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參展科別	動物學
作品名稱	Non-invasive study of the electrical activity of the brain of various chordate animals
得獎獎項	四等獎
就讀學校	Autonomous non-profit educational organization "Phystech-lyceum" named after. P.L. Kapitsa
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關鍵詞	<u>EEG</u> 、 <u>cat sharks</u>

作者照片



Abstract

Key words: EEG, cat sharks.

In clinical practice, EEG is used to diagnose a number of neurological diseases and to diagnose epilepsy. But at present, the question of the nature of EEG has not been completely resolved and is of great scientific interest. There have been no studies at all on the non-invasive study of the electrical activity of the brain of the shark superorder, which belongs to the class of cartilaginous fish. By studying the electrical activity of the brain of various gnathostomes, it is possible to obtain an answer to the question of the emergence of rhythms from the point of view of phylogenesis and evolution, and by comparing their EEG with the human EEG, one can identify similar patterns that help in the study of reactions to various influences.

During the work, for the first time, EEG indicators of spotted cat sharks, ECG, heart rate and respiratory rate of cat sharks and toads were obtained. In the future, it is planned to assemble a smaller neuroheadset for non-invasive studies of the electrical activity of the brain of small animals (sharks, toads, monitor lizards). This data can be used for evolutionary and medical research.

*No animals were harmed during or after the experiments.

Introduction

Currently, one of the most informative methods for studying the human and animal brain is the electroencephalography method.

Electroencephalography is a method for studying the functional state of the brain. The method is based on recording the total electrical activity of brain neurons removed from the surface of the scalp - an electroencephalogram (hereinafter EEG).

The electroencephalography method is widely used for scientific and clinical purposes and remains one of the most commonly used for studying the brain. EEG makes it possible to qualitatively and quantitatively analyze the functional state of the brain and its reactions under the influence of stimuli and during the performance of various types of activities.

Relevance: In clinical practice, EEG is used to diagnose a number of neurological diseases and to diagnose epilepsy. But at present, the question of the nature of EEG has not been completely resolved and is of great scientific interest. There have been no studies at all on the non-invasive study of the electrical activity of the brain of the shark superorder, which belongs to the class of cartilaginous fish. By studying the electrical activity of the brain of various gnathostomes, it is possible to obtain an answer to the question of the emergence of rhythms from the point of view of phylogenesis and evolution, and by comparing their EEG with the human EEG, one can identify similar patterns that help in the study of reactions to various influences.

This question determined the purpose of our work.

Objective: To non-invasively study the electrical activity of the brain (EEG) of various chordate species.

Tasks:

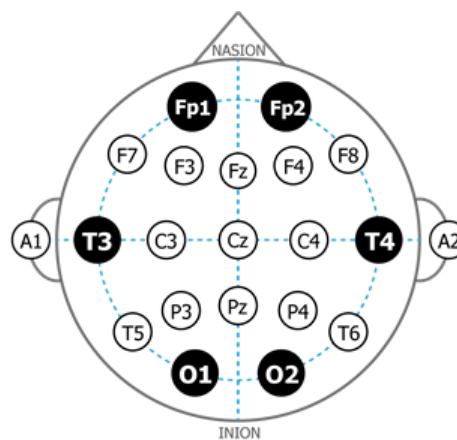
1. Select representatives of various classes of the gnathostome superclass;
2. Select sensors for non-invasive recording of EEG of the brain;
3. Develop a method for taking EEG for selected representatives, taking into account their characteristics;
4. Take and compare the EEG of various representatives at rest and upon presentation of a stimulus.

Electroencephalography is a method for studying the functional state of the brain. The method is based on recording the total electrical activity of brain neurons removed from the surface of the scalp - an electroencephalogram (hereinafter EEG).

To take readings, the NeuroPlay-6C neuroheadset and the NeuroPlayPro 1.9.0 program were used (Application 2).

The NeuroPlay-6C neuroheadset is a high-precision voltmeter that measures the electrical activity of the brain using six dry electrodes and transmits these signals via Bluetooth

The electroencephalogram experiment involved four spotted cat sharks, three Caribbean toads and a monitor lizard *Varanus salvator*. Animals of each species are similar to each other in mass and build.



2.1. Experimental part

2.1.1. Description of shark EEG measurement:

The EEG was taken on land for no more than 50 seconds. The shark was removed from the water. Recording was carried out on a rubberized surface. The shark was placed on a mat and a neural headset was installed around its upper body. The animals were in a state of stress at the time of the experiment, because the animals were not in an aquatic environment, the duration of the analysis was determined by the time that the shark could breathe outside the water. (Application 3)

Shark №1:

The EEG recording was successful. In some areas, rhythmic activity with a frequency of 15 Hz is visible, which is also visible on the spectra in the form of peaks at 15 Hz and its subharmonics at 7.5 Hz (00:03-00:04 s.) (Fig. 2)

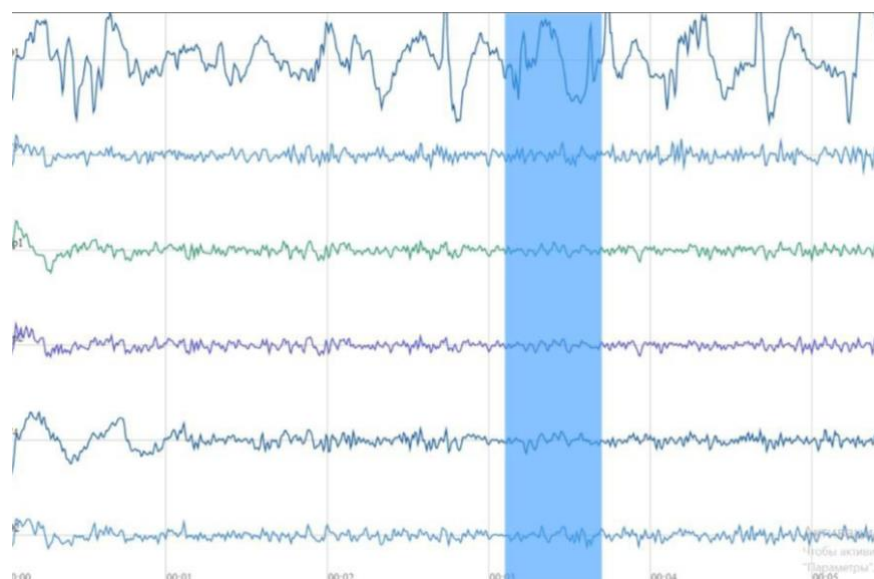


Figure 2. EEG recording of shark No. 1 - 00:00-00:05 s.

The EEG of the first shark does not have an exact structure, it is polymorphic (00:09-00:14 s.). A typical graph is shown in Figure 3, which confirms the spectrum is flat - no peaks.

