Neurovascular coupling in a rat model of ischemic stroke recovery

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Introduction

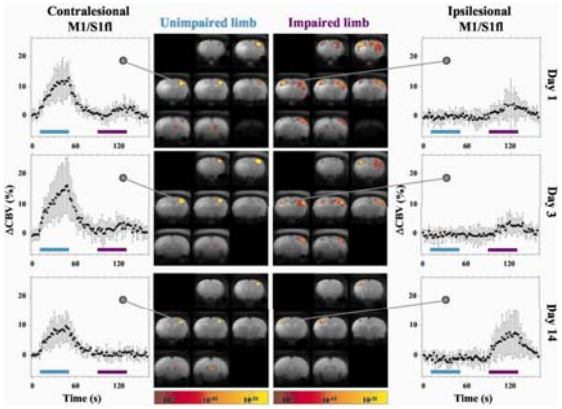
In addition to its established role in basic brain research, fMRI is beginning to make inroads into clinical applications including monitoring of stroke rehabilitation. fMRI studies in patients demonstrate remarkable plastic changes in the adult human brain weeks and months following the stroke. These 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 under this clinical condition. Here we apply optical imaging, electrophysiology immunohistochemistry and fMRI to evaluate the neurovascular coupling in stroke recovery and to relate our findings directly to the growing body of clinical fMRI studies on stroke rehabilitation. In particular, we focus on functional role of the unaffected hemisphere and reorganization of cortical maps.

In comparison with stimulation of *unaffected* limb, stimulation of *affected* limb featured: (1) reduced amplitude of hemodynamic and voltage-sensitive dyes (VSD) signals in contralateral SI; (2) spatial shift of the active contralateral region in the posterior direction; (3) bilateral VSD signals of comparable amplitude and post-stimulus delay (upon stimulation of the *unaffected* limb VSD response in the ipsilateral SI had a longer 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 abnormal shape of the local field potentials on the affected side indicative of a change in neural circuit dynamics. Multiple unit activity recorded 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 [1,2,3]. According to their results stimulation of the unimpaired forelimb invariably resulted in a significant activation-induced cerebral blood volume (CBV) response in the undamaged hemisphere (contralateral to the stimulated forelimb). Stimulation of the impaired forelimb at days 1 and 3 post-stroke resulted, in most animals, in a diminished amplitude of the CBV response in the ipsilesional hemisphere (contralateral to the stimulated forelimb) and extensive responses in the contralesional hemisphere (ipsilateral to the stimulated limb). At 14 days post-stroke, activation responses to impaired forelimb stimulation were nearly absent in the contralesional hemisphere in most, but not all, studied animals.

1. Dijkhuizen, R.M., et al., *Functional magnetic resonance imaging of reorganization in rat brain after stroke*. Proc Natl Acad Sci U S A, 2001. 98(22): p. 12766-71.
2. Dijkhuizen, R.M., et al., *Correlation between brain reorganization, ischemic damage, and neurologic status after transient focal cerebral ischemia in rats: a functional magnetic resonance imaging study.* J Neurosci, 2003. 23(2): p. 510-7.
3. Kim, Y.R., et al., *Measurements of BOLD/CBV ratio show altered fMRI hemodynamics during stroke recovery in rats.* J Cereb Blood Flow Metab, 2005. 25(7): p. 820-9.



Averaged T2-weighted images of coronal rat brain slices overlaid by statistical maps of CBV changes (data are averaged across 7 animals). Infarction areas are characterized by increased T2-weighted signal intensity. *p* values are color-coded. The graphs show the time course of CBV changes in an ROI (5 voxels) in ipsilesional (right) and contralesional (left) hemisphere. Forepaw stimulation (3Hz, 40sec) is represented by the bars under the graphs.

From: Dijkhuizen, R.M., et al., Correlation between brain reorganization, ischemic damage, and neurologic status after transient focal cerebral ischemia in rats: a functional magnetic resonance imaging study. J Neurosci, 2003. 23(2): p. 510-7.

