

New Approaches in the Study of the Neuroplasticity Process in Patients with Central Nervous System Lesions

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Received July 23, 2012

Abstract—Methods that, on the one hand, can ensure patient's mobility and, on the other hand, activate afferent inputs are the main in the rehabilitation treatment. Recent studies have shown that plasticity is the structural basis of recovery after central nervous system lesions. Reorganization of cortical areas, increase in the efficiency of the functioning of preserved structures; and active use of alternative ascending pathways, e.g., intensification of afferent input, constitute the anatomical basis of plasticity. However, sensory correction methods, without accounting of functional condition of patients, may lead to the formation of pathological symptoms: spasticity, hyperreflexia, etc. So, the main aim is to study adequate management of the neuroplasticity process. This problem cannot be solved without modern methods of neuroimaging and brain mapping. The new approach for the study of cortical mechanisms of neuroplasticity, responsible for locomotion, was developed in the present study. This approach is an integrated use of functional magnetic resonance imaging (fMRI) and navigation transcranial magnetic stimulation (nTMS). It has been shown that vast fMRI activation area in the first and second sensorimotor areas emerges with a passive sensorimotor paradigm usage that imitates backing load during walking. The Korvit mechanical stimulator of backing zones of footsteps is used to create this paradigm. The nTMS examination used after fMRI helps to localize motor representation of muscles which control locomotion more accurately. We assume that the new approach can be used for studying the neuroplasticity process and assessing neuroplasticity changes when taking rehabilitation measures to restore and correct the walking process.

Keywords: neuroplasticity, functional magnetic resonance imaging (fMRI), fMRI passive sensorimotor paradigm, navigation transcranial magnetic stimulation (nTMS), neurorehabilitation

DOI: 10.1134/S0362119713030055

Mechanisms of neuroplasticity, the capacity of nervous tissue to perform structural and functional rearrangement after its damage, are the basis of the restoration and compensation of functions of the nervous system. Neuroplasticity is the basis not only of the restoration of disturbed functions but also of memory, training, and acquisition of new skills [1, 2].

Achievements of modern neurophysiology have considerably extended our knowledge of mechanisms of neuroplasticity, permitting us to distinguish its different kinds. In several papers, definite principles of the functional–structural brain reorganization underlying neuroplasticity were formulated [3]. One of them is the principle of the overlapping of projections of afferent effects at various levels of the CNS, which leads to functional polysemy (multifunctionality) of brain formations. This principle is the basis of the hypothesis on the reorganization of cortical areas under the action of sensory inputs [4–6]. The preservation of locomotive representation of some or

another part of the body is provided by the afferent flux from it.

In the past few years, neuroplastic processes occurring in the brain are more frequently studied using method BOLD-contrasting functional magnetic resonance imaging (fMRI), the basis of which is imaging changes of hemodynamics in some sites of the brain in response to their activation by various kinds of stimulation (locomotor, verbal, thinking, tactile, etc.), which leads to an increase in the oxyhemoglobin-to-deoxyhemoglobin ratio with the subsequent increase in the homogeneity of the magnetic field and increase in the signal intensity in the series of T2* images.

The study of neuroplastic processes related to locomotion is especially difficult. For this purpose, different fMRI paradigms are used in which the subject is instructed to visualize walking [7–9] or different devices and facilities are used to exercise active or passive pedaling with lower extremities [10]. Note that the use of fMRI paradigm related to imaging walking requires definite skills and an indispensable prelimi-

nary training of the imagination. In addition, the preservation of cognitive functions of the subject, which are often impaired in patients with brain injury, is necessary for using this fMRI paradigm. As for the paradigm with the use of devices intended for pedaling, note that pedaling leads to pronounced artifacts related to a multijoint movement of the subject's legs, which causes certain problems in interpreting data obtained by fMRI.

We suggested an original sensorimotor passive fMRI paradigm simulating the backing load during walking under the conditions of magnetic resonance tomography whose use during fMRI permits obtaining visualization data of the functional organization of sensorimotor systems responsible for locomotion in the normal state and in patients with poststroke hemipareses [11]. However, when this fMRI paradigm is used, vast areas of the primary and second sensorimotor cortex involved in performing this complex locomotor act are activated; therefore, it is difficult to estimate the changes in neuroplastic processes in a specific sensorimotor area with time against the background of rehabilitation measures aimed at the restoration and correction of the motor skill.

