As a manuscript

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THE ROLE OF DIFFERENT KINDS OF AFFERENCE INFLUENCES IN ACTIVATION OF THE SOMATOSENSORY CORTEX IN NEWBORN RATS

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ABSTRACT
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The work was carried out on the basis of the research laboratory "Neurobiology" of the Department of Human and Animal Physiology of the Institute of Fundamental Medicine and Biology of Kazan (Volga Region) Federal University (Kazan).

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With the dissertation can be found in the Scientific Library named after N.I. Lobachevsky at the Kazan (Volga Region) Federal University.

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Scientific Secretary of the Dissertation Council,
Doctor of Biological Sciences, Professor Abramova Z.I.

GENERAL DESCRIPTION OF WORK

The relevance of the research theme and the degree of its development

The central question of developmental neurobiology is the question of how neurons establish specific contacts with each other in the process of ontogenesis to form a functional, "thinking" brain. Two key and complementary mechanisms are involved in this process: genes and activity. While genes determine the phenotype of neurons and the general location scheme of nerve cells and their processes, the correlated activity of neurons provides a more subtle functional tuning of neuronal networks. The role of activity is most important during the "critical" periods of development, during which the disruption of activity leads to the most dramatic and sometimes irreversible defects in the structure and function of neural networks (Fox, 1992; Erzurumlu and Gaspar, 2012). Understanding the mechanisms underlying critical periods is one of the most urgent problems of modern developmental neuroscience.

The classical model for the study of developmental neural networks during the critical period is the primary somatosensory cortex in rodents (rats and mice) (Khazipov et al., 2004), and in particular the part which provides processing of sensory information from the whiskers on the animal's muzzle - the barrel cortex (Petersen, 2007). The convenience of this experimental model is due to the fact that each of the whiskers is represented in the somatosensory barrel cortex by a specific morpho-functional module - the cortical column, and there is a barrel in its IV layer (Fox, 2008; Bosman et al., 2011). And the first week after birth in rodents is a critical period of the formation of thalamocortical somatotypic maps in the barrel cortex (Fox, 1992; Erzurumlu and Gaspar, 2012). A variety of manipulations with whiskers (Fox, 1992; Simons and Land, 1987), and also the blocking of activity in the barrel cortex (Van der Loos and Woolsey, 1973) during the first week after birth lead to significant disorders in the development of somatotypic barrel maps, which indicates the critical importance of sensory activity for the development of these maps in the postnatal period. However, the physiological processes underlying the sensory-dependent plasticity in the developing barrel cortex remain only fragmentarily studied. So, it was shown that activity in the barrel cortex during the critical period is characterized by intermittent temporary organization and unique patterns of burst oscillatory activity (early oscillatory patterns - EOP), including early gamma-oscillations (RGO) (Minlebaev et al., 2011) and spindle bursts (SB) (Minlebaev et al., 2007). The studies at the cellular level, as well as simultaneous registration of activity in the thalamus and cortex (Minlebaev et al., 2011; Yang et al., 2013) revealed the key importance of thalamic inputs in the generation of these early patterns of activity, as well as their involvement in the processes of long-term plasticity in thalamocortical synapses. It was revealed that sensory stimulation is an effective generator of SB (Khazipov et al., 2004; Minlebaev et al., 2007) and EGO (Minlebaev et

al., 2011), and isolated stimulation of a single whisker specifically causes the EGO in the corresponding cortical column of the barrel cortex (Minlebaev et al., 2011). In spite of the fact that in aggregate these data clearly indicate that the early patterns of activity, that are triggered in the barrel cortex by the sensory input from the whiskers, are involved in plasticity processes in newborn animals (Erzurumlu and Gaspar, 2012), there remains a whole range of unresolved issues related to the physiology of the developing somatosensory system during the critical period.

One of these little-known and topical questions is the question of what mechanisms provide sensory stimulation of whiskers and barrel cortex in natural conditions. It is known, that all previous knowledge about the functioning of the "whisker-barrel" system were obtained under artificial experimental conditions on isolated newborn rats (Minlebaev et al., 2011; Minlebaev et al., 2007; Yang et al., 2013; Tiriac et al., 2012; Tiriac et al., 2014; Minlebaev et al., 2009; Yang et al., 2009). In this case, the role of different types of afferent influences in the activation of the somatosensory cortex of newborn rats under natural conditions remains unknown. We suggested that there are two potential mechanisms that could contribute to stimulating the sensory inputs from the whiskers: (1) passive stimulation of whiskers from littermates and mother and (2) sensory activation during active whisker movements, including: (2a) reafferent signals that arise with free whisker movements and are proprioceptive in terms of their functional significance in the whisker system and (2b) exoafferent (tactile) signals that occur by active whisker touch with external objects (Khazipov et al., 2004; Tiriac et al., 2014; Moore et al, 2015; Tiriac et al., 2015). How important and what is the relative contribution of each of these mechanisms to the sensory activation of the barrel cortex in newborn animals in natural conditions remains unknown. This information is fundamental for understanding how the somatosensory system functions during the critical period of development.

The aim of work – to study the role of different types of afferent influences and their comparative contribution to the activation of the somatosensory cortex in newborn rats under natural conditions, including sensory inputs that are activated during active whisker movements, as well as when whiskers are stimulated by littermates.