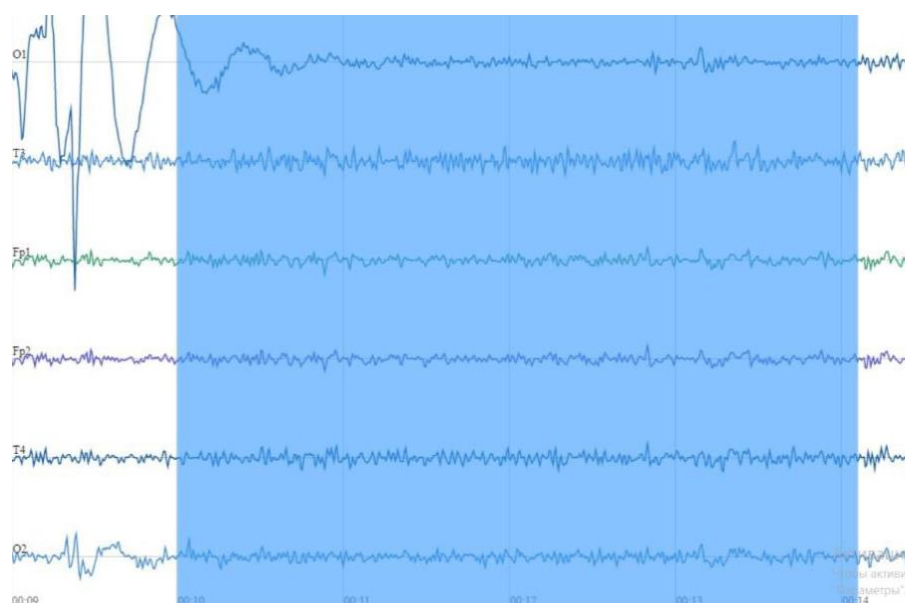


Figure 3. EEG recording of shark No. 1 - 00:09-00:14 s.

On electrode O1, which is presumably located in the area of the projection of the heart and head, a regular electrocardiogram (hereinafter referred to as ECG) is visible; in the 8-second section, 9 ECG complexes with a pronounced wave (00:02-00:10 second) are visible (Fig. 4). From this you can calculate the heart rate (hereinafter HR) of the shark, which was 67 beats per minute.

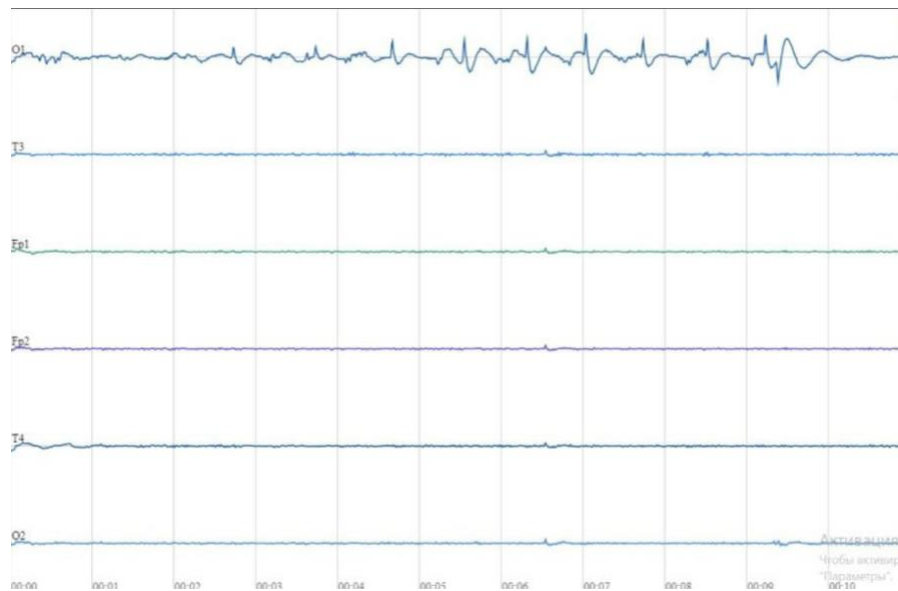


Figure 4. ECG recording of shark No. 1 (00:00-00:10 s.)

Shark №2:

Shark No. 2 shows clear peaks at a frequency of 10-12 Hz (00:15-00:17 sec.) (Fig. 5).

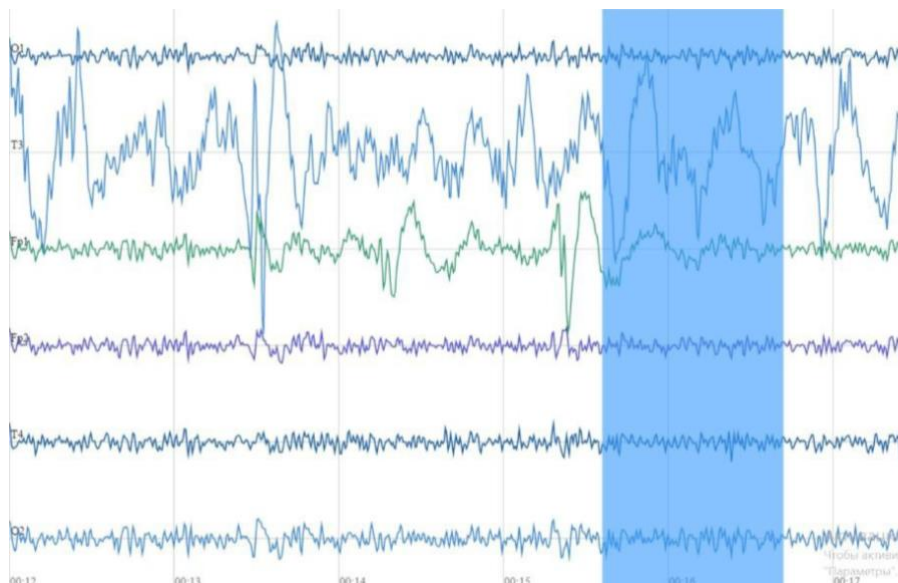


Figure 5. EEG recording of shark No. 2 (00:12-00:17 s.)

Shark №3:

Shark No. 3 failed to make a high-quality recording, as it exhibited increased motor activity. You can see a large number of artifacts. Each movement causes noise and interference in the EEG recording (00:00-00:21 seconds) (Fig. 6).

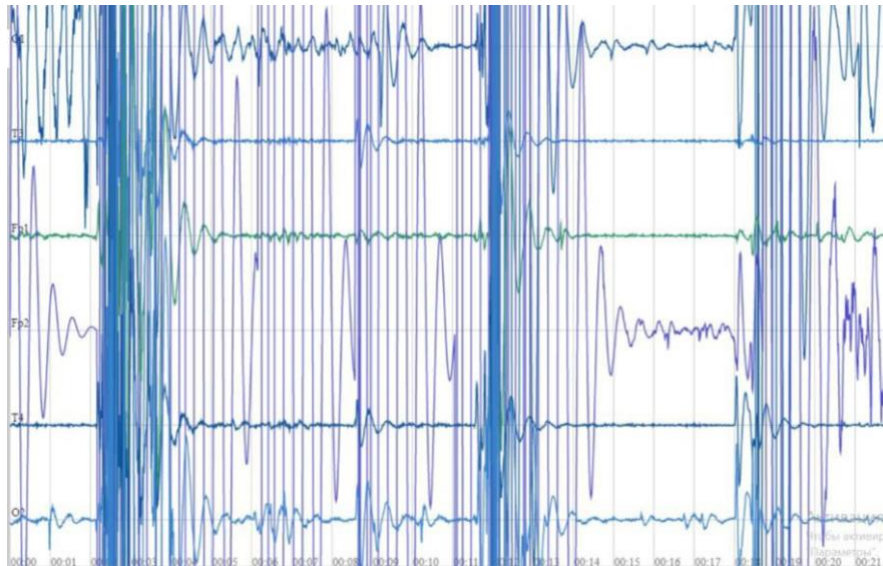


Figure 6. EEG recording of shark No. 3 - 00:00-00:21 s.