To assess the reorganization of cortical representation of motor areas, besides fMRI, other methods of neuroimaging methods are used, in particular, navigation transcranial magnetic stimulation (nTMS) [12–14]. Unlike classical transcranial magnetic stimulation (TMS), the nTMS method permits applying stimulus more purposefully and locally with connection to the individual MRT of a personality to precisely repeat the site of stimulation and obtain information on the depth of stimulation and the dose of the stimulus [15]. In addition to the standard MRT, activation areas of some sites of the brain revealed during fMRI are used to determine the area of stimulation during nTMS. There is evidence [16–18] that, in certain cases (depending on the aim and tasks of the study), navigation according to fMRI data can be more efficient than that according to the data of standard MRI. However, there was no combined use of fMRI and nTMS in any of these studies to reveal sensorimotor areas responsible for locomotion.

METHODS

Four apparently healthy volunteers (mean age, 26.50 ± 5.25 years) having no history of neurological, mental, or cardiovascular diseases participated in the study. All subjects had no contraindications to perform MRT and TMS studies, and all of them gave their written consent to voluntary participation in this work.

In this study, the passive sensorimotor fMRI paradigm simulating backing load during walking previously developed by us was used. Stimulation of backing afferentation was performed using a Korvit simu-

lator of the backing load developed together with the Institute of Biomedical Problems of the Russian Academy of Sciences and OOO TsAM. Korvit permits reproducing the physiological modes of the backing response arising during locomotion process. The simulator consists of the control block, feeding block, MRT-compatible artificial airways, and orthoses with pneumo-chambers built into the inner soles and fixed on the feet of the subject. The device operates on the principle of creating pneumomechanical pressure on the corresponding backing zones of the foot using pneumo-chambers functioning in the mode of actual locomotions.

During one session of scanning, in each subject, the paradigm (task) that was rehearsed beforehand prior to the study and directly in the scanning room was used. The paradigm had a block design consisting of six alternating blocks of the period of activation and the period of rest with a duration of 38 s each. The task began from the period of rest during which any stimulation was absent. Then, the period of activation followed—stimulation of backing zones of the foot in the mode of simulating slow walking with a pressure of 40 kPa on the feet and a frequency of 75 steps/min. During each block, ten series of brain images were obtained. The total duration of the paradigm was 3 min 53 s, during which 60 series of brain images were obtained.

The MRI data were obtained using an MR tomograph with a magnetic field intensity of 1.5 T (Siemens MAGNETOM Avanto). The study began from the standard mode of T2 turbo-spin echo in an axial projection to eliminate pathological changes in the brain substance. In order to obtain anatomical data, the study was performed in the mode of a 3D-T1 gradient echo (T1-mpr) with obtaining a set of 176 sagittal slices covering the entire brain substance volume. Then, two sets of functional data were obtained successively (for each paradigm) in the mode T2* gradient echo in a local projection (time of repetition (TR)), 3800 ms; time of echo (TE), 50 ms; inclination angle, 90 degrees; matrix, 64×64 mm; section thickness, 3.0 mm; voxel size, $3 \times 3 \times 3$ mm; 36 sections per series). Each T2* mode included 60 measurements of the entire brain tissue.

The data were assessed using the SPM5 software package for statistical processing (Wellcome Rust Centre of Neuroimaging, London, United Kingdom) separately for each paradigm at the stage of preprocessing. All arrays of functional data were aligned with respect to the first one to correct the subject's movements, after which the average functional file was linearly corecorded with the corresponding anatomical file with the subsequent spatial normalization of the first ($3 \times 3 \times 3$ mm) and of the second ($1 \times 1 \times 1$ mm) with respect to the Montreal Neurological Institute's

(MNI) standard space of coordinates. Before the statistical analysis, the transformed functional data were leveled using the Gaussian function with the kernel size of $10 \times 10 \times 10$ mm to increase the signal-to-noise ratio (by weakening high-frequency noise) and to compensate for the variation of the structure of convolutions of the brain between subjects. Statistical parametric maps were generated on the basis of voxel-to-voxel comparison using the general linear model [19]. In order to decrease artifacts caused by the subject's movement, parameters of rigid transformation during leveling were introduced as regressors during statistical processing of the first level (for each subject). In group analysis, a random-effects model with an established level of statistical significance $p < 0.001$ (without correction) to reveal significant activation areas was used. As a result, only activation areas (clusters) with $p_{\text{correct}} < 0.05$ at the cluster level are revealed.