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

1. To characterize the early patterns of whisker movements in newborn rats.
2. To investigate the activity in the somatosensory cortex of newborn rats arising as a result of whisker movements, as well as to reveal differences in cortical activity caused by free whisker movements and movements accompanied by tactile contact with passive external objects.
3. To characterize the responses caused in the somatosensory cortex by contact with littermates at their location, simulating the natural location of the animals in the nest.

4. To study the effect of acute sensory deprivation caused by the infraorbital nerve cut on the cortical activity in the somatosensory cortex of newborn rats to assess the spontaneous activity of the thalamocortical oscillator.
5. To evaluate the comparative contribution of various afferent influences and spontaneous activity of the thalamocortical oscillator to the activity of the newborn rat somatosensory cortex under conditions mimicking the natural environment.

Scientific novelty of the work

For the first time, was characterized the activity in the barrel cortex of newborn rats under conditions mimicking the natural environment, and was performed detail analysis of the mechanisms providing sensory stimulation of the somatosensory cortex in the region of the whisker representation during the critical period of development of somatosensory maps. It was shown for the first time that both passive whisker stimulation by the littermates and sensory signals that occur during active whisker movements are effective generators of bursts of early oscillatory activity in the barrel cortex in newborn rats. At the same time, was revealed a greater efficiency of the active whisker movements, accompanied by their tactile contact with external objects. Comparative analysis of different types of afferent influences allowed for the first time to determine the equal importance of passive stimulation and active whisker movements under conditions as close to natural as possible, and also revealed that these two mechanisms provide almost three times the higher level of cortical activity than that supported by the thalamocortical oscillator, devoid of sensory input.

Theoretical and scientific-practical significance of the work

The theoretical significance of the results of the research consists in revealing various types of afferent influences in the activation of the somatosensory cortex on the "whisker-barrel" system model and in describing the functions of the somatosensory cortex during the critical period of topographic somatosensory map formation in conditions as close to natural as possible. The role of passive whisker stimulation by the littermates, as well as reafferent and exoafferent signals arising during active whisker movements, has fundamental importance for understanding the nature of sensory input, which is critical for the normal development of thalamocortical somatosensory maps in the postnatal period. The obtained data also indicate a significant role of the environment for providing a physiological sensory input from whiskers of newborn rats, which is fundamentally distinguishes the way of somatosensory system works during the critical period from the visual and auditory systems, the functioning of which does not depend on sensory inputs from the external environment and is provided by a spontaneous activity at the level of the sensory periphery.

These conclusions can be of great practical importance for the development of optimal conditions for the development of premature newborns, who often have disorders

in the development of sensory systems. Early stages of the nervous system development, including the formation of somatosensory maps, in humans occur *in utero* during the second half of gestation. In this case, the activity patterns which homologous to the oscillatory bursts of activity, observed in the newborn rat cortex, are caused in the somatosensory cortex of premature newborns by external sensory stimulation and spontaneous physiological twitches. However, there is a significant difference in the conditions in which movements occur *in utero* and *ex utero*: if *in utero* a fetus is compact and almost all parts of its body are in contact with each other and the wall of the mother uterus, and the fetus movements are accompanied by tactile contact, accordingly, then *ex utero* premature baby is located with freely positioned arms and legs in the cuvette, which reduces the probability of tactile contact. This can lead to a decrease in sensory activation of the cortex, and, as a consequence, to disturbances in the somatosensory system development. Thus, the observations, obtained on the model of newborn rats and evidencing the important role of tactile feedback in the activation of the somatosensory cortex during spontaneous movements, raise the question for perinatologists about providing such tactile contact in the preterm neonate management.

The thesis to be defended:

- The afferent activation of the primary somatosensory cortex of newborn rats under natural conditions is provided by a set of mechanisms that include: (1) re-afferentation - sensory feedback that is activated by free whisker movements, (2) exoafferentation - tactile feedback that is arised by whisker movements during its contact with external objects and (3) external tactile stimulation, which is provided by spontaneous movements by the littermates.

Realization of research results

11 published works were published on the aim of the dissertation. Of these, 5 articles were published in peer-reviewed scientific journals included in the Higher Attestation Commission list.

BASIC CONTENT OF WORK
MATERIALS AND METHODS OF RESEARCH

Animal preparing for experiment. This work has been performed in accordance with ethical requirements for animal experiments, and all animal-use protocols were approved by the Institutional Animal Care and Use Committee of Kazan State Medical University (N9-2013). Newborn rats of both sexes from postnatal days (P) 1–7 were used. Preparation of the animals (surgery) for electrocorticogram (ECoG) recording was performed under isoflurane anesthesia. Recordings from the nonanesthetized newborn rat were performed 1 day after surgery. A chlorided silver wire, placed behind the somatosensory cortex, served as a ground electrode.

Transection of the infraorbital nerve was performed under deep isoflurane anesthesia.

Sensory stimulation of whiskers. For mechanical whisker stimulation, the whiskers were trimmed to a length of 0.8–1.5 mm. The tip of the whisker was inserted 0.5 mm into the blunt tip of a needle glued to the end of piezo actuator. To induce deflection of the piezo actuator, square 30–40 V pulses of 2 or 10 ms duration were applied at 10–20 s intervals.

