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ELECTRICAL ACTIVITY OF MOTOF AND SENSORY ZONES OF SPINAL CORD DURING SPONTANEOUS MOVEMENTS IN NEONATAL RATS

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General description of the work

The relevance of the research and its degree of development

The early stages of animal development are characterized by special forms of movements, including spontaneous twitches, startles and more complex movements. (Blumberg et al., 2010; Hafez, 1975; De Vries et al., 1982). It is considered, that this early forms of movements are involved in the formation of neuronal networks ensuring the coordinated working of the sensory and motor systems (Sanes et al., 1999; Petersson et al., 2003; Mendelsohn et al., 2016). An important property of this primitive motor patterns is that the underlying motor commands are independently generated in the spinal cord (Robinson et al., 1999). It has also been shown that spontaneous primitive movements trigger early oscillatory patterns of activity at higher levels of the sensory-motor system, including the thalamus, as well as in the somatosensory and motor cortex (Khazipov et al., 2004; Tiriac et al., 2014; Akhmetshina et al., 2016). Based on these data, as well as as a result of behavioral studies and computer modeling of the development of nociceptive reflexes (Petersson et al., 2003), it was suggested that the activation of somatosensory pathways due to primitive movements is provided by the activation of sensory feedback circuits (including tactile and proprioceptive sensory feedback). This hypothesis suggests a coordinated activity of motor neurons and neurons in the sensory zone of the spinal cord, which is provided by sensory feedback during primitive movements. However, the physiological patterns of neuronal activity in the motor and sensory zones of the spinal cord, as well as the mechanisms underlying the coordinated activity of neurons in the spinal cord during primitive movements, are still poorly understood. This is primarily due to the fact that the basis of modern knowledge about the activity of neural networks of the spinal cord in the early stages of ontogenesis is the result of studies using an isolated spinal cord preparation, or slices of the spinal cord of newborn rats and mice in vitro. These studies revealed spontaneous bursts of activity in the motor zones of the isolated spinal cord in newborn animals. Such bursts spread to the ventral roots, and are also accompanied by the depolarization of afferent fibers and activation of the dorsal roots of the spinal cord. Since the activation of afferents involves the transfer of excitation to neurons of the sensory zone of the spinal cord, it was suggested that this mechanism of “efferent copy” can provide coordinated activation of the motor and sensory zones of the spinal cord even in the absence of connections between the spinal cord and muscles and the sensory feedback (Bos et al., 2011). However, it should be noted that the simultaneous registration of neural activity in the sensory and motor zones of the isolated spinal cord of newborn animals has not yet been performed, and the mechanism of the “efferent copy” also remains only hypothetical. Moreover, it remains unknown which physiological patterns characterize the activity of the sensory and motor zones of the spinal cord in intact newborn animals in vivo, how these patterns of neuronal activity in the spinal cord correlate with early forms of motor activity, and what mechanisms underlie coordination sensory-motor activity in the developing spinal cord. Information on the organization of spinal cord activity during spontaneous movements is key to understanding the early functioning and development of both the neural networks of the spinal cord and the sensory-motor system as a whole.

The aim and tasks of the research

The aim of this work was to study the patterns of electrical activity and the study of mechanisms for synchronizing the activity of neurons in the sensory and motor zones of the spinal cord of newborn rats during spontaneous movements.

In accordance with the goal, the following tasks were set:

1. To carry out functional mapping of the sensory and motor zones of the lumbar segment of the spinal cord of newborn rats.
2. To classify patterns of spontaneous motor activity of the hind limbs in newborn rats.
3. To study the patterns of electrical activity of neurons in the dorsal and ventral horns of the lumbar segments of the spinal cord of newborn rats during various types of spontaneous movements of the hindlimbs.
4. To evaluate the effect of deafferentation on the electrical activity of neurons in the dorsal and ventral horns of the spinal cord of newborn rats during spontaneous movements.
5. To conduct a comparative analysis of the patterns of spontaneous electrical activity in the dorsal and ventral horns of the spinal cord of newborn rats in vivo and in the isolated spinal cord in vitro.
6. To identify mechanisms for the generation of spontaneous electrical activity of neurons in an isolated spinal cord in vitro using pharmacological methods

Scientific novelty of the research

- The spatiotemporal properties of electrical activity in the sensory and motor zones of the lumbar segments of the spinal cord of newborn rats during spontaneous movements of the hindlimbs were first described.
- The sequential activation of neurons in the motor and sensory zones is shown, in which activation of motor neurons is preceded, and activation of neurons in the sensory zone follows the beginning of movements
- It was shown for the first time that deafferentation of the spinal cord by cutting dorsal roots does not lead to significant changes in electrical activity in the motor zone and in the motor activity of the hind limb, but eliminates the activation of neurons in the sensory zone of the spinal cord during spontaneous movements.
- The spatiotemporal properties of spontaneous activity have been characterized, and a low level of correlation between neuronal activity in the sensory and motor zones of the isolated spinal cord of newborn rats in vitro has been shown.
- The direct evidence is presented for the first time that sensory feedback underlies the coordinated activation of neurons in the motor and sensory zones during spontaneous movements