An eXimia Nexstim system of navigation transcranial magnetic stimulation (nTMS) (Finland) was used. This system includes a transcranial magnetic stimulator, an electromyograph for recording evoked motor responses to magnetic stimulus, and a navigation system for localizing the magnetic field in the patient's MRT. Stimulation was performed using a BiPulse Nexstim eight-shaped double-pulse coil. The duration of the magnetic impulse was 280 μs and the maximal magnetic field intensity was 199 V/m.

The study algorithm is given below:

(1) The subject's MRT examination in the T2–VI modes to eliminate pathological changes in the brain substance, T1 MPR (multiplanar reconstruction), and fMRI using the paradigm simulating bearing load in the mode of slow walking (Korvit device) by means of a 1.5 T Siemens MAGNETOM Avanto magnetic resonance tomograph.

(2) Input of the T1 MPR and fMRI data into the NBS eXimia Nexstim system; construction of an individual three-dimensional brain model of the subject, with the fMRI activation areas mapped.

(3) Comparison of actual anatomical formations with the data obtained using MRT in the T1 MPR mode.

(4) Application of electromyographic (EMG) electrodes on the studied muscles—m. gastrocnemius, m. soleus, and m. tibialis anterior—according to Livenson and Sinel'nikov's atlas.

(5) Preliminary magnetic stimulation (nTMS) of the brain activation area obtained using fMRT, with determination of the evoked motor responses (EMRs) of the studied muscles with an amplitude of 100–500 μV and a magnetic field intensity in the stimulation point of 80–110 V/m.

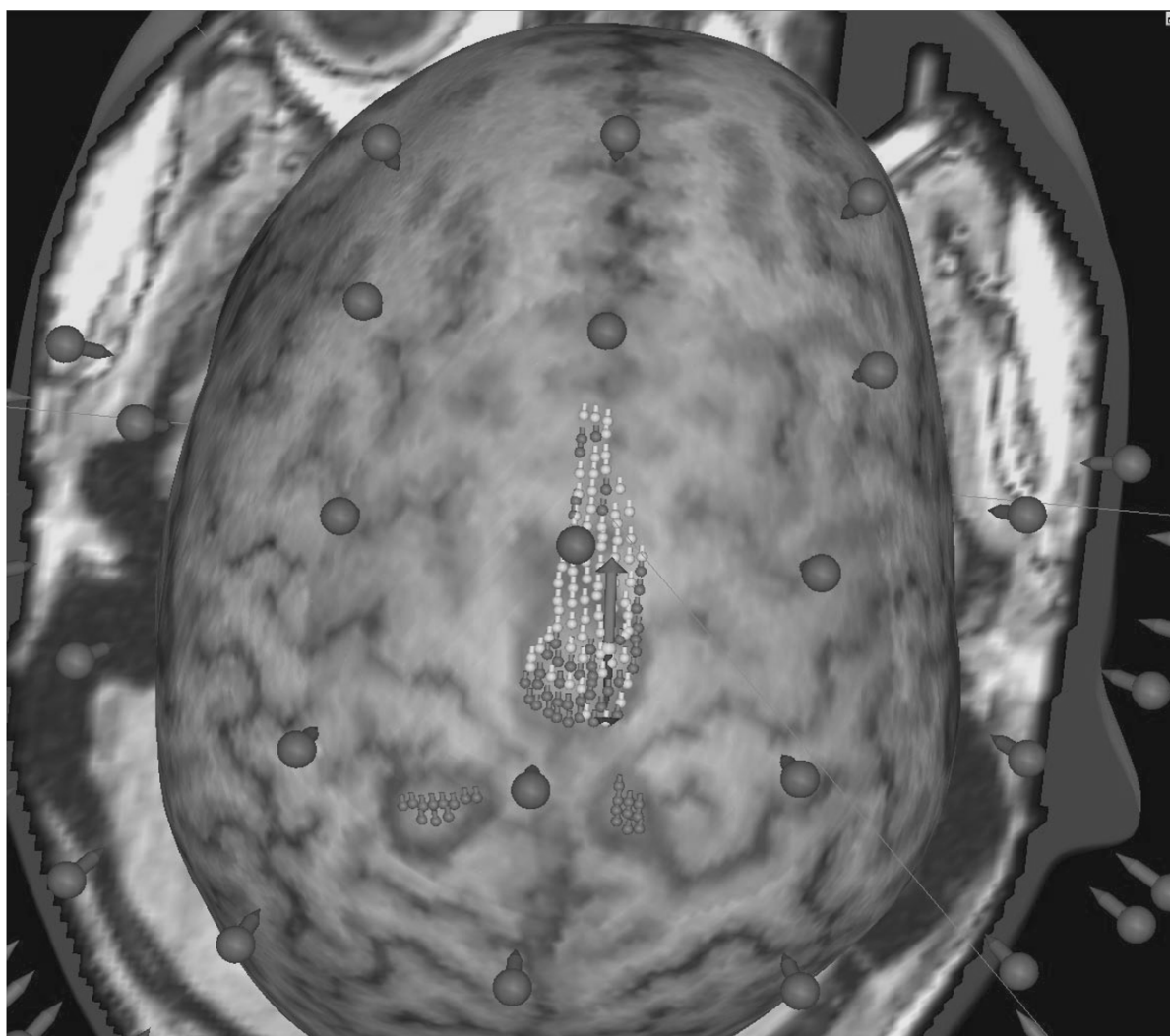
(6) Determination of the point in the individual three-dimensional brain reconstruction with maximal amplitude of EMR.