The experiments with middle branch of the motor facial nerve (*bs, ramus buccolabialis superior*) stimulation were performed in urethane-anesthetized rat pups (1.5 g/kg, i.p.). The *ramus buccolabialis superior* was exposed by a 1- to 2-mm-long incision above the its middle part and stimulated by bipolar electrodes at a frequency of 0.1–0.2 Hz. The stimulus amplitude and duration were selected individually for each animal and did not exceed 50 V and 2 ms, respectively (Figure 7).

Extracellular recording of brain activity and subsequent analysis. Extracellular recording of neuronal activity in barrel cortex was performed on anesthetized and nonanesthetized newborn rats P1-7. As an anesthetic, urethane with a final concentration (1.5 g/kg, i.p.) was used. Extracellular recordings of the local field potential (LFP) and the multiple unit activity (MUA) were performed from a principal barrel column. Principal whisker (PW) was determined by the minimum delay between the stimulus and the induced response in the recorded barrel. The whisker was deflected by the piezo actuator in the caudal direction. Multichannel linear electrodes, with 16 channels, 50 μm separation distance, on a silicon substrate (Neuronexus Technologies, USA) we used in the experiments. Electrodes were vertically inserted into principal cortical column at a depth of 680-1100 μm from the cortical surface (depending on the animal age), to level IV layer. The signals were amplified and filtered (x10,000; 0.1 Hz to 10 kHz) using a DigitalLynx (Neuralynx, USA) amplifier with 256 channels, then digitized at 32 kHz and saved on a PC for post hoc analysis. Raw data were preprocessed using custom-written functions in Matlab (MathWorks, USA).

Video registration of whisker movements in newborn rats and subsequent analysis. Gouache paint was applied to the trimmed tips of the principal whisker (PW) and neighboring whiskers. One or two additional gouache marks were made on the whisker pad skin to serve as reference points. A high speed digital camera (Promon 501, AOS Technologies, Switzerland) with a TEC-M55 objective (Computar, USA) was placed along the PW axis to record whisker movements at 100 frames/s and 480 x 320 resolution in two consecutive 15-min-long recordings (Figure 1). The analysis of the video recordings was performed using ProAnalyst 1.5.7.4 (Xcitex, USA).

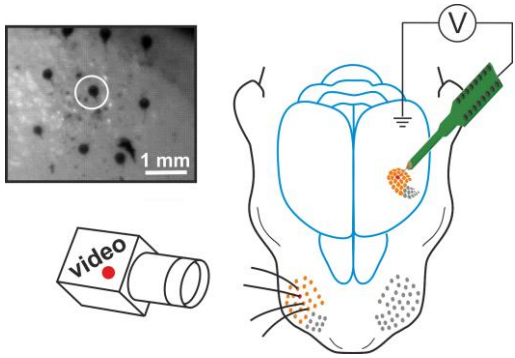

Figure 1 - The scheme of simultaneous video registration of whisker movements and recording of extracellular activity of the barrel cortex in the left hemisphere using a linear 16-channel electrode. Note: a photo of the whisker pad region with the C2 principal whisker (the calibration segment corresponds to 1 mm, top left), a multichannel is inserted into the C2 principal cortical column (right).

Video registration of muzzle movements of two littermates and subsequent analysis. The littermate was cotton wrapped and positioned closely (≤ 3 mm) to the recorded rat pup (Figure 12). The muzzle movements of two littermates were recorded with Promon 501 (AOS Technologies, Switzerland) и QICAM Fast 1394 (QImaging, Canada) digital cameras. The line (1-D) method was used to detect the movements of the whisker pad region of each pup.

Video registration of the rat pups behavior in natural conditions (in the nest). The observation of the pup's interactions with the littermates, mother, and passive objects in the nest was performed using a C270 (Logitech, Switzerland) camera with 3 h/d video recordings of 1 or 2 rat pups per litter in their home cage in the habitual environment of the animal house (n = 6 litters, 8–12 rat pups per litter, the behavior of 13 rat pups was investigated; P0–5).

Statistical analysis was performed using the Matlab Statistics toolbox and Origin. Statistical comparisons between the groups were performed using the Wilcoxon tests (Wilcoxon rank sum test and paired sample Wilcoxon signed rank test). The significance level was set at $p < 0,05$. Group data are expressed as mean ± standard error (SEM), unless otherwise indicates.

RESULTS OF RESEARCH AND THEIR DISCUSSION

1. Whisker movement patterns in neonatal rats

Spontaneous free whisker movements were recorded in nonanesthetized newborn rats at the age of P2–7 (P - postnatal day, P0 - day of the birth). One of the video recording frames of the P5 experimental animal's snout with the whisker tips marked with a black paint is shown on Figure 2A. Figure 2B shows the example traces of the four whisker movements labeled in Figure 2A. As can be seen from the presented example, the whisker movements are characterized by different types of movements on amplitude and duration. To quantify the whisker movements, their directions, amplitude-time parameters and the degree of synchronism of movements were analyzed. As a result of the analysis, the main types of whisker movements in newborn rats were two main groups: (1) short-duration unidirectional (preferentially rostral or caudal) events, illustrated in Figures 2B, 3 and marked with green and red asterisks, and (2) longer-lasting episodes of complex movements, marked with a blue asterisk (Figures 2B, 3). The overwhelming majority of whisker movements in newborn rats was collective, and whiskers deviated simultaneously, in unison (Figures 2B, 3).