Theoretical and scientific-practical significance of the research

The results obtained in this research are mainly having a fundamental importance, which consists in obtaining new data on the principles of the organization of neuronal activity in the spinal cord of mammals in the early stages of development. The results obtained allow us to form a complete picture of the processes occurring in the spinal cord of newborn rats and to correlate early patterns of neuronal activity in the motor and sensory zones of the spinal cord with spontaneous movements. The data obtained on the correlated activity in the topographically connected sensory and motor zones of the spinal cord, which is provided by reverse sensory activation during spontaneous movements, are of fundamental importance both for understanding the plasticity and development of local sensory-motor neuronal networks in the spinal cord and for establishing topographic connections between the spinal cord and muscles, as well as between the spinal cord and higher structures of the central nervous system. Studies in the framework of this research carried out on rats of age P5 – P7 (P0 corresponds to the birthday), in a period that, in terms of the level of development of the central nervous system, corresponds to the end of the second - the beginning of the third trimester of intrauterine development in humans. Thus, the results can be transmitted to the fetus who has primitive patterns of locomotor activity similar to newborn rats. In the future, an experimental model developed for this study for registration limb movements and neuronal activity in the spinal cord can also be used to study early activity and to study the pathophysiological mechanisms of both a number of congenital and acquired diseases of the spinal cord.

Methods of the research

In vivo experiments were performed on Wistar rats of age P5 – P7 under conditions of fixation of the spinal column. Preparation of animals for the experiment was carried out using isoflurane anesthesia. To record electrophysiological activity (local field potentials and multiple unit activity), linear multichannel extracellular electrodes were used in the lumbar segments of the spinal cord. At the same time, the motor activity of the limbs of the animal was recorded using piezoelectric devices. In vitro experiments were performed on isolated spinal cord preparations of Wistar rats of P5 – P7 age using the same linear multichannel extracellular electrodes as in the in vivo experiments.

The thesis to be defended:

1. The electrical activity of neurons in the lumbar segments of the spinal cord of newborn rats is characterized by correlated bursts of activity in the sensory and motor zones that occur during movements of the hind limbs.
2. Activation of neurons in the sensory zone of the spinal cord during spontaneous movements of newborn rats is provided by sensory feedback, which is activated during these movements.

The reliability of the data

The reliability of the results is confirmed by a sufficient amount of experimental data and statistical analysis methods.

The research approbation

The materials of the work were presented at the International Scientific Conference “Science of the Future” (St. Petersburg, 2014); IX International Forum of Neuroscience FENS (Milan, Italy, 2014); VI Russian International Conference on Motion Control “Motor control 2016” (Kazan, 2016); International Scientific Conference “Translational Medicine, Present and Future” (Kazan, 2016), II International Scientific Conference “Science of the Future” (Kazan, 2016

Dissertant's personal contribution to the research

This scientific work was carried out with the personal participation of the dissertation in the analysis of literature, the formulation of the aims and objectives of the study, performing experiments, analyzing and discussing the results, and preparing publications.

The main content of the work

Materials and research methods

The experimental procedures in this study were carried out in accordance with protocols approved by the ethics committee of the Kazan State Medical University for the protection of the rights and use of animals for experimental purposes (No. 9-2013), as well as in accordance with EU Directive 2010/63 / EC on work with laboratory animals. All animal use protocols have also been approved by the French National Institute of Health and Medical Research INSERM (protocol No. 007.08.01). For experiments, Wistar rats of the age P5 – P7 days after birth were used (P0 corresponds to the birthday).

In vivo experiments

To solve the formulated problems, we used the method of simultaneous recording of electrical activity in the spinal cord and registration of hind limb movements in newborn rats. During surgery and for a number of experiments, isoflurane anesthesia (1.5%) was used. Local anesthesia in the area of surgical intervention -was carried out by subcutaneous injection of lidocaine. Throughout the experiment, body temperature was maintained at 37C using thermostatted plate on which the experimental animal was located. The spinal cord was covered with silicone oil and was protected by a metal ring attached to the spine using cyanoacrylate adhesive, and the ring itself was attached to the stereotactic frame, which ensured the mechanical stability of the drug (Fig. 1A). Electrical activity was recorded in the lumbar segments of the spinal cord, which are functionally connected to the hind limbs. To record the electrical activity of the spinal cord, we used a linear 16-channel matrix of electrodes with a distance of 100 μm between the electrodes (A1x16-5mm-100-703-A16, Neuronexus Technologies, USA). The matrix of electrodes was placed 0.3–0.5 mm lateral to the spinal veins and sank in the medial-lateral direction at an angle of 17 degrees (Fig. 1B). Registration of electrical activity began 30 minutes after the introduction of the electrode array. Electrophysiological signals were amplified 1000 times and recorded at a sampling frequency of 10 kHz (Digidata, Axon Instruments, USA). Tactile stimulation was carried out by a thin tube mounted on a piezoelectric device (PAB-4010, Nihon Ceratec, Japan), which was driven by short-term rectangular pulses with a frequency of 0.2 Hz (Master 8, AMPI, Israel). For each stimulation point, 100 stimuli were applied. Spontaneous activity was recorded within 30-60 minutes. The movements of the limbs were recorded using a piezoelectric sensor (KPEG165, Kingstate, UK). For the purpose of electrical stimulation, a bipolar electrode of nichrome wire 50 μm thick was immersed in the spinal cord under isoflurane anesthesia. The electrode was lowered in increments of 100–200 μm to a depth value corresponding to the location of the lower recording electrode. On a given depth, the value of the stimulating current was selected in such a way as to cause a registered movement of the limb. Stimulation was carried out by electric pulses with a duration of 50 μs and a frequency of 0.2 Hz. At each selected depth value, 50 stimuli were applied. Before the deafferentation procedure, which was carried out under isoflurane anesthesia, the electrode matrix was removed from the spinal cord, and then the dura mater was opened to access the dorsal roots in the region of the segment in which electrical activity was recorded. The roots were separated with thin tweezers and cut with the help of micro scissors. After that, the matrix of electrodes was again introduced along the initial trajectory to the same depth as before deafferentation. The efficiency of dorsal root transection was monitored to eliminate the sensory-induced response during mechanical stimulation of the corresponding limb.

