sitting position (observation № 1) and in the relaxed wakefulness state when lying down before falling asleep (observation № 2) in individuals under the age of 30 years (Student's *t*-test, KS-test norm < 0.01)

Region	O1		O2		P1		P2	
	1	2	1	2	1	2	1	2
Observation	1	2	1	2	1	2	1	2
M	10.3	10.7	10.3	10.6	10.2	10.3	10.2	10.3
$\sigma$	1	1.4	1	1.4	1.2	1.3	1.2	1.3
Mo	9.6	10.5	9.6	10.5	8.2	9.5	8.2	9.5
Me	10	10.6	10	10.6	10	10.5	10	10.5
<i>p t</i> -Student	0.357		0.525		0.789		0.857	

**Table 3.** Pairwise comparison of the alpha activity frequency characteristics (Hz) in occipital and parietal areas in the relaxed wakefulness state in the sitting position (observation № 1) and in the relaxed wakefulness state when lying down before falling asleep (observation № 2) in individuals over the age of 30 years (Student's *t*-test, KS-test norm < 0,01)

Region	O1		O2		P1		P2	
	1	2	1	2	1	2	1	2
Observation								
M	10.3	9.8	10.3	9.8	10.5	9.8	10.5	9.8
$\sigma$	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9
Mo	10.2	9	10.2	9	10.5	9	10.2	9
Me	10.2	9.6	10.2	9.6	10.4	9.6	10.4	9.6
<i>p t</i> -Student	0.07		0.1		0.02		0.02	

**Table 4.** The data on the rate of EEG activity recording (%) over certain Brodmann fields acquired by solving the EEG inverse problem for the model of eight individual EEG microstates in the general group (Wilcoxon signed-rank test, KS-test norm > 0.5)

Subject's position	Brodman areas 23, 29, 30, 31	Brodman areas 17, 18, 19
Sitting	18.3%	34.2%
Lying down	41.7%	25.0%
<i>p (t</i> -Wilcoxon)	0.01	0.4

**Table 5.** Age-related features of the rate of EEG activity recording (%)over certain Brodmann fields acquired by solving the EEG inverse problem for the model of eight individual EEG microstates in various age groups (Wilcoxon signed-rank test, KS-test norm > 0.5)

RSC (Brodman areas 23, 29, 30, 31)		
Age	1–30 years	> 30 years
Sitting	7.4%	12.5%
Lying down	28.7%	19.8%
<i>p (t</i> -Wilcoxon)	0	0.05
VC (Brodman areas 17, 18,19)		
Sitting	20.1%	15.6%
Lying down	27.1%	23%
<i>p (t</i> -Wilcoxon)	0.03	0.5

activity produced by the RSC structures; according to our observations, these can be considered as related phenomena.

The use of advanced mathematical methods for EEG signal analysis enables clear differentiation of alpha activity and determining the sources in various brain structures [39]. Under the conditions of targeted functional load this makes it possible to link repetitive EEG recording fragments to the activity of certain neural networks of the brain [40, 41] involved in production of alpha activity not only by the cerebral visual cortex structures [42–49]. Such observations can be considered as formation of stable links and growing significance of visual cortex as the main source of information in individuals over the age of 30 years, including information about the position of the body in space, as previously reported for other cortical areas [50–52].

Thus, alpha activity identified when performing conventional EEG tests is not the common “basic” rhythm typical for brain structures, but the combination of several rhythms with similar frequency and amplitude characteristics. The above bioelectric activity is produced by various brain structures, particularly the RSC, which is confirmed by conclusions of several studies [5–9] suggesting the alpha rhythm cortical origin. This makes

it possible to re-interpret the findings of the studies [53] showing heterogeneity of alpha rhythm spectra in individuals with various mental deviations, as well as the results of earlier studies [54], especially that focused on the multimodal alpha rhythm variants.

CONCLUSIONS

The use of the brain bioelectric activity frequency analysis within the framework of conventional technology is not an effective method for assessment of higher nervous functions. Modern EEG tests require using the combination of mathematical methods for extraction of individual EEG microstates and EEG inverse problem solution, thereby making it possible to obtain a simple and cost-effective tool for assessment of the brain structures’ functional activity. The use of such technology will allow us to expand the scope of the research focused on assessing the brain functional activity in both research and clinical centers, thereby paving the way for understanding the features of the brain structures’ activity in physiologically normal conditions and in individuals with mental disorders.

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