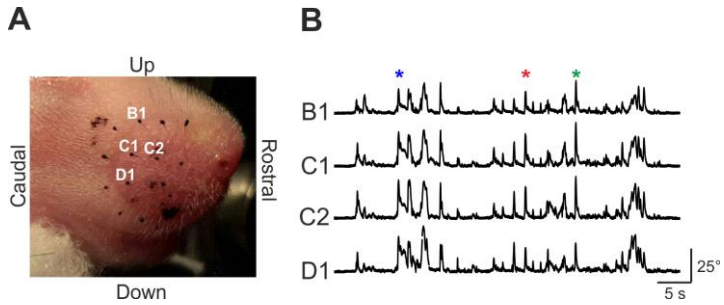

Figure 2 - Whisker movement patterns in neonatal rats. (A) Snapshot of a P5 rat snout with the whisker tips marked with a black paint. (B) Example traces of the four whisker movements labeled on (A). Note: example traces reflect the angle of whisker deflection from the rest position.

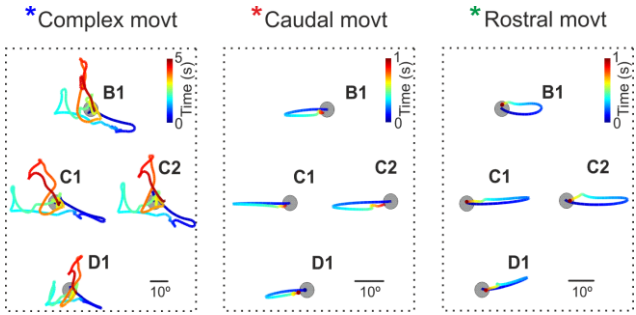

Figure 3 - Whisker movement patterns in neonatal rat P5. Note: time-colored traces of the four whisker movements marked with asterisks on 2B. Blue color - the beginning of movement, red color - the end of movement. Grey circle - the rest position of whisker.

Thus, the movement patterns in the neonatal rat pups share some similarities with the movement patterns in adult animals, including the predominance of collective movements in the forward-backward (protraction-retraction) direction (Hill et al., 2008; Welker, 1964; Bermejo et al., 2002). However, the neonatal whisker movements are not yet organized in active whisking (Landers and Philip, 2006; Grant et al., 2012; Welker, 1964) and include primitive intermittent patterns of brief twitchy events and more complex movements (Tiriac et al., 2012), which are also observed in the skeletal musculature during the neonatal period (Khazipov et al., 2004; Tiriac et al., 2015; Gramsbergen et al., 1970). We further explored how these primitive whisker movements correlate with the activity in the somatosensory barrel cortex.

2. Activity in the neonatal rat barrel cortex during free principal whisker movements and active touch

In the next series of experiments on newborn rats, the correlation between spontaneous whisker movements and activity in the barrel cortex was investigated. Registration of extracellular electrical activity in all layers of one cortical column in the barrel cortex was carried out using a 16-channel electrode. This type of intracortical recording allows to obtain information about two key parameters of cortical activity, including (1) the local field potential (LFP) and (2) the multiple unit activity (MUA), wherein the current source density (CSD) analysis also makes it possible to characterize the main synaptic pathways that are involved in the generation of cortical activity. An important aspect of the simultaneous recording of the whisker movements and barrel cortex activity was that for each recorded cortical column was identified its principal whisker (PW). The PW was identified by a comparative analysis of sensory responses induced in the cortical column by stimulation of individual whiskers by two main criteria: (1) the maximal amplitude of sensory evoked potential (SEP); (2) short latency IV layer MUA followed by early

gamma oscillations (EGO) and spindle bursts (SB) in response to a brief mechanical deflection (Minlebaev et al., 2011; Mitrukhina et al., 2015).

Correlation analysis between cortical column activity and PW movements was performed in two experimental configurations: (1) free whisker movements and (2) movements with touch, when an object was placed into the PW trajectory (Figure 4).

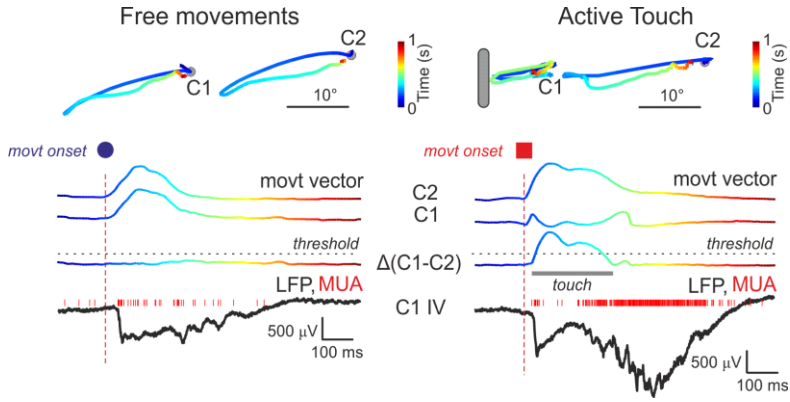

Figure 4 - The color-coded example traces free movement of C1 and C2 whiskers (top left) and a movement associated with C1 whisker contact with an external object (top right) obtained from a P4 nonanesthetized rat pup. Note: the amplitude vectors of the whisker deflections reflect the angle of deflection from the rest position (top). Corresponding movement amplitude vectors and the result of their subtraction - Δ(C1–C2) (bottom). The color encodes the time between the movement onset and the end. Simultaneous LFP (black traces) and MUA (red bars) recordings from IV layer of C1 barrel column (bottom). Vertical dashed lines indicate movement onsets.