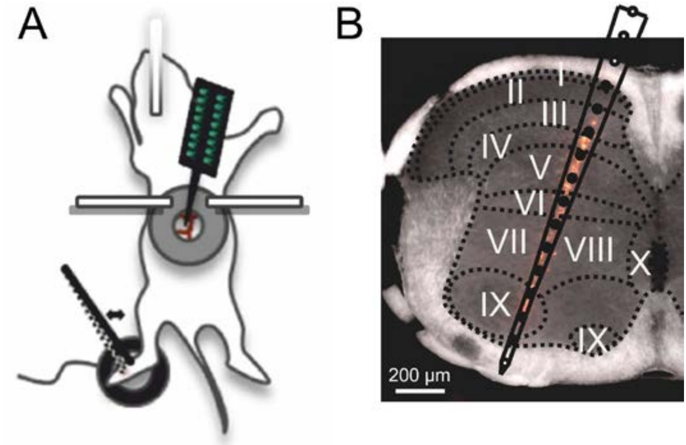

Figure 1 - A. In vivo experimental setup. 1) a newborn rat, mounted on a stereotactic frame using a metal ring; 2) a multi-channel matrix of electrodes for registration of local field potentials and multiple unit activity; 3) a piezoelectric device for detecting movements; 4) a metal tube on a piezoelectric plate for mechanical stimulation of the limbs; B. Epifluorescence image of a slice of the spinal cord and superimposed reconstruction of the position of the recording electrodes. The dashed lines indicate the assumed boundaries of the Rexed laminae.

In vitro experiments

To isolate the spinal cord, the animal was decapitated under isoflurane anesthesia, and after evisceration, the body of the animal was placed in chilled carbogenized (95% oxygen, 5% carbon dioxide) artificial cerebrospinal fluid (ACSF). Then a spinal laminectomy was performed from the sacral to the cervical segment. The dura mater was removed, and the spinal cord, along with the dorsal and ventral roots, was placed in a chamber filled with carbogenized ACSF, where it was kept for at least one hour. Then, the spinal cord was fixed with the dorsal side up in the modified interface type perfusion chamber and perfused with carbogenized ACSF at a rate of 3-4 ml / min at room temperature. The introduction of the matrix of electrodes was carried out similarly to in vivo experiments. The experimental data were analyzed using MiniAnalysis software (Synaptosoft, USA) and the Matlab software package (MathWorks, USA). To compare the samples, nonparametric tests from the Statistics Toolbox for Matlab were used: paired and non-paired Wilcoxon tests. Samples were considered significantly different at $p < 0.05$.

Results of the research and the discussion

Functional mapping of the spinal cord of newborn rats in vivo

To solve the tasks at the initial stage, functional mapping of the sensory and motor zones of the spinal cord of newborn rats was carried out (n = 15). During sensory mapping using tactile stimulation of various parts of the corresponding hind limb, the following were determined: 1) a portion on the hind limb that is topographically connected with the sensory zone of the recorded segment of the spinal cord; 2) channels of the matrix of electrodes located in the sensory zone of the studied segment of the spinal cord. At the same time, topographic stimulation of the hind limb caused maximum values of the negative deflection of local field potentials (LFP) and the frequency of multiple unit activity (MUA) in the surface laminae of the dorsal horn of the corresponding segment of the spinal cord (Fig. 2A).

