Neurovascular coupling in a rat model of ischemic stroke recovery

Anna Devor1, Elizabeth M. C. Hillman1, Young R. Kim1, Haihuo Zhu2, John B. Moore1, Suresh N. Narayanan1, Eng H. Lo2, Bruce R. Rosen1, Anders M. Dale3 and David A. Boas1

Martins Center for Biomedical Imaging, and Department of Radiology, MGH, Harvard Medical School, Charlestown, MA, Departments of Neurosciences and Radiology, University of California, San Diego, CA

Introduction

In addition to its established role in basic brain research, fNIRS is beginning to make inroads into clinical applications including monitoring of stroke rehabilitation. fNIRS studies in patients demonstrate remarkable plastic changes in the adult human brain weeks and months following the stroke. These changes occur in areas immediately adjacent to the lesion, but also in areas remotely connected to the lesion such as the contralateral (unaffected) cortex. The functional significance of these plastic changes, however, is poorly understood, largely due to the lack of understanding of the interplay of neuronal and cerebrovascular mechanisms underlying this clinical condition. Here we apply optical imaging, electrophysiology, immunohistochemistry and fNIRS to evaluate the neurovascular coupling in stroke recovery and to relate our findings directly to the growing body of clinical fNIRS studies on stroke rehabilitation. In particular, we focus on functional changes in the unaffected hemisphere and reorganization of cortical maps.

In comparison with stimulation of affected limb, stimulation of affected limb featured: (1) reduced amplitude of hemodynamic and vasogenic-sensitive dyes (VSD) signals in contralateral SI; (2) spatial shift of the active contralateral region in the posterior direction; and (3) bilateral VSD signals of comparable amplitude and post-stimulus delay (upon stimulation of the unaffected VSD response in the ipsilateral SI had a larger delay due to signal traveling via corpus callosum). In the majority of cases the ipsilateral VSD signals were unaccompanied by any detectable hemodynamic response. Electrophysiological recordings showed an aberrant shape of the local field potentials on the affected side indicative of a change in neuronal circuit dynamics. Modified VSD signals from the affected hemisphere showed that an increase in neuronal firing was lacking a subsequent decrease characteristic of control conditions. These findings suggest a possible reduction in number of inhibitory interneurons.

Motivation

Previous studies have characterized the pattern of evolution of non-invasive imaging signals during post-stroke recovery in an animal stroke model (1,2). According to their results, focal stimulation of the unaffected forelimb led to increased reorganization-specific activation-induced cortical blood volume (CVB) response in the unaffected hemisphere (contralateral to the stimulated SI). Stimulation of the impacted forelimb at days 3 and 3 post-stroke resulted, in most animals, in a diminished amplitude of the CVB response in the ipsilateral hemisphere (contralateral to the stimulated forelimb) and contralateral responses in the contralateral hemisphere (ipsilateral to the stimulated SI). At 3-4 days post-stroke, activation responses to impacted forelimb stimulation were nearly absent in the contralateral hemispheres in most, but not all, studied animals.

Methods

Rats were anesthetized by continuous intravenous infusion of α-chloralose at 40 mg*kg-1*h-1. An area of skull overlying SI was exposed and thinned until transparent. During experiments involving lumbar arterial electrodes and voltage-sensitive dyes (VSD) the thinned skull and dura matter were removed.

Homogeneous imaging. Spatial light from a tungsten-halogen source was directed through a 6-position rotating filter wheel (HbO, Hb, Hb and HbT) using a modified Beer-Lambert law. Differential pathlength correction was applied.

Spectral imaging. Light from a tungsten-halogen source was directed through a 6-position rotating filter wheel (785 nm, 40 mW) was expanded to illuminate the cortex at an angle ~ 30°. The laser was coupled into a 600-µm diameter silica optical fiber and a collimating lens. Images were acquired by 8 bit CCD camera at ~200Hz. The raw speckle images were converted to speckle contrast to estimate blood flow [4]. A dichroic beam splitter was used to divert the 780 nm speckle contrast of blood oxygenation and flow. Electrophysiology. Filtered between 500-5000 Hz to record MUA, and between 0.1-500 Hz to record LFP. Voltage-sensitive dyes imaging. Hemodynamic imaging. After the recovery of the cortex, the video was filled with a buffered saline containing 115 mM NaCl, 5 mM KCl, 15 mM HEPES, 1.8 mM CaCl2, and 1 mM MgCl2. Voltage sensitive dyes dye 1691 (Optical Imaging Ltd, Mountainside, NJ) was used in the buffered saline. The dye was left for 2 hours in the incubation plate. The incubation dye was washed with fresh buffered saline. The data was acquired using a commercial CCD-based VSD imaging system (Optical Imaging Ltd, Mountainside, NJ).

Stroke induction. Rats were anesthetized with 1-1.5% isoflurane in N2O/02 (70:30) under spontaneous respiration. Transient focal cerebral ischemia was induced by 8-5.2 mm occlusion of the right middle cerebral artery (MCA) with an intraluminal filament using 4.0 nylon filament coated with silicone inserted into the internal carotid artery.

Immunohistochemistry in normal controls

We used immunolabeling to investigate luminal distribution of inhibitory neurotransmitters and used rats were anesthetized with α-chloralose [5]. After the animals were sacrificed, the brains were removed, and coronal sections were prepared for immunohistochemical staining using antibodies against GAD65, parvalbumin, calbindin-D28K, and calretinin.

In our experimental model the stroke affects the right hemisphere in all animals. Data are shown for two animals: 9824805 (on the left) and 9828905 (on the right). We show the time course of CVB changes in A (5 voxels in ipsilateral right and contralateral left hemispheres). Farupo stimulation (60 Hz, 40µsec) is represented by bars under the graph.

Comparison of VSD and hemoglobin oxygenation

A. Spatiotemporal evolution of stimulus-induced response following stimulation of the unaffected (top row) and affected (bottom row) limb. Each image represents signal detected at post-stimulus time indicated above the image divided by the prestimulus baseline. 100 stimulus presentations were averaged. B. Comparison of hemodynamic maps (signal from 1-3 sec post-stimulus divided by prestimulus baseline, top row) to VSD maps (50sec post-stimulus divided by the prestimulus baseline, bottom row). The colors correspond to Hb and HbT signal. Each panel shows that the VSD results are consistent with the hemodynamic maps: Hb in the contralesional hemisphere and VSD maps in the homologous contralateral stimulated paw. However, hemodynamic response upon stimulation of the affected paw partially overlaps with VSD signals. C. VSD response timecourses extracted from each homologous paw (ipsilateral paw: blue-contralateral, red-unaffected hemisphere; affected paw: green-contralateral, black-unaffected hemisphere). D. Hemodynamic signals from each homologous extracted from B. Color conventions the same as in C.

Electrophysiological recordings of local field potential (LFP)

LFP recordings from the unaffected (blue) and affected (red) hemispheres. Data are shown for two animals: 9824805 (on the left) and 9828905 (on the right). Two electrodes were inserted bilaterally, and recordings from both hemispheres were performed simultaneously. A-B. Stimulus-evoked response. 100 stimuli presentations were averaged. C. Spontaneous LFP activity from the unaffected (blue) and affected (red) hemispheres by the time course of blood flow in the contralesional hemisphere (green arrow). This is in contrast to normal response starting with a large downward deflection.