A quantitative analysis of the correlation between the whisker movements and activity in the IV layer of the corresponding barrel column revealed a number of significant differences between cortical activity that occurs during free movements and movements with touch:

(1) MUA frequency. During free whisker movements, IV layer MUA increased from 6.6 ± 2.8 spikes/s of baseline activity to 49.1 ± 40.1 spikes/s within a 1 s time window after the movement onset. Movements with touch were associated with an almost twofold stronger increase to 98.5 ± 55.1 spikes/s (n = 5 pups; P4–7; p = 0.031; paired sample Wilcoxon signed rank test; Figure 5).

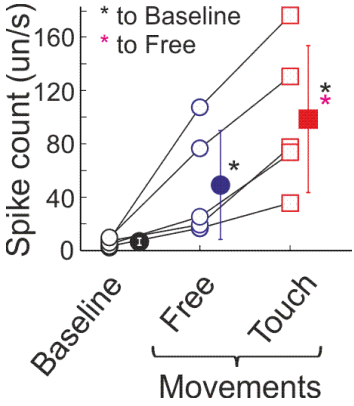

Figure 5 - Group data from the five P4-7 nonanesthetized rat pups on the total spike counts in IV layer of principal barrel column evoked by free PW movements and movements with touch. Note: baseline MUA frequency was assessed within 1 s of baseline average (p = 0,031; paired sample Wilcoxon signed rank test).

(2) LFP power in the spindle burst (8–29 Hz) and gamma (30–90 Hz) frequencies showed an increase during free movements to 41.6 ± 11.1 μV^2/Hz and 4.9 ± 1.3 μV^2/Hz, respectively, whereas during movements with touch, it attained 71.9 ± 18.5 μV^2/Hz (spindle bursts) and 7.3 ± 1.7 μV^2/Hz (gamma oscillations) (n = 5 pups; P4–7; p = 0.031; paired sample Wilcoxon signed rank test; Figure 6).

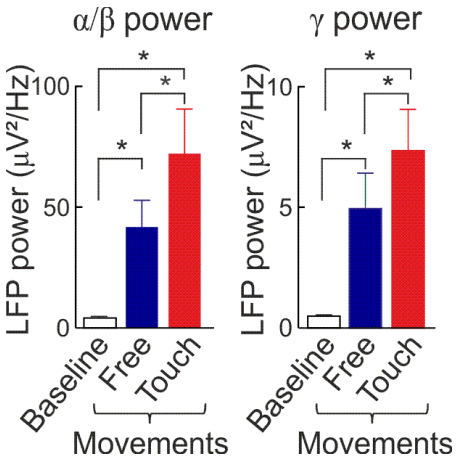

Figure 6 - Group data from the five P4-7 nonanesthetized rat pups on the LFP power in α/β and γ frequency bands in IV layer of principal barrel column evoked by free PW movements and movements with touch. Note: baseline LFP power was assessed within 1 s of baseline average (p = 0,031; paired sample Wilcoxon signed rank test).

The obtained data indicate that whisker movements are a rather effective trigger for barrel cortex activation in newborn rats, but movements accompanied by tactile contact with the external object are more effective than free movements. To estimate the impact of tactile contact during whisker movements on the total ongoing activity in the barrel cortex, we compared the mean IV layer MUA from 30 min recording sessions of free whisker movements with 30 min recording sessions of whisker movements in con-

tinuous contact with various naturalistic passive objects. We found that overall the IV layer MUA increases from 10.9 ± 1.8 spikes/s during free whisker movements to 13.3 ± 2.2 spikes/s during continuous contact with the passive objects (n = 4 pups; P2–6; p = 0.029; Wilcoxon rank sum test; all passive objects pooled together; Figure 16).

3. Barrel cortex activity in newborn rats during artificial whisker movements

To provide accurate whisker movement control, which is difficult in behaving animals, we induced artificial muscle-driven (evoked) whisker movements in urethane-anesthetized rat pups by applying electrical stimulation to the middle branch of the motor facial nerve - *bs* (Szwed et al., 2003; Zucker and Welker, 1969) (Figures 7, 8). Artificial whisker movements were composed of active protraction (angle 1.2 ± 22.2 °, amplitude 20.6 ± 9.9 °; n = 4 pups; P5-6) and passive retraction (Figures 8, left). When an object was introduced into the whisker trajectory, the whisker touched it and bent so that the whisker tip moved in a backward direction, and then bent back and retracted (Figures 8, right). Since the animals were in a state of deep anesthesia, spontaneous whisker movements in this series of experiments were not observed.

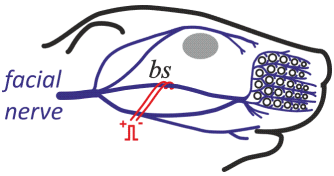

Figure 7 - The scheme of bipolar electrodes placement for stimulation of the middle branch of the motor facial nerve (*bs*, *ramus buccolabialis superior*)

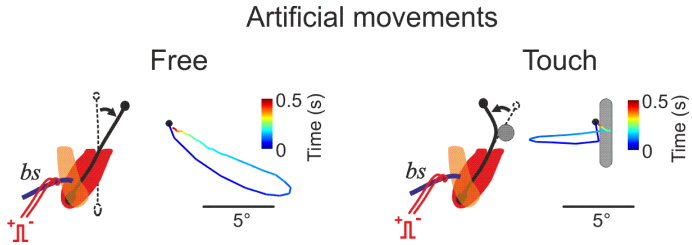

Figure 8 - Schematic drawing of the motor facial nerve (*bs*) stimulation evoked protractions. Note: free (left) and with an object (right) introduced in the whisker path and corresponding examples of time color-coded D2 principal whisker movement trajectories in a urethane-anesthetized P6 rat pup. The amplitude vectors of the whisker deflections reflect the angle of deflection from the rest position.