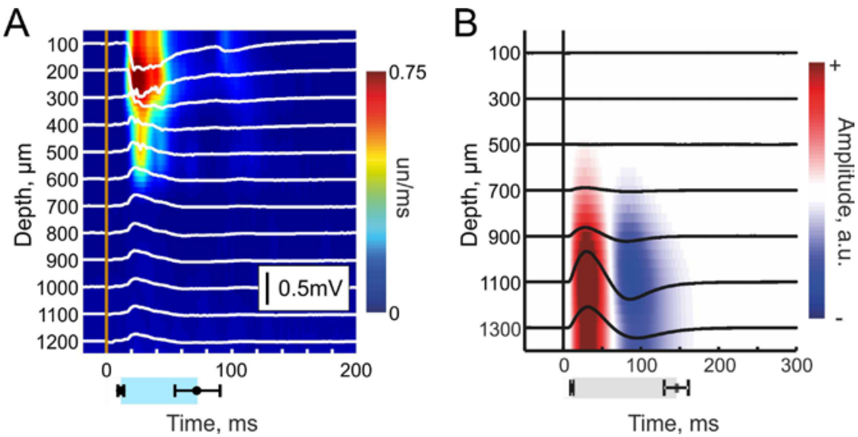

Figure 2 - Functional mapping of the sensory and motor zones of the spinal cord.

A. The frequency of MUA and LFP of sensory-induced responses in the spinal cord of a newborn rat in the presence of 0.5% isoflurane (averaging was carried out over 100 stimuli). LFPs are presented in the form of horizontal white lines, the vertical orange line corresponds to the stimulus time. Dark red color code corresponds to the maximum value of the MUA frequency, dark blue to the minimum. The diagram below shows the average start and end times of a sensory-evoked response ± standard deviations in all experimental animals (n = 15); B. Mechanograms of the movements of the hind limb that occur in response to electrical microstimulation of the spinal cord at various depths. Dark red and dark blue colors correspond to the maximum amplitudes of movements, white color - to the absence of motor activity. The chart below shows the average values of the beginning and end of movement ± standard deviations for all experimental animals (n = 15).

For the purpose of functional mapping of the motor zone, we used the technique of electrical microstimulation of the spinal cord (EMSC) at different depths relative to the surface of the spinal cord using a bipolar electrode with simultaneous recording of movements of the hind limb induced by EMSC. Based on the analysis of the amplitude of motor responses, depending on the depth of the EMSC for each animal, the motor zone was determined, the stimulation of which caused the maximum motor response, and the channels of the recording matrix of electrodes located in the motor zone were determined (Fig. 2B).

General characteristics of the motor activity of the hind limbs in the preparation of newborn rats with spinal fixation.

The motor activity of the hind limbs in animals with spinal fixation was characterized by various types of movements, including short-term twitches and complex non-stereotypic motor events. Based on the analysis of mechanograms of the hind limbs, within the framework of this work, we classified the movements in duration into short-term myoclonic twitches and longer complex movements. For short-term myoclonic twitches, biphasicity was characteristic, with the first fast dominant clonic phase and the subsequent relaxation phase. About half of all analyzed movements were classified as myoclonic twitches (53 ± 15%) with a duration of 252 ± 122 ms, occurring against the background of atony in the absence of previous and subsequent motor activity. Of the remaining movements were episodes of activity with a duration of more than 900 ms were identified, which were classified as complex movements (1.7 ± 1.4 s). Thus, to characterize motor activity in newborn rats with spinal fixation used two main classes of movements - short-term myoclonic twitches and complex movements that characterize motor activity in animals during active sleep and wakefulness, respectively. Patterns of electrical activity of neurons of the spinal cord of newborn rats during myoclonic twitches and complex movements. At the next stage, the spatio-temporal organization of activity in the lumbar segments of the spinal cord was studied, obtained on the basis of registration of the LFP and MUA using a multi-channel matrix of electrodes during myoclonic twitches and complex movements. It was found that electrical activity in the sensory and motor zones of the spinal cord has an intermittent temporal organization, and bursts of activity in both zones correlate with movements of the hind limb. Spontaneous electrical activity was characterized by MUA bursts in motor and sensory zones with frequencies of 4.3 ± 1.0 / min and 3.6 ± 0.9 / min, respectively (n = 15 rats). Analysis of network dynamics in the spinal cord during the time of myoclonic twitching along the profile of the current source density (CSD) and the frequency of MUA revealed that the movements are preceded by short bursts in the motor zone, and bursts of activity in the sensory zone arise after the start of movements (Figs. 3 and 4). It should also be noted that an increase in the frequency of MUA was observed during the entire time course of myoclonic twitches.

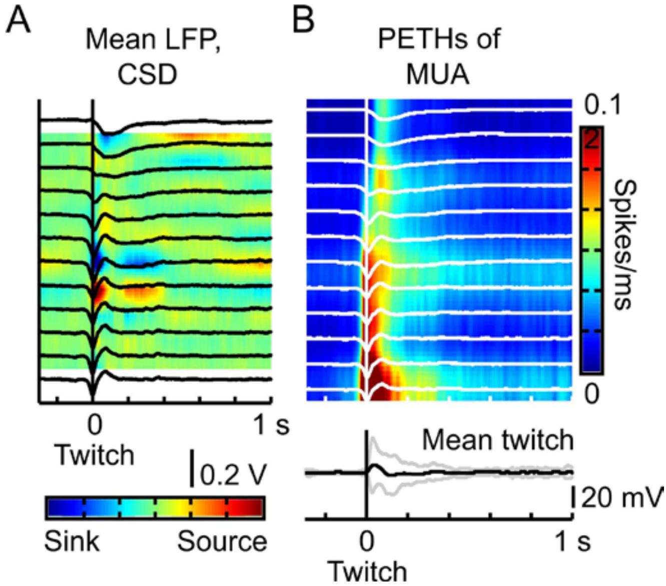

Figure 3 - Electrical activity in the lumbar segment of the spinal cord of newborn rats during myoclonic twitches of the hind limb.