Shark №4:

The recording shows an example of an ECG signal. In this section, there are 7 beats in 10 seconds, which corresponds to a heart rate of 42 beats per minute (00:18-00:28 s.) (Fig. 7).

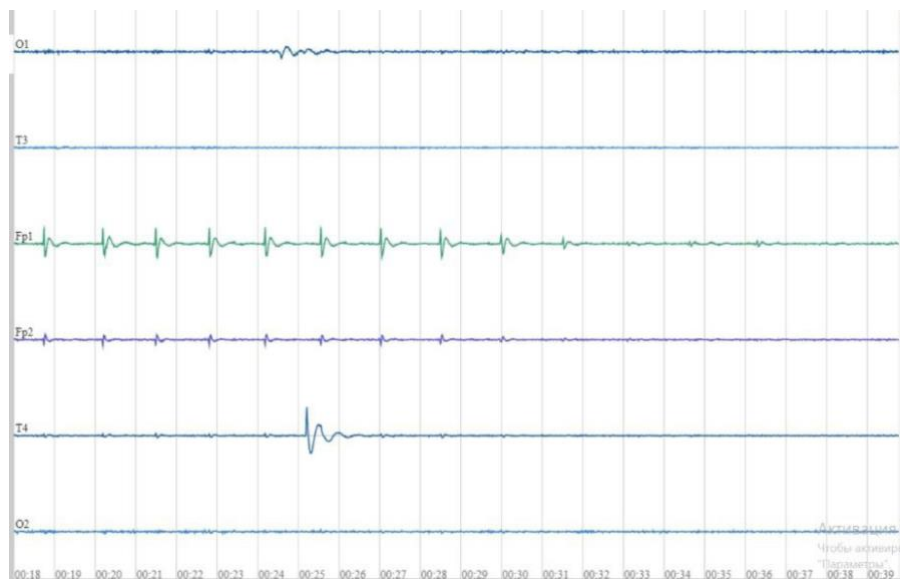


Figure 7. ECG recording of shark No. 4 - 00:18-00:39 s.

Shark No. 4 (like shark No. 1) has a polyphasic spectrum - there are no peaks, the spectrum is flat. This shark has a visible rhythm at a frequency of 10 Hz (00:43-00:44 s.) (Fig. 8).

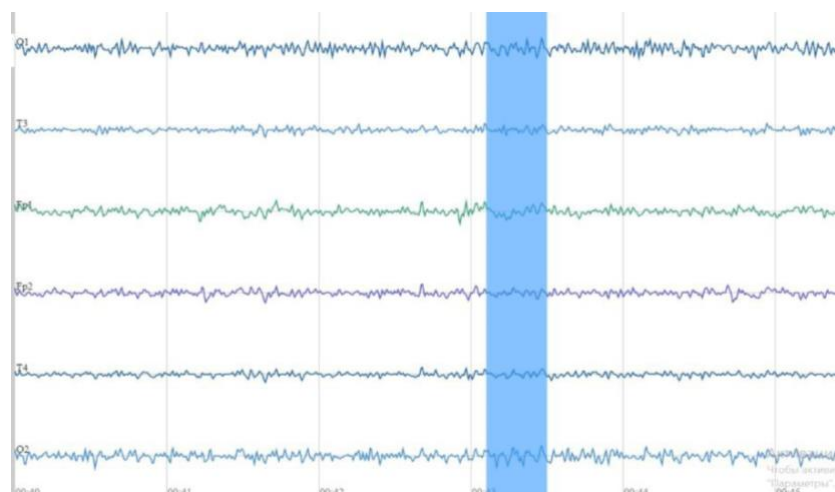


Figure 8. EEG recording of shark No. 4 - 00:40-00:45 s.

The results of the experiments are combined into a summary table of EEG and heart rate of sharks (Table 1).

	Rhythmic activity at frequency: (Hz)	Rhythm	Heart rate
shark 1	15	beta	67
shark 2	10	alpha	-
shark 3	-	-	-
shark 4	10	alpha	42

Table 1. Summary table of EEG and heart rate of sharks.

Analysis of the data obtained from the examination of 4 sharks shows that the main problem is the fixation of the shark. For further research, it is necessary to develop a shark-sized headset. The determination of the rhythm type was made by analogy with the rhythm frequency on the human EEG. The alpha rhythm was determined for two sharks, and the beta rhythm for one. For one of the sharks, it was not possible to clear the EEG of artifacts. The heart rate was determined for two sharks.

EEG of sharks was obtained for the first time.

2.1.2. Description of EEG measurement of toads:

The EEG was taken on land for 120 seconds. The toad was removed from the terrarium. Recording was carried out in a plastic container with low sides. The toad was placed in it, and a neuro-headset was placed around its body. The first toad was active and trying to escape. It was noticed that if the toad lies on its back, then it is calm, and accordingly, readings can be taken. During the experiment, the toads lay on their backs. (Application 4)

A key feature of taking EEGs in toads is their small size, and this complicates the fixation of the neuroheadset and leads to possible noise and artifacts.

Toad №1:

ECG activity is visible on all channels. At 10 seconds there are 13 contractions, which corresponds to 78 beats per minute (00:15-00:25 s.) (Fig. 9).

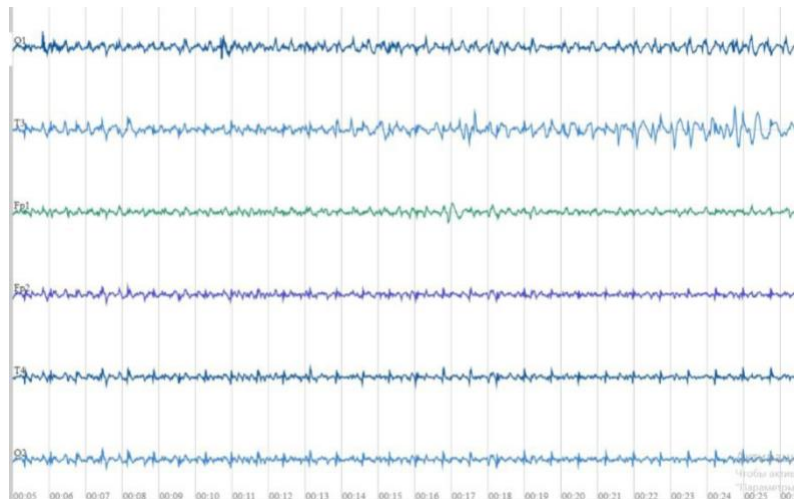


Figure 9. ECG recording of toad No. 1 - 00:05-00:26 s.

Toad №2:

An ECG is also observed on all channels. Heart rate was 72 beats per minute (00:19-00:22 s.) (Fig. 10).