(7) Determination of the passive motor threshold at the point with the maximal amplitude of EMR using a special Stimulus Repetition mode of the NBS eXimia Nexstim. This mode permits applying a stimulus to the selected point to an accuracy of 2 mm. The motor threshold is the minimal intensity of magnetic stimulation (in percent) at which, in more than half of the repeated stimuli, EMR with an amplitude of more than 50 μV is recorded.

(8) Mapping using nTMS of motor representation of muscles at the intensity of 110% from the selected motor threshold in the individual three-dimensional brain reconstruction, and drawing an individual map of the representation in the brain cortex of sensorimotor areas responsible for locomotion.

RESULTS AND DISCUSSION

During fMRI, we used the passive sensorimotor paradigm—mechanical stimulation of backing zones of feet in the mode of slow walking imitation—to locate, in the group of volunteers, areas of activation of the first sensory cortex (S1) comprising vast bilateral activation areas in the paracentral lobules, as well as in the primary motor cortex (M1), which proves the efficiency of this paradigm for imitating precisely active movements. It is important to note the bilateral activation of Brodmann's area (BA) 6, to which the lateral (premotor) area, frequently referred to as the supplementary motor area (SMA), belongs. It is considered that SMA is activated upon the intention to perform an action (in this case, the actual action does not necessarily follow) and its planning, as well as during stimulation of the movement without its performance, which was the case in our task. Activation was also obtained of posterior parietal areas representing a complex associative cortex, namely, in the region of inferior parietal lobules (IPLs) (more to the left) (BA 40). These sites play an important role in assessing cognitive strategies and motor programs involved during successive movements by legs. In addition, activation of the dorsolateral prefrontal cortex (DLPFC, BAs 9 and 46) was recorded. This associative area represents the highest level of planning, organization, and regulation of movement and plays an important role in the integration of sensory and mnemonic information, as well as in the processes of the working memory. The bilateral activation of the insular lobe can be related to cognitive control, coordination of the task, and involvement of the working memory. In addition, activation of cerebellum occurs, which is related to the control of its tone during any locomotive act, maintaining tone, and integration of the corresponding sites of the cortex of cerebral hemispheres, as well as in performing stereotype movements. Thus, during the use in the subjects of the passive sensorimotor fMRI paradigm, a vast activation of the region of the primary



Comparison of areas of activation of the cerebral cortex obtained at fMRI using sensorimotor passive paradigm imitating bearing load during walking and activation map at navigation TMS in apparently healthy volunteers aged 28 years (active electrodes at m. tibialis ant. dex et sin.). White dots designate areas with the presence of evoked motor responses (EMR), gray points, without response.

and secondary sensorimotor cortex participating in locomotion control is recorded.