Methods

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

Hemodynamic imaging. ***Spectral*****: light from a tungsten-halogen source was directed through a 6-position rotating filter wheel (560, 570, 580, 590, 600 and 610 nm) and coupled into a 12 mm fiber bundle. Images of ~ 6x6 mm were acquired by cooled 12 bit CCD camera at ~15Hz. The spectral data were converted to percent change maps for oxy-, deoxy- and total hemoglobin (HbO, Hb and HbT) using a modified Beer-Lambert law. Differential pathlength correction was applied.** ***Speckle*****: diode laser (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 image to the 8 bit CCD camera and pass the 560-610 nm light to the 12 bit CCD camera for simultaneous recording of blood oxygenation and flow.**

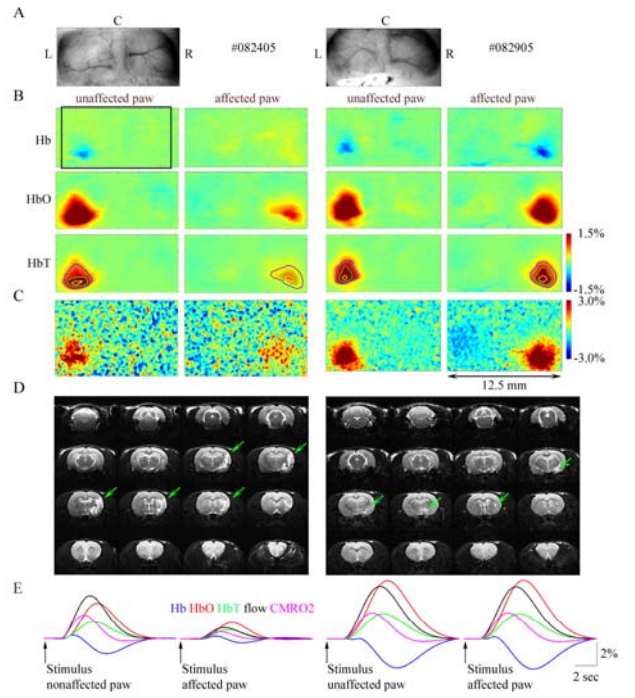
Electrophysiology. Single metal electrodes (2-5 MW, FHC) were inserted in cortical layer II/III. The signals were amplified and filtered between 500-5000 Hz to record MUA, and between 0.1-500 Hz to record LFP.

Voltage sensitive dyes (VSD) imaging. Immediately after the exposure of the cortex the well was filled with a buffered saline containing 135 mM NaCl, 5 mM KCl, 5 mM Hepes, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$. Voltage sensitive dye 1691 (Optical Imaging Ltd, Mountainside, NJ) was dissolved in the buffered saline. The dye was left for 2 hours to impregnate the brain. The unbound 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% halothane in N2O/O2 (70:30) under spontaneous respiration. Transient focal cerebral ischemia was induced by 0.5 or 2hr occlusion of the right middle cerebral artery (MCA) with an intraluminal filament using 4.0 nylon monofilament suture coated with silicone inserted into the external carotid artery.

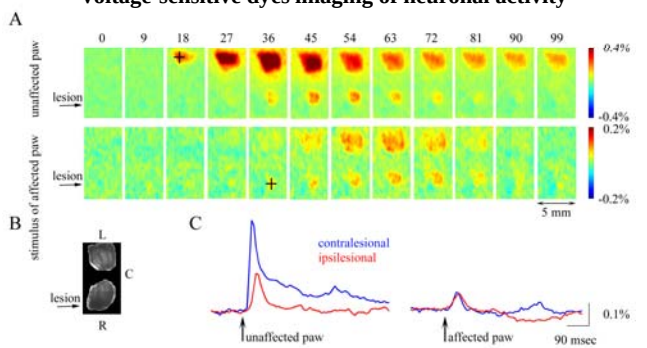
4. Dunn, A.K., et al., *Dynamic imaging of cerebral blood flow using laser speckle.* J Cereb Blood Flow Metab, 2001. 21(3): p. 195-201.

Optical imaging of hemoglobin oxygenation and blood flow, and calculation of CMRO2



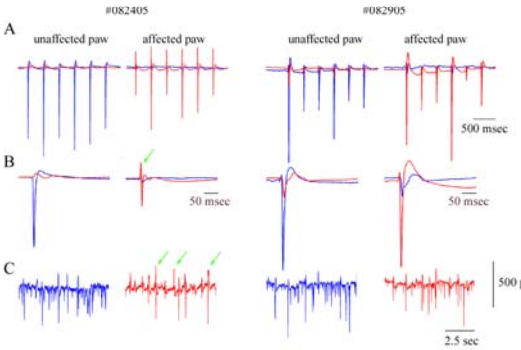
In our experimental model the stroke affects the right hemisphere in all animals. Data are shown for two animals: #082405 (on the left) and #082905 (on the right). A. An image of the bilateral cortical exposure for each one of the animals corresponding to functional ratio maps in B-C. L=left, R=right, C=caudal. B-C. Spatiotemporal evolution of Hb, HbO and HbT (B) and blood flow (C) response following stimulation of the unaffected and affected forepaw. Black rectangle denotes area corresponding to blood flow images. Each image corresponds to the signal 4.2 sec following the stimulus onset divided by the prestimulus baseline. 100 stimulus presentations were averaged. D. T2-weighted structural MRI scans showing the extent of the lesion. E. Response timecourses extracted from 2x2mm ROI centered on the earliest detectable HbT response.