A. Dynamics of local field potentials and the profile of current source density during myoclonic twitches. B. Frequency dynamics of multiple unit activity in the spinal cord during myoclonic twitches. The mechanogram of movements is presented in the form of an average value and standard deviation. Averaging was carried out on the basis of myoclonic twitches (vertical orange line, n = 330 movements).

Cross-correlation analysis of MUA in the lumbar segments of the spinal cord of newborn rats also revealed a high degree of correlation between activity in all laminae of the spinal cord with myoclonic twitching, activation of the motor zone of the spinal cord before movement, and the activation of neurons in the sensory zone occurs with a significant time delay after activation of neurons in the motor zone.

Complex movements were also accompanied by an increase in the level of activity in the sensory and motor zones of the spinal cord, with activation of motor neurons preceding the onset of movements, and the sensory zone was activated with a delay from the beginning of movements. An increase in the MUA frequency in all layers was also observed during the entire duration of complex movements, although a decrease in this activity was observed towards the end of the movements. A comparison of spinal cord activation during myoclonic twitches and complex movements did not reveal any fundamental differences in the spatiotemporal organization of activity in different zones of the spinal cord, with the exception of the fact that complex movements were accompanied by longer episodes of both motor and neuronal activity in the spinal cord the brain. Considering that the two types of movements studied characterize the functional states of sleep and wakefulness, the data obtained indicate that the organization of the network activity of the spinal cord does not fundamentally differ in these two states.

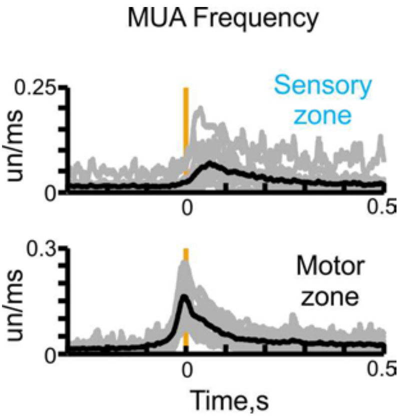

Figure 4 - Group data on the dynamics of the MUA frequency in the sensory and motor zones of the spinal cord of newborn rats during myoclonic twitches of the hind limb.

Data are presented as mean values (indicated in black) and individual values for each animal (indicated in gray). The vertical orange line corresponds to the start of movement (n = 15 rats).

The effect of deafferentation on electrical activity in the spinal cord of newborn rats.

At the next stage, it was studied how deafferentation of the lumbar segments affects the activity in the sensory and motor zones of the studied segments during spontaneous movements. It was assumed that in the case of activation of sensory zones neurons by an efferent copy, activation of the sensory zones during movements would have to be maintained after deafferentation. If activation of the sensory zone during movements is associated with sensory feedback, then deafferentation should lead to the elimination of neuron activation in the sensory zone during movements and to the separation of activity in the sensory and motor zones. The sequence of experimental procedures included functional mapping and registration of spontaneous activity under control conditions with subsequent withdrawal of the recording sample from the spinal cord. Then, using isoflurane anesthesia (1.5%), ipsilateral dorsal roots were cut at the level of lumbar enlargement and nearby segments. Following this, a recording probe was introduced back into the spinal cord in the same position as before deafferentation, and spontaneous electrical activity and movements of the hind limb were again recorded. After transection of the dorsal roots, a variety of patterns of motor activity of the limbs was observed, including myoclonic twitching and complex movements, the parameters of which did not differ significantly before and after deafferentation. After deafferentation, LFP signals and bursts of the MUA frequency in the motor zone associated with movements were also preserved, and no significant change in the frequency of bursts and the frequency of MUA in the motor zone was found (Figs. 5 and 6). However, in the sensory zone, activity underwent significant changes due to deafferentation. So, after transection of the dorsal roots in the sensory zone, a significant decrease in the frequency of bursts (by 66 ± 30%) and the level of the MUA frequency by 61 ± 28% was observed (Fig. 5), as well as the complete suppression of neuron activation in the sensory zone during myoclonic movements (Fig. 6). Similar the picture with the suppression of activity in the sensory zone and its conservation in the motor zones was also observed during complex movements. Cross-correlation analysis of APs also showed that after deafferentation, the correlation between APs in the motor and sensory zones was significantly reduced both during myoclonic twitches and during complex movements. The data obtained show that the prevailing paradigm about the functioning of the spinal cord of newborn rats is the generation of spontaneous outbursts of activity in the motor zone, which cause myoclonic twitching and complex movements, and then, through the sensory feedback mechanism, neurons are activated in the sensory zone. The complete suppression of activation of neurons in the sensory zone during movements due to deafferentation is direct evidence of the hypothesis of the dominant role of sensory feedback in the coordination of sensory-motor networks of the developing spinal cord.