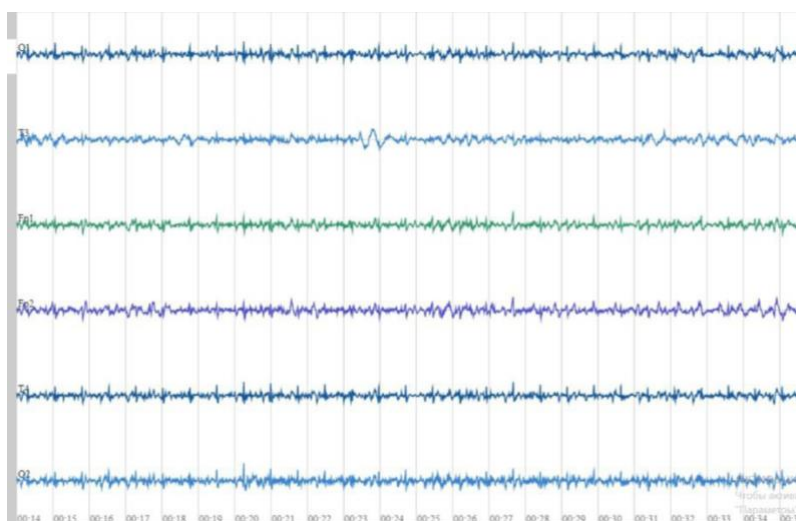


Figure 10. ECG recording of toad No. 2 - 00:14-00:35 s.

Toad №3:

Heart rate was 60 beats per minute (visible on channel Fp1), (00:13-00:20 s.) (Fig. 11).

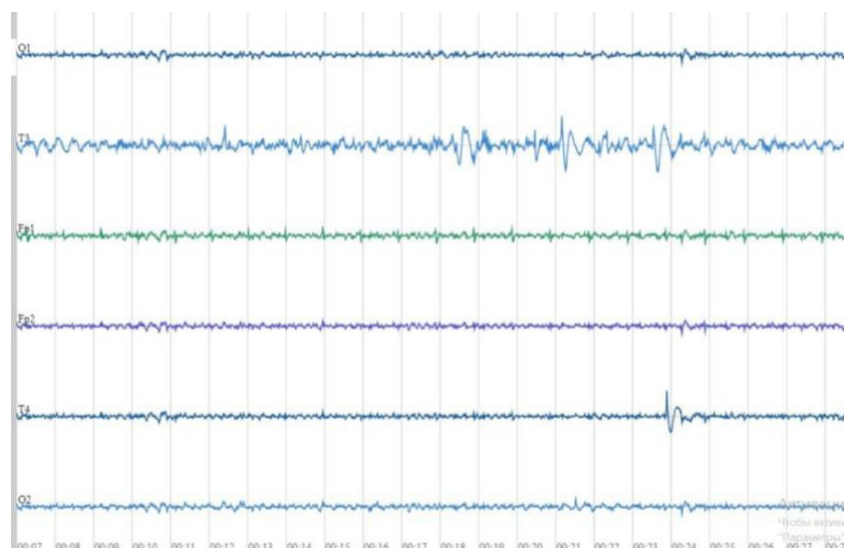


Figure 11. Recording the heart rate of toad No. 3 - 00:07-00:28 s.

In the section 00:03-00:09 s. artifacts are visible, which may be caused by breathing. The respiratory rate (hereinafter referred to as RR) was 120 per minute (Fig. 12), which is the norm for representatives of this species [10].

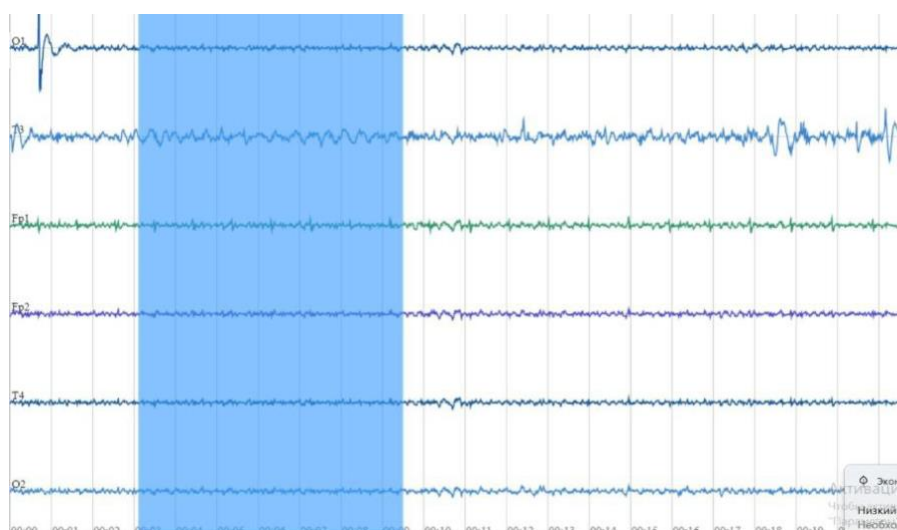


Figure 12. Recording of the respiratory rate of toad No. 3 - 00:00-00:21 s.

The results of the experiments are combined into a summary table of the heart rate and respiratory rate of toads (Table 2).

	Heart rate	NPV
toad 1	78	-
toad 2	72	-
toad 3	60	120

Table 2. Summary table of heart rate and respiratory rate of toads

2.1.3. Description of monitor EEG measurement:

The EEG was taken on land for 3 minutes. The monitor lizard was removed from the terrarium. The monitor lizard was placed on a table, and electrodes were installed around its body. The monitor lizard was lying on his stomach. During the recording, the monitor lizard had to be held tightly; it resisted. (Application 5)

The first recording was made using three separate electrodes. The electrode clamps were removed from the electrode system. The electrodes were wiped with damp alcohol wipes. Next, the gel was poured into the fixative and the electrode was attached. The electrodes were glued to the skin of the monitor lizard using a medical plaster. Two electrodes were installed on the back, one in the area of the brain, presumably above the third eye. The electrodes were connected by wires to an analog converter. Taking an EEG failed, probably because there was poor contact between the skin and the surface of the electrodes; also, the monitor lizard's skin has a high resistance, it acted as a dielectric..