As a result of the nTMS procedure, for each subject, upon three-dimensional brain reconstruction, an individual map of motor representation of the target muscles (sensorimotor areas) most important for locomotion (m. gastrocnemius, m. soleus, and m. tibialis anterior) was plotted. It was shown that at most points, EMRs are caused immediately in the three muscles (82%). The latent periods of EMR in m. gastrocnemius, m. soleus, and m. tibialis anterior were close (29.09 ± 1.60 , 29.83 ± 0.22 , and 28.91 ± 0.68 ms, respectively). Such a high percentage of coincidence of values of the latent periods of EMR possibly indicates first a mosaic location of neurons innervating the examined muscles and second that for mapping motor

area, a derivation from only one muscle, for instance, m. tibialis anterior can be used (table).

It was also shown that the map of the motor representation of target muscles involved in locomotion obtained using nTMS coincides in part with the motor areas of activation emerging during the use of the fMRI paradigm imitating bearing load during walking (figure). These data can be assessed first as a proof of the fact that at least a part of the vast area of activation of the primary sensorimotor cortex emerging during sensorimotor passive fMRT paradigm imitating walking is localized in the area of the motor representation of muscles involved in locomotion; second, an incomplete coincidence of motor areas of activation of fMRI and TMS has been shown also in other studies [20]; the distance between the motor areas may be up to

Parameters of evoked motor responses (ENR) in the course of performing nTMS

Subjects	Sex	Age, years	Evoked response threshold, %	Parameters	Studied muscles (M ± SD)		
					m. gastrocnemius	m. soleus	m. tibialis anterior
K	F	28	65	EMR amplitude, μV	167.83	138.72	165.20
				Latency, ms	26.99	30.10	30.30
Kr	M	23	58	EMR amplitude, μV	316.91	150.48	210.11
				Latency, ms	29.30	30.30	28.50
S	M	29	62	EMR amplitude, μV	713.12	377.04	250.23
				Latency, ms	29.77	29.05	28.68
G	F	26	58	EMR amplitude, μV	615.18	445.28	380.61
				Latency, ms	30.30	29.85	28.16
Average values		26.50 ± 5.25	60.75 ± 8.69	EMR amplitude, μV	453.26 ± 220.04	277.88 ± 132.51	251.54 ± 80.36
				Latency, ms	29.09 ± 1.60	29.83 ± 0.22	28.91 ± 0.68

21.7 mm (on average, 3.70 ± 4.85 mm). Some authors consider that this may be related to the fact that the nTMS method maps the areas of motor representation in the cortex more accurately than fMRI [21–23].

CONCLUSIONS

Walking is known to be one of the most important locomotor skills in whose performance various regions of the central nervous system are involved. The main locomotor step pattern is generated at the spinal brain level, while walking is controlled by centers located higher up, including the motor cortex, cerebellum, and trunk [24]. Despite the considerable number of experimental and clinical studies dedicated to the study of mechanisms of corticospinal interaction during walking, many issues of the supraspinal control of locomotion in humans remain insufficiently studied. This is mainly explained by difficulties in studying the central mechanisms of walking control using the available methods of neuroimaging (fMRI, PET, EEG, nTMS, etc.). Apparently, none of these methods alone can completely reflect the complex, hierarchically organized system of locomotion control, and only the combination of several methods of brain mapping—functional, structural, and electrophysiological—can permit solving this problem. From this standpoint, the integrated use of fMRI methods with the use of the sensorimotor passive paradigm imitating bearing load during walking suggested in this paper is a fundamentally new approach to study of the mechanisms of cortical control of locomotion, which subsequently can

be used for studying neuroplasticity related to locomotion and for assessing changes in the course of rehabilitation measures aimed at restoring and correcting walking.

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Translated by I. Pogoyants