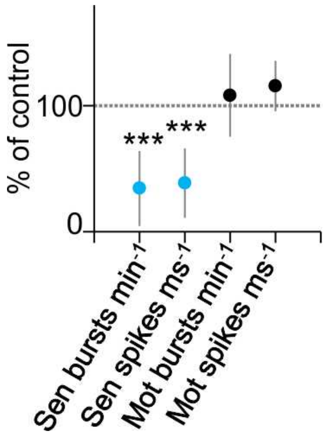

Figure 5 - Change in the MUA frequency and bursts frequency in the sensory and motor zones of the spinal cord as a result of deafferentation. The control values for each of the parameters are taken as 100%. Parameters for the sensory and motor zone are presented (blue and black color-coded, respectively) (n = 3 rats).

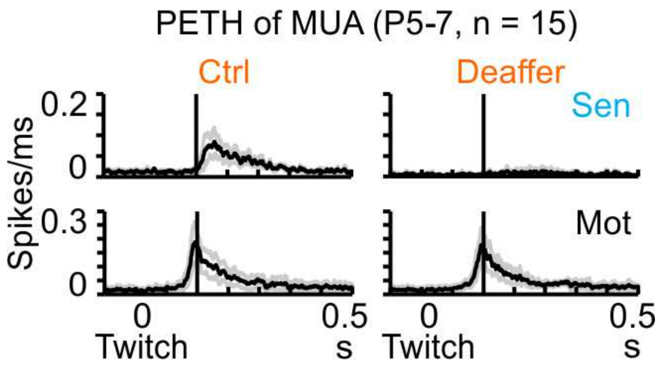

Figure 6 - Group data on the dynamics of the MUA frequency in the sensory and motor zones of the spinal cord of newborn rats during myoclonic twitches before and after deafferentation.

Data are presented as mean values (indicated in black) and individual values for each animal (indicated in gray). The vertical black line corresponds to the start of movement (n = 3 rats).

Electrical activity in the isolated spinal cord of newborn rats in vitro

The next step was to study the activity in the lumbar segments of an isolated spinal cord in vitro - in a preparation in which both sensory inputs and motor efferents were cut, but the connections inside the spinal cord were preserved, which could provide coordinated activity neurons of the sensory and motor zones through an efferent copy. The position of the recording electrodes in the lumbar segment of the spinal cord in rats of age P5-P7 was similar to the position of the electrodes when recorded in vivo. An example of the recording of electrical activity in the lumbar segment of an isolated spinal cord in vitro is presented in Figure 7.

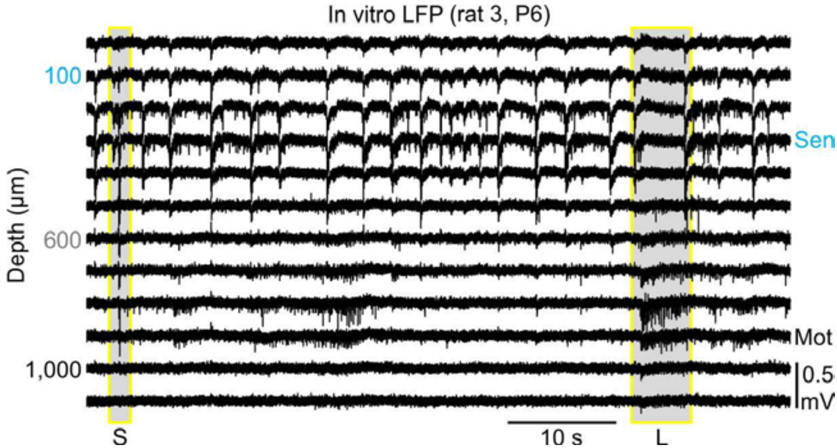

Figure 7 - An example of recording electrical activity in the lumbar segment of the isolated spinal cord of a newborn rat.

The presented LFPs demonstrate various types of spontaneous activity. Yellow frames highlight examples of, respectively, short-term and long-term bursts in the motor zone.

Activity in the dorsal horns in the isolated spinal cord was organized in the form of frequent short bursts occurring with a frequency of 15 ± 4.9 / min and a duration of 119 ± 28 ms (n = 5 rats, 6860 flashes). Bursts of activity in the sensory zone were characterized by local negative deflections of the LFP and an increase in the frequency of MUA in the dorsal horns and, as a rule, the absence of associated activity in the ventral horns (Fig. 8). An analysis of CSD revealed during these bursts the presence of a powerful single sink in the dorsal horns. Short bursts in the sensory zone were similar in characteristics to bursts that were observed during myoclonic twitching in vivo, but the frequency of bursts in vivo was significantly lower. In addition, unlike data obtained in vivo, these bursts in the dorsal horns had a fundamentally different temporal organization with respect to activity in the ventral horns. In particular, bursts in the sensory zone were not significantly preceded by activity in the motor zone (within 100 ms interval), and only 3.7 ± 1.9% of sensory bursts were associated with subsequent activity in the ventral horns. Another difference of activity in dorsal horns in isolated spinal cord in vitro there was a lack of prolonged episodes of activity that were observed during complex movements in vivo.