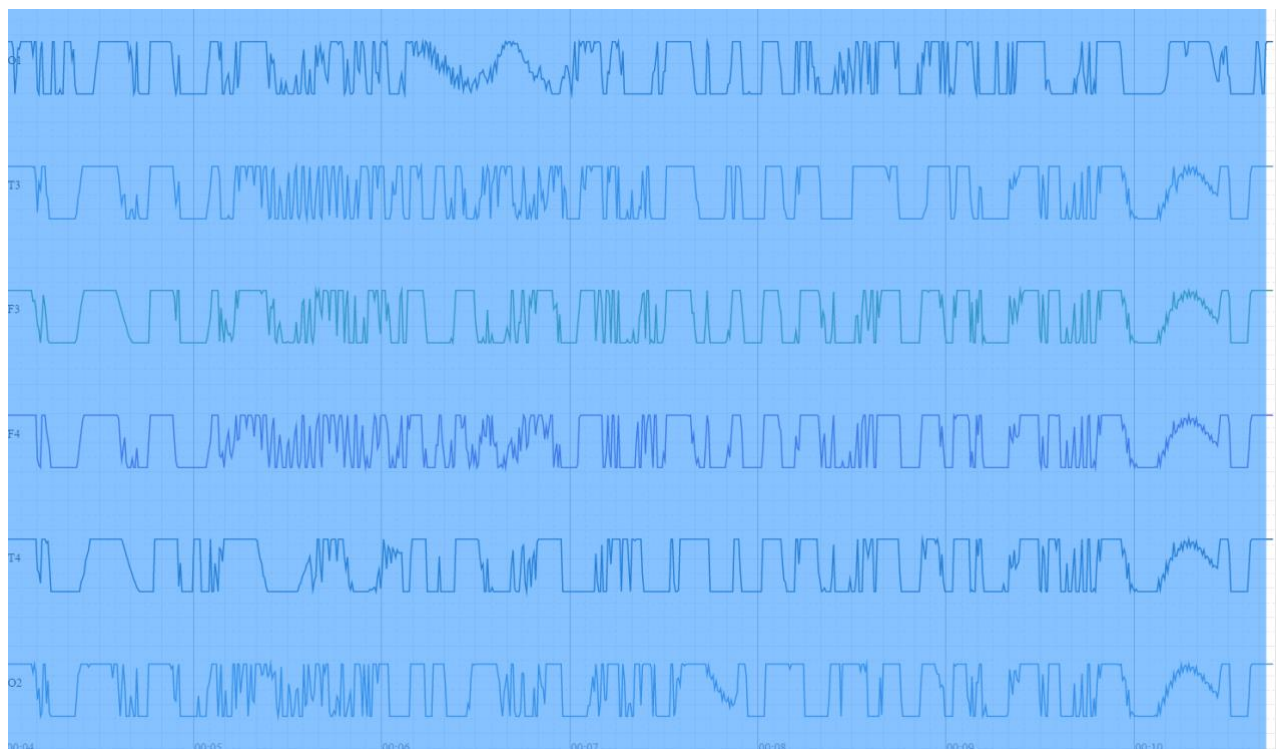


Fig. 13 EEG monitor lizard, recording No. 1 – 00:04 – 00:11 s.

This recording shows an example of an unsuccessful signal caused by an external factor - lack of contact of the electrodes. Noise at 50 Hz. Straight sections – no signal. Multi-frequency sections - noise (00:04 – 00:11)

A key feature of taking an EEG in a monitor lizard is the small size of the monitor lizard's head, and this complicates the fixation of the neuroheadset and leads to the appearance of noise and artifacts.

The second EEG recording was made using the NeuroPlay-6C neuroheadset. The registration was successful. The neuro-headset was installed around the upper part of the monitor lizard's body or applied to the head.

Another feature of taking an EEG in a monitor lizard is the small size of its head, which complicates the fixation of the neuroheadset and leads to the appearance of noise and artifacts.

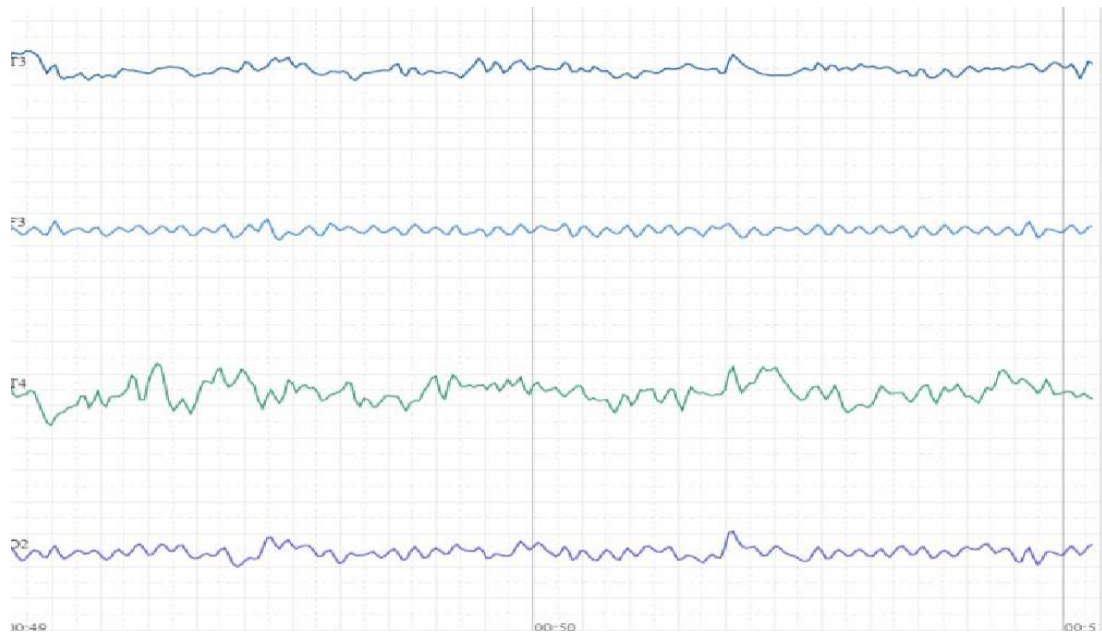


Fig. 14 EEG of the monitor lizard, recording No. 2 – 00:49 – 00:51 s.

In this section of the monitor's EEG recording, no clear structure of the graph is visible. Each electrode transmits its own structure. Similar areas can be traced - probably a heartbeat around 49.5 seconds and 50.5 seconds. The heart rate of monitor lizards usually varies from 40-60 beats per second. The monitor lizard under study exhibits ~58 beats per second. The increased heart rate is explained by the state of stress (00:49 –