Free artificial movements evoked an SEP of 311 ± 65 μV with a delay of 47 ± 8 ms after the stimulus with the main sink and a MUA burst of 10 ± 3 spikes/response in IV

layer, but they barely evoked gamma/spindle bursts (n = 4 pups; P5–6; Figures 9, 10A). When an object was introduced into the PW's path, artificial whisker movements evoked an almost twofold larger SEP of 750 ± 168 μV and associated IV layer MUA of 17 + 2 spikes/response (Figures 9, 10A).

Artificial movements with touch much more reliably triggered the gamma/spindle burst component than free movements as evidenced by an increase in IV layer MUA determined within a time window from 25 to 700 ms after SEP (161 ± 22 vs 30 ± 7 spikes/response) (Figures 9, 10A). And power of gamma and spindle burst oscillations was higher in case of artificial movements with touch than free artificial movements.

We further compared the responses evoked by artificial whisker movements with the responses to brief mechanical deflection of the stationary passive PW, with the stimulus intensity evoking an SEP of the same amplitude as during artificial whisker movements with touch. The afterdischarge properties of these purely tactile-induced responses were largely similar to the responses evoked by the artificial whisker movements with touch including the magnitude of IV layer MUA (204 ± 46 spikes/response) and the power of spindle burst and gamma oscillations (Figures 9B, 10). The only difference between the external touch and artificial movement with touch evoked responses was a longer afterdischarge delay in the case of artificial movements with touch that may be due to more synchronous activation of sensory afferents during external mechanical deflection (Figure 9).

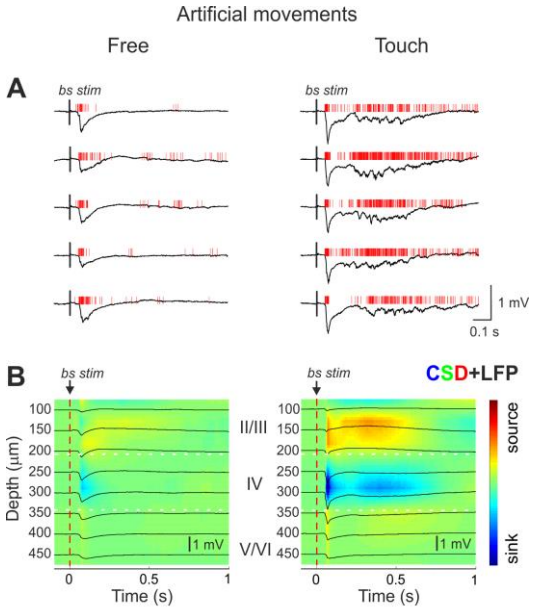

Figure 9 - Cortical activity in the IV layer of D2 principal barrel column during artificial whisker movements in P6 newborn rat. (A) Example responses evoked by artificial whisker movements in IV layer of the D2 cortical barrel column. (B) The stimulus-triggered averages (n = 100) of LFP (black traces) overlaid on color-coded current source density (CSD).

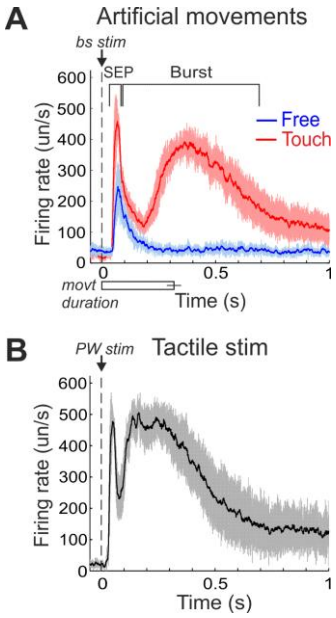

Figure 10 - Statistical plots of (A) IV layer MUA peristimulus time histograms (PSTHs) during free and touching artificial movements and (B) brief PW deflection in the cortical IV layer normalized to the PW deflection-evoked responses. Pooled data from four urethane-anesthetized P5–6 newborn rats.

Together, the results obtained during spontaneous and artificially evoked movements indicate that (1) the sensory feedback resulting from the free whisker movements and movements with tactile contact is an efficient source of cortical activation in neonatal rats, (2) but also that movements with contact of passive objects are more effective than free whisker movements.

4. Influence of conditions, as close to natural as possible, on the cortical activity in newborn rats

Passive tactile stimulation provided by the littermate and mother movements is another potential source of somatosensory input. Before electrophysiological recordings, we estimated the amount of time the rat pups spent with their snout in contact with the littermates and mother. Video recordings of the rat pup behavior in their home cage environment revealed that the rat pups spend 74 ± 6% of their time in the nest with the whisker pad in a close contact with the littermates and mother. The mother (adult rat) left the nest for 6 ± 2 min with 19 ± 5 min intervals (21 ± 5% of total recordings time). During these periods, the rat pups mainly stayed in contact with each other. Feeding periods of 27 ± 5 min occurred with 17 ± 4 min intervals (56 ± 6% of the time mother spent in contact with the pups). During feeding, the pups' whiskers were continuously in contact with the mothers' fur. In total, the rat pups' whiskers were in contact with the external objects, including littermates, mother, or nest lining 94 ± 1% of time, and only 6% of the total registration time was due to the lack of contact between the whiskers of newborn rats with

environmental objects (n = 6 litters, 8-12 rat pups per litter, 3 hours of video recording for each nest, the behavior of 13 rat pups was investigated, P0-5; Figure 11).