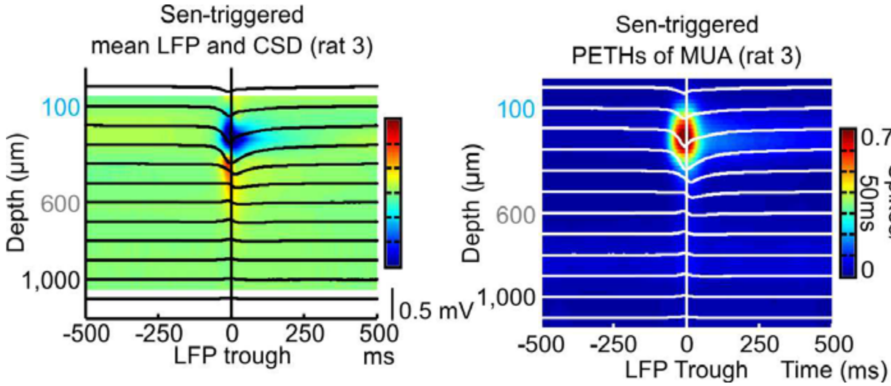

Figure 8 - The depth profile of electrical activity in the isolated spinal cord of newborn rats during bursts of activity in the sensory zone.
The average CSD profile (left) and the dynamics of the MUA frequency (right) are presented. Average was carried out over the peak of the LFP in the sensory zone (vertical orange line).

In the ventral horns of the spinal cord, activity was represented by two main patterns, which, in the first place, differed in their duration and included short bursts with a duration of 62 ± 14 ms and long discharges lasting 3.0 ± 0.3 s. Short bursts occurring at a frequency of 1.9 ± 0.7 / min were similar to short bursts observed during twitch in vivo. However, unlike in vivo experiments, bursts in the ventral horns in the isolated spinal cord in vitro fundamentally differed in their temporal correlation with activity in the dorsal horns. While in vivo activation of neurons in the dorsal horns followed by short bursts in the ventral horns, in vitro this sequence was inverted, and activity in the dorsal horns was preceded by short bursts in the ventral horns. Wherein 16.4 ± 9.7% of short bursts of activity in the motor zone were preceded by bursts in the sensory zone. Averaging of LFP and CSD, and the MUA frequencies at the peak of the LFP of short bursts in the motor zone showed the presence of sink and an increase in the frequency of MUA in the ventral horns, as well as the preceding sink and bursts of MUA in the dorsal horns (Fig. 9). In addition to short patterns, activity in the ventral horns was also represented by rare (1.7 ± 0.3 / min) and prolonged bursts of activity organized in oscillations with a frequency of 6.0 ± 0.1 Hz, but not accompanied by activation of sensory zone. Thus, the spatiotemporal organization of activity in the isolated spinal cord of newborn rats in vitro significantly differed from in vivo activity by a significantly lower and time-inverted correlation of neuronal activity in the sensory and motor zones.

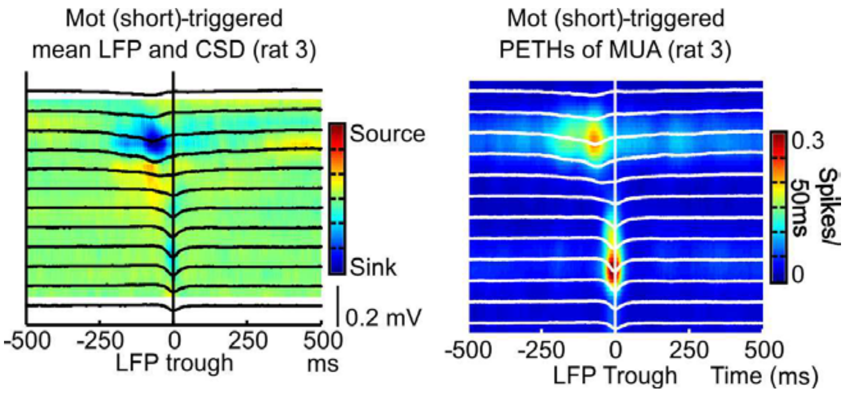

Figure 9 - The depth profile of electrical activity in the isolated spinal cord of newborn rats during short bursts in the motor zone.

The average CSD profile (left) and the dynamics of the MUA frequency (right) are presented. Averaging was carried out over the peak of the LFP in the motor zone (vertical orange/white line).

Pharmacological analysis of the mechanisms of spontaneous activity in the isolated spinal cord of newborn rats

The next step was an analysis of the mechanisms of functioning of the networks of the isolated spinal cord in vitro using pharmacological methods. For this, glutamate and GABA-A receptor antagonists were applied. Pharmacological analysis showed that the combined use of ionotropic glutamate receptor antagonists CNQX (20 μM) and APV (50 μM) completely suppresses spontaneous activity in both sensory and motor zones (Fig. 10A), which indicates the key role of glutamatergic mechanisms in the generation of spontaneous bursts in the sensory and motor zones in the isolated spinal cord of newborn rats.