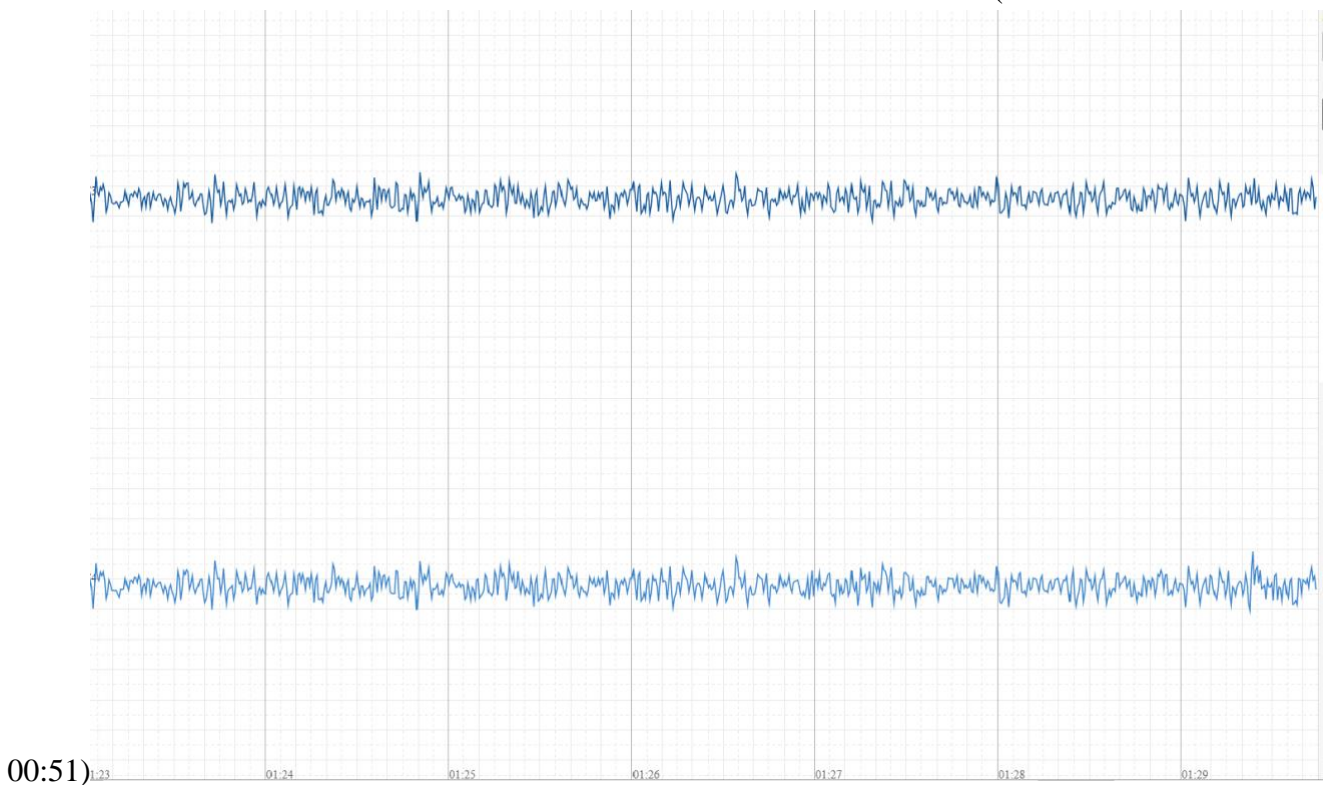


Fig. 15 EEG of the monitor lizard, recording No. 3 – 00:23 – 00:30 s.

The EEG of the monitor lizard is polymorphic and does not have an exact structure. Spectrum - no peaks. Gamma and beta rhythms dominate on both electrodes(00:23 – 00:30 s).

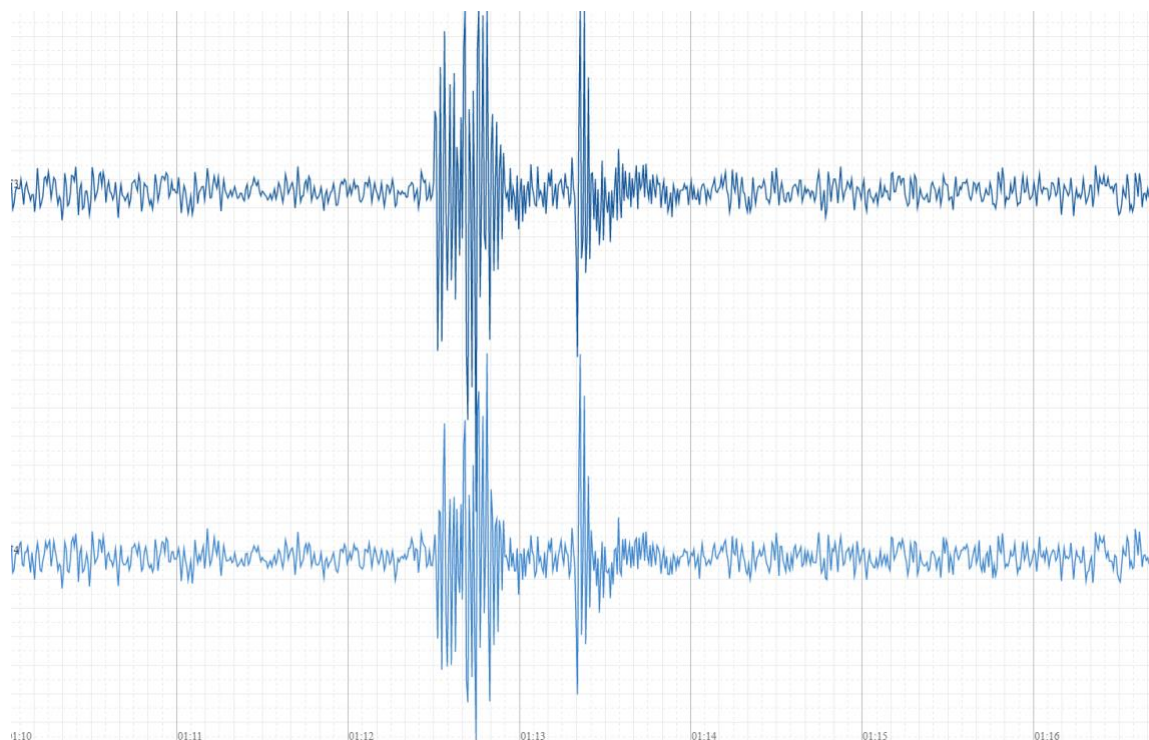


Fig. 16 EEG of the monitor lizard, recording No. 4 – 01:10 - 01:18 s.

In this section of the monitor's EEG recording, one can see an artifact caused by the monitor's movements, namely the movement of the tongue. Such artifacts are observed throughout the recording. Since the reptile was in an unfamiliar environment, it explored the territory, contracted the muscles of its tongue, grabbing air particles with it. Muscle contractions created interference on the graph of total electrical activity (01:10 - 01:18)

Analysis of the data obtained during the examination of the monitor lizard shows that the main problem is fixation of the monitor lizard. For further research, it is necessary to develop a headset based on the size of the monitor lizard. The determination of the rhythm type was made by analogy with the rhythm frequency on the human EEG. Gamma and beta rhythms were determined for the monitor lizard. Heart rate was determined.

2.1.4. Description of human EEG measurement:

EEG was taken while sitting on a chair for no more than 3 minutes. Two people took part in the experiment. The course of the experiment is to close the eyes for 20 seconds, open the eyes for 20 seconds and repeat. These actions help the subject to calm down and relax. After opening the eyes, the subject heard 2-3 pops at intervals of 3-4 seconds, and again closed his eyes for 10 seconds. The next irritant is quickly turning off and turning on the lights in the room. Light acted as a stimulus for 10-12 seconds.

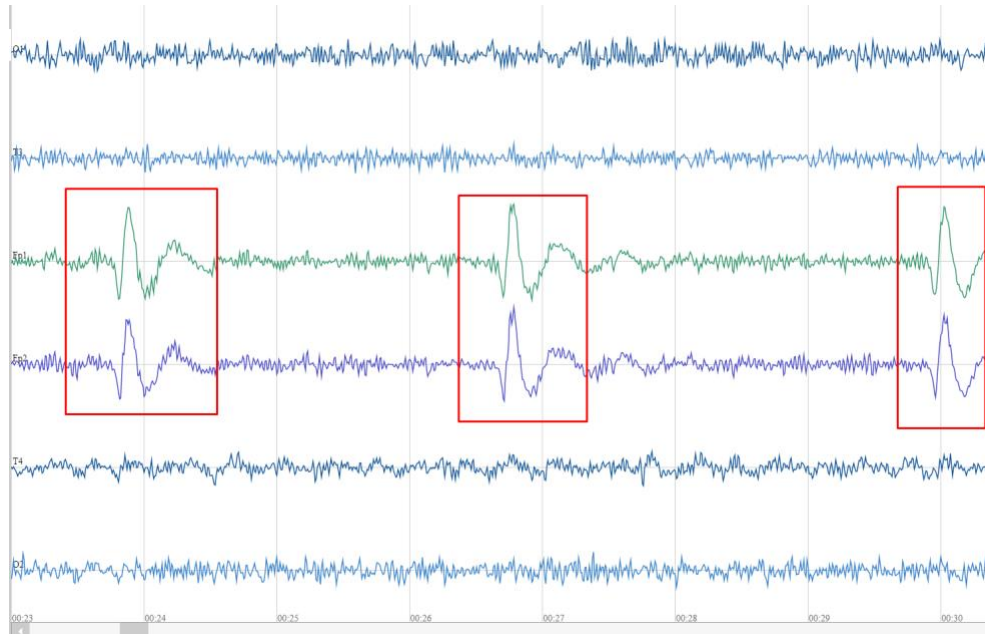


Fig. 17 EEG of a person with open eyes

In this electroencephalogram, the beta rhythm, characteristic of active wakefulness, dominates at all electrodes. Average frequency – 24.52Hz. The peaks highlighted in the figure are human blinking.

