Diffusion fMRI can detect neural activation when the BOLD fMRI response is abolished by nitroprussiate

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Target audience: Researchers and clinicians with interest in fMRI

Purpose

Diffusion-weighted functional MRI (DfMRI) has been shown to be sensitive to neural activation, [1,2] however, its vascular (BOLD like) or non-vascular origin has been a subject of controversy. [3] We have compared the DfMRI and BOLD responses in the rat brain during forepaw electrical stimulation (ES) with and without nitroprussiate, a neurovascular coupling inhibitor, and compared these responses with neural activity.

Methods

(1) ***fMRI***: MRI experiments were performed on a 7T imaging system (Bruker, Ettlingen, Germany) using a dedicated surface coil. Sixteen male wistar rats were anesthetized with 1.5% isoflurane in air (30% oxygen). BOLD images were acquired using a Gradient echo (GE)-EPI sequence (TE/TR = 20/3000 ms) and DfMRI images were acquired using the diffusion-sensitized double spin echo-EPI sequence (TE/TR = 38.9/3000 ms, b=10 (equivalent to a spin-echo (SE) BOLD sequence), 250, 1000, 1800 or 2600 s/mm^2). For both sequences, the following parameters were used: matrix size = 100 x 100, FOV = 32 mm, slice thickness = 1.2 mm, 3 slices. Anatomical images were acquired for spatial correction using multi-slice rapid acquisition with relaxation enhancement (RARE): TE_{eff}/TR = 60/2500 ms, RARE factor = 8, matrix size = 256 x 256, FOV = 32 mm, slice thickness = 1.2 mm, 3 slices. The protocol consisted of 5 blocks of forepaw ES (2 mA, 7 Hz, 30 seconds) followed by a 30 seconds resting: total 5.5 min. To increase the SNR in DfMRI images, we averaged 3 and 5 acquisitions for b=250 and for b=1000, 1800 and 2600, respectively. After the spatial correction by SPM5 (Welcome Trust Center for Neuroimaging, UK), the signal changes were calculated using a script written in Matlab (Mathworks, MA) in a region of interest (ROI) in the somatosensory cortex contralateral to the stimulated hand, corresponding to the location of the tip of the recording electrode in the electrophysiological study (Fig. 1). The colocalization of the electrode and the ROI was obtained from a rat brain atlas. [4] An apparent diffusion coefficient was calculated as $ADC=\ln(S_{b1000}/S_{b2600})/1600$ in the baseline and activated conditions to remove confounding T2 relaxation effects induced by changes in hemodynamics.

(2) ***Electrophysiology***: The electrophysiological experiments were performed independently from the MRI experiments. Eight male wistar rats were anesthetized with 1.5% isoflurane in air. To compare the neural activity with MRI signal changes, we recorded local field potential (LFP) in layer 2/3 of the somatosensory cortex with a single tungsten microelectrode, whose tip was positioned as shown in Fig. 1. The LFP signals were acquired at a 1 kHz sampling rate using data acquisition software (Cheetah 5.6.3, Neuralynx, MT). The reference electrode was positioned on the scalp. LFPs were recorded twice and then averaged. The amplitude of evoked LFP was calculated as the difference between the positive and negative peaks. [5] To compensate for the low time resolution of MRI (1.5s), a "ΣLFP" response was then calculated by summing LFP amplitudes within intervals of 1.5s. The ES parameters were identical to those used for MRI experiments.

(3) Nitroprussiate injection: Nitroprussiate infusion was started 10 min before the beginning of experiment at the rate of 0.05 g / min / kg body weight and continuously infused throughout the experiment.

Results

Without nitroprussiate, the ΣLFP response rapidly increased upon stimulation and sharply decreased to baseline (Fig. 2a) reflecting known neuronal adaptation effects. [5] The DfMRI signal peaked at about the same time as the BOLD signal in contrast to earlier findings in the human visual cortex, [1,2] but decreased immediately after the peak, similarly to the ΣLFP response, while the BOLD response more slowly decreased (Fig.2b). ADC was found to decrease by 6% (Fig.3).

With nitroprussiate, GE-BOLD and SE-BOLD responses were abolished or slightly inverted but the peak value of the ∑LFP was not affected by nitroprussiate (Fig.2b and 3), in agreement with a previous study. [6] In contrast, the DfMRI response remained high with only a partial amputation. Furthermore, the DfMRI response amplitude significantly increased with the degree of diffusion weighting (Fig.2b). The peak amplitude of ADC decrease was also not affected (Fig.3).

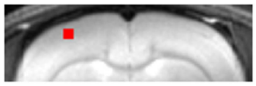

Fig. 1 ROI of the somatosensory cortex where ∑LFP was recorded (red zone).

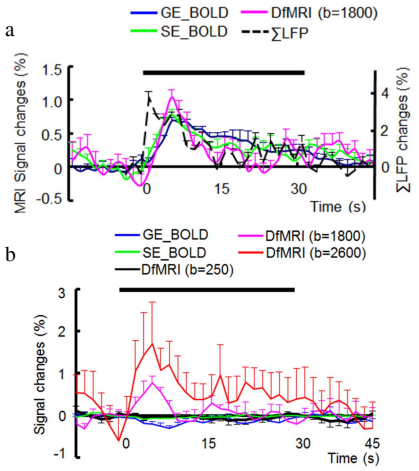

Fig. 2 Percent changes in the BOLD and DfMRI responses (a) without / (b) with nitroprussiate infusion. Black bar, electrical stimulation

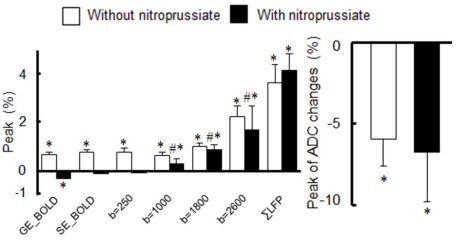

Fig. 3 Peak amplitude of BOLD signal, DfMRI signal, ΣLFP and ADC changes with/without nitroprussiate. Data are expressed as mean ± S.E.M. * p< 0.05, compared with baseline; # p<0.05, compared with GE and SE_BOLD.

Discussion

These results show that, while the BOLD response is completely quenched under nitroprussiate, the neuronal response remains intact and the DfMRI response persists. The DfMRI signal is the sum of a genuine diffusion component and a residual relativity (T2') component. [2] Only the T2' part is suppressed by nitroprussiate, a neurovascular coupling inhibitor, while the diffusion component is maintained, increasing with the b value, (constant ADC change). Altogether these results demonstrate that the DfMRI response contents a component which is not of vascular origin and closely relates to the neuronal response (sharing adaptation effects and resisting neurovascular coupling modulation). As diffusion MRI exquisitely reflects how molecular water displacements in tissues are affected by obstacles, in particular cell membranes, the observed diffusion changes point out to a modulation of the neural tissue structure upon activation.

Conclusion

The direct observation of such functional structural changes through DfMRI represents a very promising approach to carry out fMRI studies in conditions where the integrity of neurovascular coupling and the hemodynamic response could be altered, as a result of anesthesia, the presence of interfering drugs or pathology.

References

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