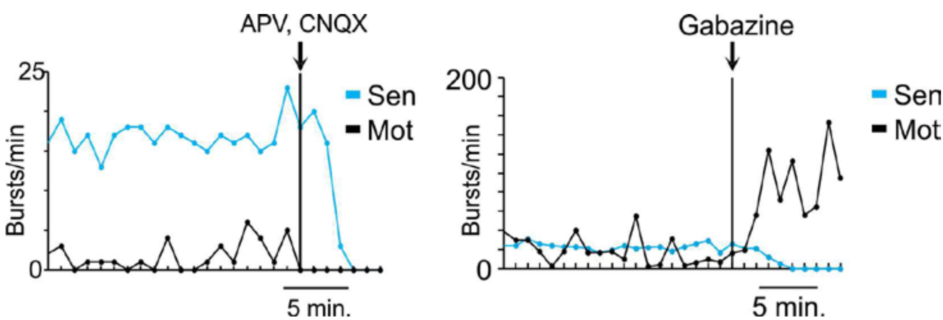

Figure 10 - Dynamics of changes in the frequency of bursts in the motor and sensory zones of the isolated spinal cord in vitro under control conditions and after application

of APV and CNQX (A) and gabazine (B). The interval for calculating the parameters is 1 minute. The vertical black line corresponds to the APV and CNQX / gabazine application times.

Application of the GABA-A receptor antagonist of gabazine (5 μM) led to the elimination of regular flashes in the sensory zone and significantly increased the LFP power and the MUA frequency during long bursts in the motor zone (Fig. 10B) with the elimination of 6-Hz oscillations, which indicates inhibitory effect of GABA-A receptors and the participation of GABAergic interneurons in the generation of oscillations in the neuronal networks of the ventral horns of an isolated spinal cord.

The presented data show that even highly synchronized activity in the ventral horn caused by blocking inhibition does not lead to activation of neurons in the primary sensory zone of the dorsal horn, which again indicates the absence of intraspinal connections that can provide coordinated activity in the sensory-motor networks of the spinal cord brain in newborn rats through an efferent copy. At the same time, the elimination of activity in the dorsal horns of the spinal cord during blocking of GABA-A receptors indicates the participation of GABAergic mechanisms in the synchronous excitation of neurons in the sensory zone during activity, which is probably due to the stimulating effect of GABAA on neurons in the dorsal horns of the spinal cord brain of newborn rats in vitro.

Conclusion

In this work, we studied the spatiotemporal dynamics of activity in sensory and motor networks of the spinal cord of newborn rats in vivo during spontaneous movements of the hind limbs, as well as spontaneous electrical activity in the isolated spinal cord of newborn rats in vitro. In in vivo experiments, during myoclonic twitches and complex movements, a high degree of correlation of activity in the sensory and motor areas of the spinal cord was observed according to a consistent time pattern: motoneurons - movements - neurons of the sensory zone. Deafference by the method of local dorsal root cutting suppressed activity in the sensory zone, and thereby eliminated sensory-motor coordination, without significantly affecting the characteristics of movements and activity parameters in the motor zone. In contrast to in vivo experiments, in vitro activity in the isolated spinal cord was characterized by much weaker interactions between the sensory and motor zones. In addition, sensory bursts observed in an isolated spinal cord in vitro preceded motor bursts, which is fundamentally different from the data obtained in in vivo experiments. A pharmacological analysis of network activity in an isolated spinal cord in vitro revealed the key role of glutamatergic mechanisms in the generation of activity in both the motor and sensory zones, as well as the multidirectional effects of GABA - inhibitory in the motor zone and excitatory in the sensory zone. Together, the data obtained in vivo and in the isolated spinal cord in vitro are direct evidence of the crucial role of sensory feedback in coordinating activity in the sensory and motor zones of the spinal cord during spontaneous movements.

Findings

1. The developed method of functional mapping using combined local tactile stimulation of the limbs and electrical microstimulation of the spinal cord made it possible to localize sensory and motor areas in the lumbar segments of the spinal cord of newborn rats.
2. An analysis of the motor activity of the hind limbs revealed short myoclonic twitches characteristic of the state of active sleep and long complex movements characteristic of the waking state of newborn rats.
3. Spontaneous movements of the limbs of newborn rats during sleep and wakefulness are accompanied by coordinated bursts of neuron activity in the motor and sensory zones corresponding lumbar spinal cord segment. In this case, activation of motor neurons precedes the beginning of movements, and the beginning of activation of neurons in the sensory zone follows the beginning of movements.
4. Deafferentation eliminates the activation of sensory zone neurons during spontaneous movements without changing the activity of motor neurons and movement characteristics, which is direct evidence of feedback sensory connection as the main mechanism of activation of sensory zone neurons during spontaneous movements.
5. The spatiotemporal organization of activity in the isolated spinal cord of newborn rats in vitro differs significantly from the in vivo activity of a significantly lower and time-inverted correlation of neuronal activity in the sensory and motor zones.
6. In vitro glutamatergic and GABAergic mechanisms are involved in the generation of activity in the sensory and motor zones of the isolated spinal cord of newborn rats in vitro.

Publications

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