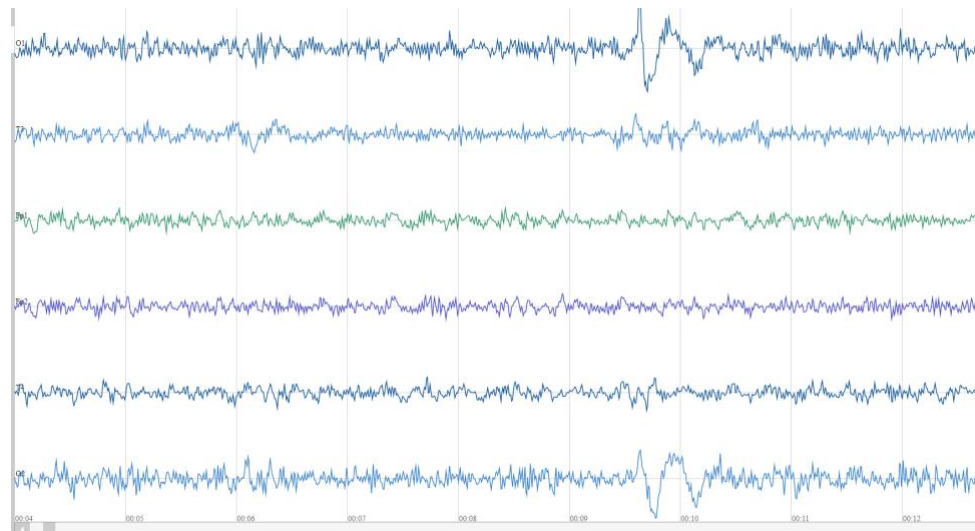


Fig. 18 EEG of a person with eyes closed

On electrodes T3 and Fp1, an alpha rhythm is observed, characteristic of the state of closed eyes and passive wakefulness. Average frequency – 9.76Hz.

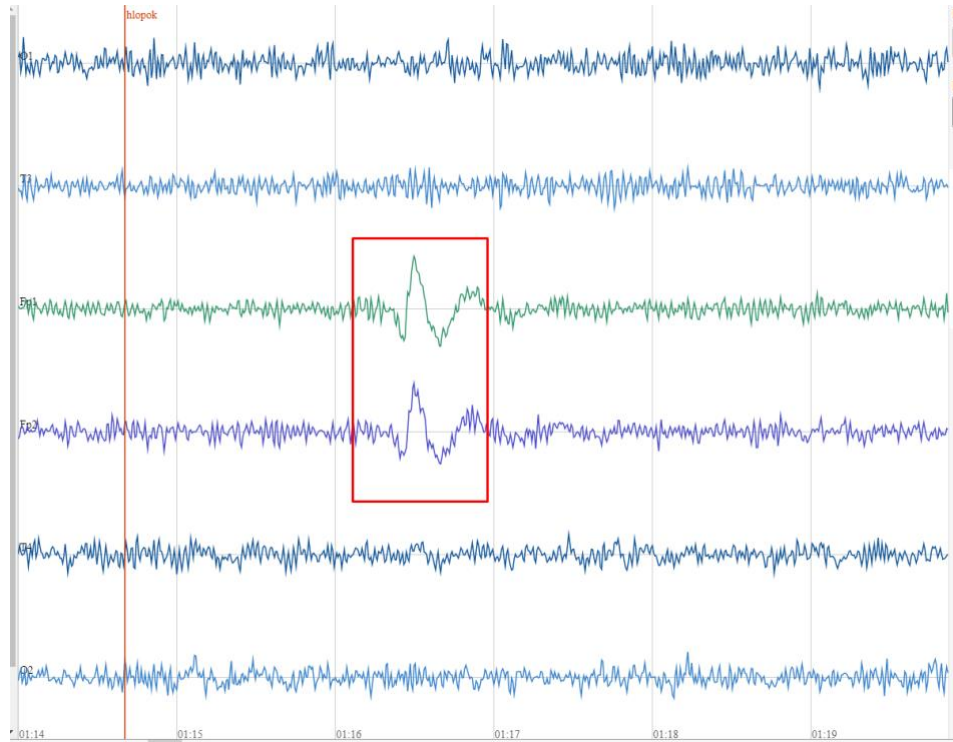


Fig. 19 EEG of a person hearing pops

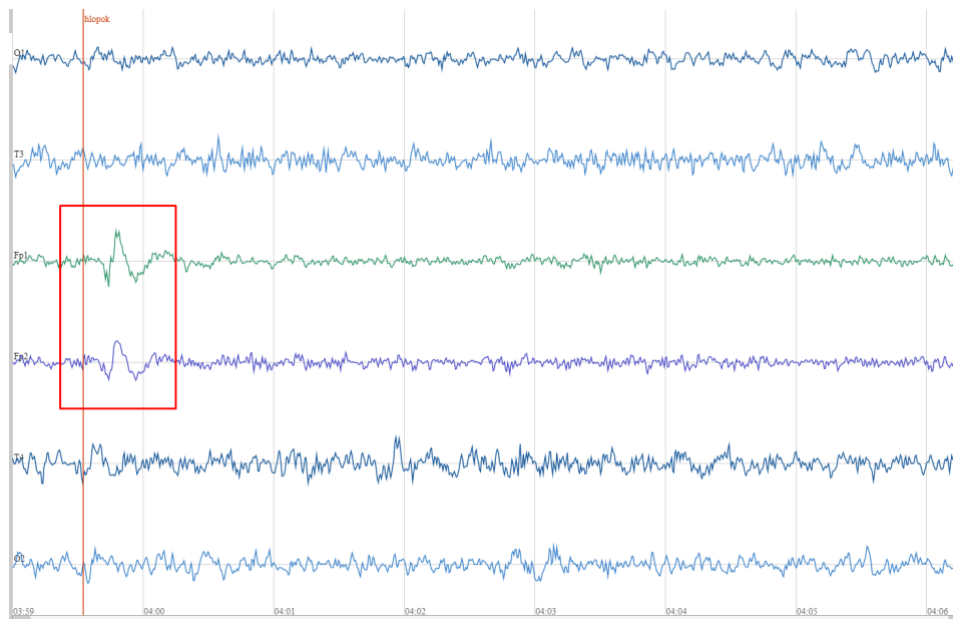


Fig. 20 EEG of a person hearing pops

Frontal electrodes Fp1 and Fp2 responded to clap in two subjects. This reaction is expressed by the rapid opening and closing of the subject's eyes (blinking). Peaks are detected thanks to Fp electrodes, which respond to muscle contraction.