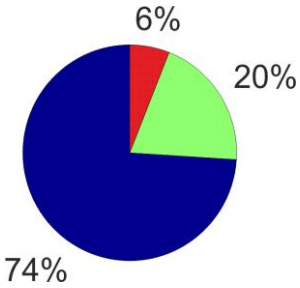

Figure 11 – A pie chart describing the contact time of the whiskers of newborn rats with various active and passive external objects under natural conditions. Note: 74% - is the total time of recording of close contact between the rat pups' whiskers with littermates, mother and nest underlay, 20% - with littermates and nest underlay, 6 % - absence of contact with objects of the external environment. Polled data: n = 6 litters, 8-12 rat pups per litter, 3 hours of video recording for each nest, the behavior of 13 rat pups was investigated, P0-5.

Next, we explored the impact of contact with the littermate on the activity in the barrel cortex. To achieve this aim, a littermate was placed in contact, snout to snout, to the recorded animal so that the PW was continuously touching the littermate (Figure 12).

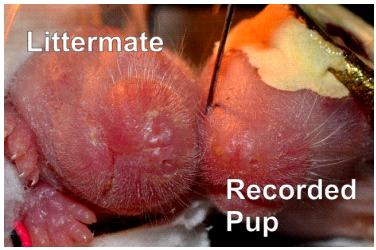

Figure 12 - P3 littermate (left) is placed snout to snoutpad to the recorded P3 rat pup (right). Note: both animals are not anesthetized.

Movements of the recorded rat pup and littermate, detected from a change in the distance between the pups' snout contours, reliably evoked cortical responses as evidenced by an increase in IV layer MUA (Figures 13, 14) as well as in α/β and γ power (Figures 13, 15). On average, IV layer MUA increased from 11.2 ± 5.6 spikes/s of baseline to 102.9 ± 54.1 spikes/s within a 1 s time window after the movement onset (n = 6 pairs of pups; P2–6; p = 0.016; paired sample Wilcoxon signed rank test). Gamma and spindle burst power also showed an increase during littermate movements from 0.5 ± 0.2 μV^2/Hz to 9.7 ± 3.3 μV^2/Hz, and from 8.8 ± 2.7 μV^2/Hz to 94.6 ± 35.5 μV^2/Hz, respectively.

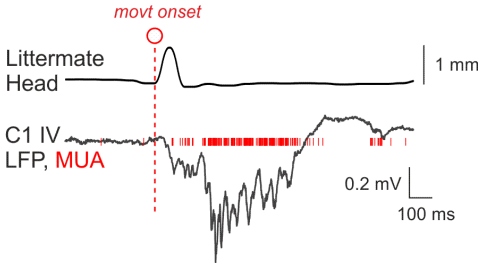

Figure 13 - Example response evoked by the littermate head movement (top) in the IV layer of C1 cortical column (bottom)

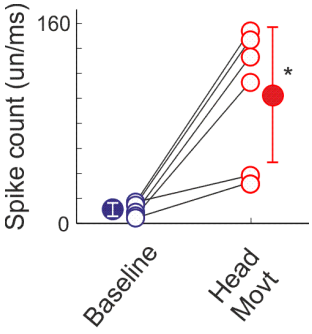

Figure 14 - Group data on littermate movement triggered IV layer MUA total spike counts within a 1 s period before and after littermate's movements. Pooled data from six pairs of P2–6 nonanesthetized rats (p = 0.016; paired sample Wilcoxon signed rank test).

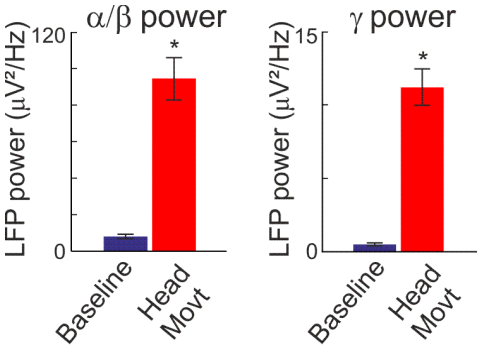

Figure 15 – Group data on littermate movement triggered IV layer LFP power in α/β and γ frequency bands within a 1 s period before and after littermate's movements. Pooled data from six pairs of P2–6 nonanesthetized rats (p = 0.016; paired sample Wilcoxon signed rank test).

The obtained data indicate that the tactile contact that occurs during the movements of newborn rats at their location, simulating the natural location of the animals in the nest, is an effective trigger mechanism for activating the barrel cortex in newborn animals.

To estimate the impact of tactile contact with the littermate on the total ongoing activity in the barrel cortex, we compared the mean IV layer MUA from 30 min recording sessions of free whisker movements with recording sessions of whisker movements in continuous snout-to-snout contact with the littermate. The total count of IV layer MUA under the conditions of contact with the littermate attained 19.9 ± 7.4 spikes/s, which was

almost twofold higher than in the isolated animal (10.9 ± 1.8 spikes/s; n = 4 pups; P2–6; p = 0.029; Wilcoxon rank sum test; Figure 16).