Voltage-sensitive dyes imaging of neuronal activity



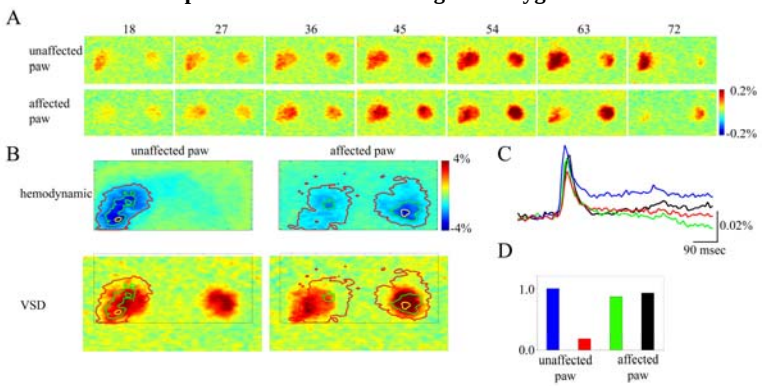
A. Spatiotemporal evolution of stimulus-induced response following stimulation of the unaffected (top row) and affected (bottom row) forepaw. A corresponding image of the bilateral cortical exposure is shown in B. C. Response timecourses extracted from 1x1mm ROI centered on the earliest detectable response (crosses in A). 100 stimulus trials were averaged.

Electrophysiological recordings of local field potential (LFP)



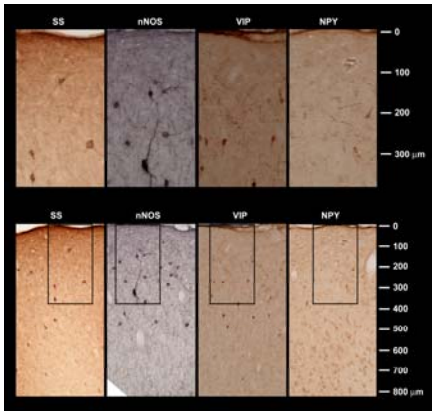
LFP recordings from the unaffected (blue) and affected (red) hemispheres. Data are shown for two animals: #082405 (on the left) and #082905 (on the right). Two electrodes were inserted bilaterally, and recordings from both hemispheres were performed simultaneously. A-B. Stimulus-evoked response. 100 stimulus presentations were averaged. C. Spontaneous LFP activity from the unaffected (blue) and affected (red) hemispheres. On the lesioned side LFP showed a fast transient response starting with an upward deflection to stimulation of the contralateral (affected) limb (green arrow). This is in contrast to normal response starting with a large downward deflection.

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 trials were averaged. B. Comparison of hemodynamic maps (signal from 1-3sec post-stimulus divided by prestimulus baseline, top row) to VSD maps (54msec post-stimulus divided by the prestimulus baseline, bottom row). The contours correspond to 90, 60 and 30% of 580 nm signal peak Note that stimulation of each limb produces colocalized hemodynamic and VSD maps in the hemisphere contralateral to the stimulated paw. However, ipsilateral hemodynamic response upon stimulation of the affected paw only partially overlaps with VSD signals. C. VSD response timecourses extracted from each one hemispheres (unaffected paw stimulus: blue=contralateral, red=ipsilateral hemisphere; affected paw stimulus: green= contralateral, black=ipsilateral hemisphere). D. Hemodynamic signals from each hemispheres extracted from B. Color conventions the same as in C.

Immunohistochemistry in normal controls



We used immunolabeling to investigate laminar distribution of inhibitory iterneurons that were shown to affect blood vessel diameter [5]. The following Ab were used: somatostatin-14 (SS) (T-4103.0050, Bachem), nNOS (07-571, Upstate), VIP (T-5030.0050, Bachem), NPY (AB1915, Chemicon).

5. Cauli, B., et al., *Cortical GABA interneurons in neurovascular coupling: relays for subcortical vasoactive pathways.* J Neurosci, 2004. 24(41): p. 8940-9.