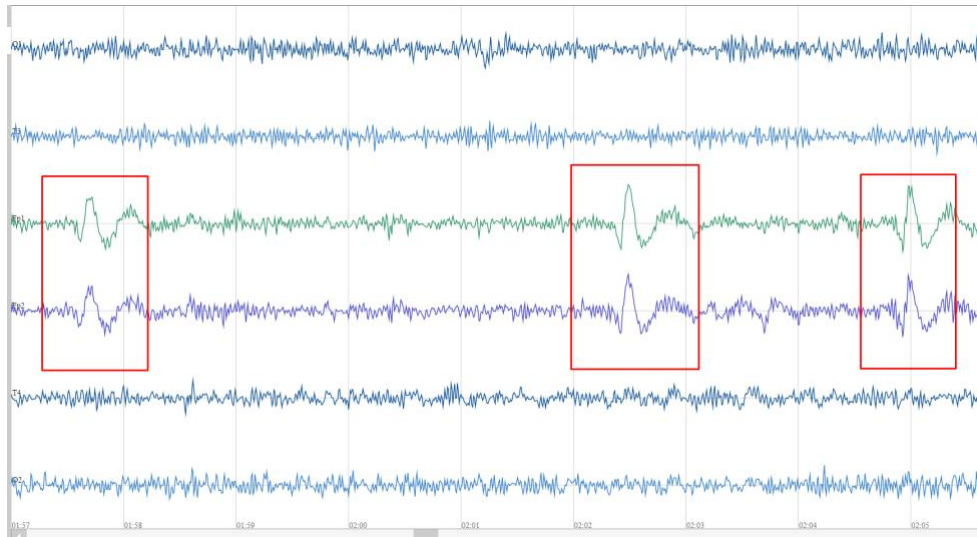


Fig. 21 EEG of a person seeing a flashing light

Frontal electrodes Fp1 and Fp2 responded to the flashing light. This reaction is expressed by the rapid opening and closing of the subject's eyes (blinking). Peaks are also detected thanks to Fp electrodes, which respond to muscle contraction.

Conclusions

1. A unique method of recording EEG in sharks and toads has been tested.

2. The optimal position for taking EEG has been selected: the sharks are on a damp rubber mat on the ventral side of the body for 1 minute. The toads are laid on their backs, the neuroheadset is fixed so that one of the electrodes is located on the neurocranium of the shark, one of the electrodes is attached to the pectoral fin/hind leg, and the remaining electrodes are located randomly, including in the area of the heart.

3. Using this method, we were able to obtain the following physiological data: rhythmic activity of sharks - at frequencies of 10 and 15 Hz. Two sharks have a frequency of 10 Hz, one shark has a frequency of 15 Hz. A shark with a frequency of 15 Hz had a heart rate of 67 beats per minute, with a frequency of 10 Hz - 42 beats per minute.

4. Heart rate, respiratory rate and ECG signals were recorded for toads. There are no EEG results for toads.

5. In the class cartilaginous fishes of the subclass elasmobranchs of the superorder sharks, the same rhythms were revealed as in the class mammals.

6. EEG signal and monitor heart rate recorded

Conclusion:

During the work, for the first time, EEG indicators of spotted cat sharks, ECG, heart rate and respiratory rate of cat sharks and toads were obtained. In the future, it is planned to assemble a smaller neuroheadset for non-invasive studies of the electrical activity of the brain of small animals (sharks, toads, monitor lizards). This data can be used for evolutionary and medical research.

*No animals were harmed during or after the experiments..

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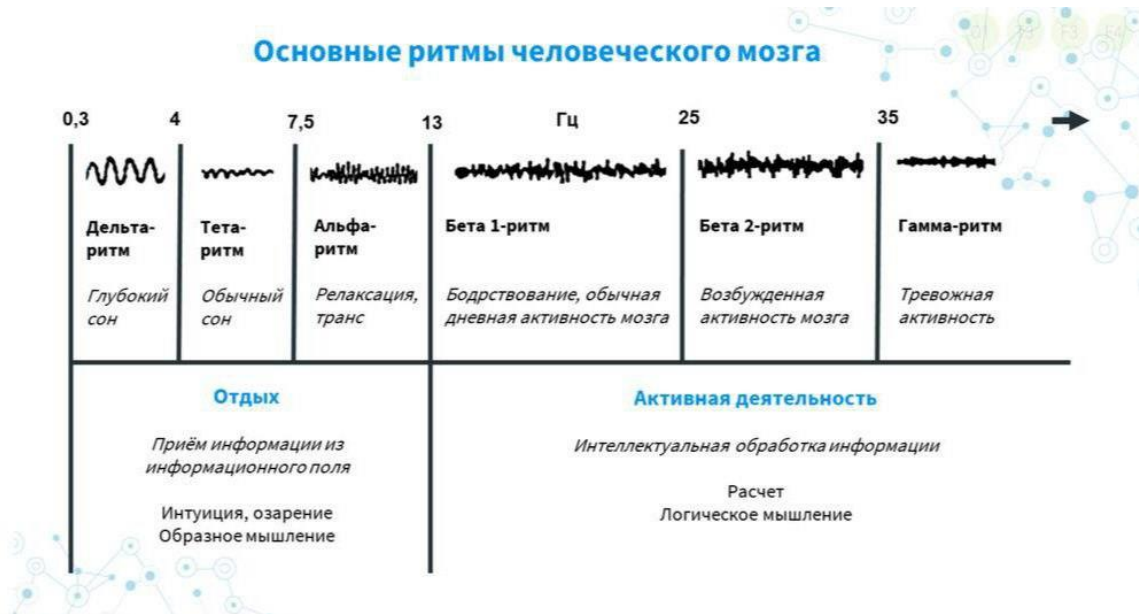
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Applications

Application 1



Application 2



Application 3



Application 4



Application 5



【評語】 050020

The research in question aims to conduct a non-invasive examination of the electrical brain activity in various chordate animals using a traditional EEG device. While the researcher has successfully recorded EEG responses from different vertebrates, there are significant concerns regarding the conditions of the animals involved. This lack of uniformity in the animal conditions makes it challenging to compare the results effectively.

Furthermore, the study does not clearly articulate its specific research objectives beyond the collection of EEG data from diverse animal brains. Therefore, it is essential to formulate a well-defined scientific question and establish a working hypothesis to provide clearer direction and purpose to the research.

Moreover, the variation in brain sizes among different animals, along with differences in brain regions, poses a challenge when using a standard EEG device with fixed electrodes. To address this issue in future studies involving EEG measurements from various animal species,

modifications to the EEG device, especially regarding electrode placement and adaptability, should be considered.

In summary, these comments offer constructive recommendations for enhancing the current research focused on EEG measurements in various chordate animals. Prioritizing animal welfare, controlling experimental conditions, defining research objectives, and adapting equipment are all critical aspects to address in future studies in this field.