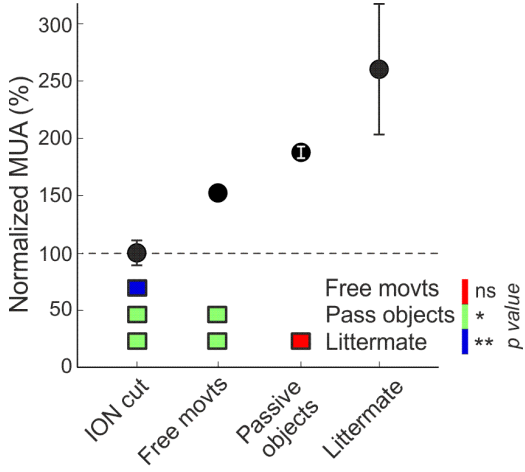

Figure 16 - Overall IV layer MUA frequency in the barrel cortex in newborn rats during free whisker movements, continuous PW contact with the passive objects (artificial fur, mesh, urethane-anesthetized littermate (1.5 g/kg, i.p.)) and a PW contact with the nonanesthetized littermate normalized to the level of activity after deafferentation by cutting the ION (100 %). Note: color-coded "p" value map for statistical comparisons between different conditions; ns - not significant.

5. Activity in the barrel cortex in conditions of total absence of sensory input into the cortex

Earlier it was shown that bursts of activity in the somatosensory cortex of newborns are self-organized thalamocortical oscillations that can be triggered by the sensory input. However, the presence of sensory input is not a prerequisite for the occurrence of such oscillations, because of sensory deprivation does not lead to their elimination, although it causes a reduction in the frequency of their occurrence (Khazipov et al., 2004; Yang et al., 2013; Yang et al., 2009). Finally, to estimate the level of spontaneous activity in barrel cortex, supported by completely deafferented somatosensory networks, we cut the infraorbital nerve (ION), which conveys all sensory input from the whiskers. Recordings before and after the ION cut were performed in nonanesthetized animals under conditions of free whisker movements, whereas ION transection was performed under deep isoflurane anesthesia. We found that overall, IV layer MUA reduced from 10.2 ± 2.1 spikes/s to 6 ± 1 spikes/s 1 h after the ION cut (n = 7 pups; P1–6; Figure 16). The level of suppression of cortical activity to 66 ± 7% of the control values after deafferentation obtained here in nonanesthetized animals was less

pronounced than reported previously (Khazipov et al., 2004; Yang et al., 2009), where the activity was suppressed to 30–50 % of the control values, that probably reflects the use of anesthetics in previous studies.

CONCLUSION

In the present study, various kinds of afferent influences in the activation of the somatosensory barrel cortex of the brain in newborn rats have been investigated during the critical period of development of somatosensory maps (it is the first week after birth in rats). It has been found that two mechanisms of comparable effectiveness provide this activation: (1) active whisker movements accompanied by reafferent and exoafferent signals, and (2) passive tactile stimulation of whiskers by the littermates. It has also been shown that tactile contact with external objects significantly increases the probability of triggering of cortical activity bursts during active whisker movements. A comparative analysis of somatosensory cortex activity in various maximally close to natural conditions revealed that the tactile signals, generated by whisker movements with the touch of external objects, as well as stimulation by the littermates, support a twofold higher level of cortical activity than that in isolated rats, and a threefold higher level of activity as compared with activity, maintained by the thalamocortical oscillator after sensory deprivation caused by the infraorbital nerve cut. Together, these results indicate that spontaneous whisker movements of newborn rats and passive stimulation provided by the littermates' movements cooperate, with comparable power, in afferent activation of the somatosensory barrel cortex in newborn rats. The results also suggest that the natural environment plays the important role in providing of physiological level of cortical activity during the critical period of somatosensory map formation.

FINDINGS

1. Early patterns of newborn rat whisker movements are diverse and include unidirectional short-duration movements with a predominant protraction-retraction direction, as well as longer-lasting complex movements. Common to all types of primitive whisker movements is a predominantly collective character with synchronous movements.

2. Whisker movements effectively trigger early oscillatory patterns of activity in the somatosensory cortex rat pups. In this case, the afferent signals that arise in the case of whisker movements, accompanied by the whisker tactile contact with the external object, are a more effective source of sensory activation of the barrel cortex in newborn rats than the afferent signals that arise during the free whisker movements.

3. Tactile contact arising by spontaneous movements of newborn rats at their location, simulating the natural location of animals in the nest, is an effective trigger

mechanism in the burst generation of early oscillatory activity in the somatosensory cortex of rat pups.

4. Complete sensory deprivation by the infraorbital nerve cut does not eliminate, but only leads to a decrease in the frequency of burst occurrence of oscillatory activity in the somatosensory cortex in newborn rats.

5. Tactile signals resulting from the whisker movements with touch of external objects, as well as from stimulation by the littermates, provide a twofold higher level of cortical activity than that in isolated animals. Together with the refferent signals, generated during the free whisker movements, these tactile signals support a threefold higher level of somatosensory cortical activity in rat pups as compared with activity maintained by the thalamocortical oscillator, deprived from the sensory input.

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