COMBINING FMRI WITH OPTICAL CA^{2+} RECORDINGS TO DEFINE THE IMPACT OF BRAIN STATES ON BOLD

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Purpose:

Furthering our understanding of the neuronal underpinnings of fMRI requires the monitoring of suprathreshold spiking. We implemented the combination of spatio-temporally well defined optical Ca^{2+} recordings with BOLD fMRI in rat somatosensory cortex (S1). With the aim of investigating the interrelation of different states of the neural network and BOLD-fMRI, our experiments were carried out at two different brain states: *persistent Up state* induced by medetomidine and characterized by fast and low amplitude network oscillations, high excitability upon sensory stimulation and relatively depolarized membrane potentials; and the oscillatory state of *Up/Down state*, induced by isoflurane, resembling slow wave sleep in which cortical neurons are in a state of low excitability and generally do not reach the action potential initiation threshold. Optic-fiber-based Ca^{2+} recordings enabled us to characterize those two brain states[1,2] and to directly monitor stimulation induced neuronal spiking activity on the population level. Depending on stimulus frequency and duration, sensory stimulations are known to cause adaptation of suprathreshold neuronal spiking[3]. With independent sets of experiments of varying stimulation frequencies and pulse train durations we explored the impact of suprathreshold neuronal activity on the BOLD response.

Methods:

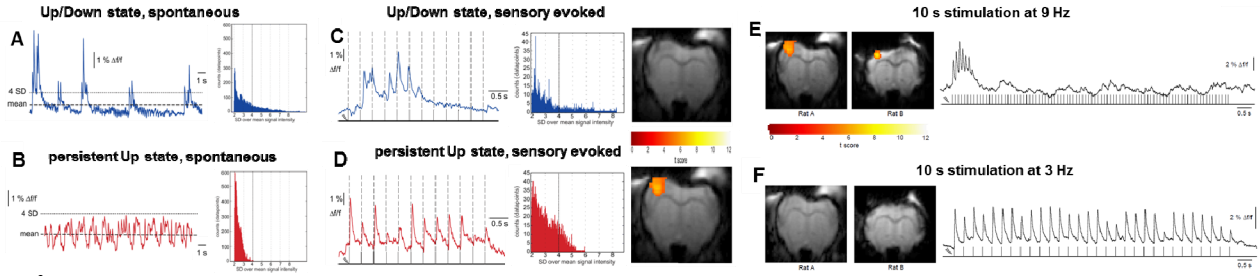
For optic fiber based Ca^{2+} recordings, anesthetized rats were placed in a stereotactic frame and a craniotomy was conducted at the level of S1 of the right hemisphere. Oregon green 488 BAPTA-1 AM (OGB-1) was injected at cortical depths of 300 and 700 µm and an optical fiber was implanted. A custom made optical laser setup was used to excite the Ca^{2+} dye and to record changes in fluorescence. For electric stimulation, two needle electrodes were inserted into the left forepaw and different stimulation paradigms were conducted. Simultaneous functional MRI was performed in a 9.4 T Biospec (Bruker) with an in-house built RF coil with lead-through for the optical fiber. fMRI data analysis was performed with SPM8.

Results:

We could identify *Up/Down state* and *persistent Up state* reliably by quantifying the Ca^{2+} recordings during spontaneous activity (Fig. A, B). In *Up/Down state* high-amplitude stereotypical Ca^{2+} waves were identified (A) versus in same animal in *persistent Up state* only baseline fluctuations could be detected (B). In *Up/Down state* electric forepaw stimulation reliably evoked *Up states* (Fig. C, left panel), with a mean latency of 143 ms ± 7 ms (3 animals, 30 events), in reasonable agreement with studies in mice using visual or auditory stimulation[1,2]. Subsequent pulses within a given pulse train did not evoke additional waves, suggesting refractory behavior of those waves, as previously shown in mice[2]. In *persistent Up state*, we detected neuronal Ca^{2+} responses with a significantly shorter latency of 20 ms ± 4 ms (3 animals, 30 events) and a significantly shorter duration of 55 ms ± 3 ms versus 959 ms ± 36 ms in *Up/Down state* (3 animals, 30 events each).The primary responses also exhibited significantly shorter rise times suggesting a different mechanism of activity initiation. Intensity histograms of traces recorded during sensory stimulation in *Up/Down state* (Fig. C, middle panel) showed an almost identical signal distribution compared to spontaneous activity (Fig. A, right panel). Yet, in *persistent Up state*, we detected signal intensities exceeding 4 SDs (Fig. D, middle panel), in sharp contrast to the spontaneous signal fluctuations (Fig. B, right panel). Notably, in the simultaneously acquired BOLD fMRI measurements, we found drastic differences between the two brain states upon sensory stimulation: in *Up/Down state* sensory stimulation did not lead to reliable BOLD activation (Fig. C, right panel), despite the reliable initiation of slow Ca^{2+} waves (Fig. D, left panels). In *persistent Up state*, we recorded primary neuronal Ca^{2+} responses (Fig. D, left panels), and robust BOLD activation (Fig. D, right panel). Notably, the duration of the BOLD response was clearly dependent on the duration of high-frequency stimulation pulse trains, even though Ca^{2+} transients revealed fast adaptation of the neuronal spiking response (Fig. E, F): while at higher stimulation frequency BOLD response was reliably detected (7 of 7 animals), the much longer neuronal spiking activity (30 versus < 10 primary responses) at lower frequency was accompanied by no or only weak BOLD responses (1 of 7 animals).

Conclusion:

Our data supports the notion that BOLD responses cannot be interpreted as a surrogate of neuronal spiking activity and provide evidence for a predominant contribution of sub-threshold neuronal activity to BOLD.



A, B. Left: Ca^{2+}recordings of spontaneous activity within the scanner. Dashed line indicates 4 standard deviations (SD) over mean level of fluorescence signal intensity. **Right:** Fluorescence intensity histograms of corresponding traces.
C, D. Left: Sensory-evoked responses: long-latency Ca^{2+} wave in *Up/Down-state* (C) short-latency primary responses in *persistent Up state (D)*. **Middle:** Fluorescence intensity histogram of corresponding traces. **Right:** Typical BOLD maps of sensory stimulation in the two brain states.
E, F. Typical BOLD maps and Ca^{2+} responses at 9 Hz versus 3 Hz stimulation in *persistent Up state*.

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