The sensitivity of diffusion MRI in direct detection neuronal activity: an in-vitro assessment

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Synopsis

Diffusion MRI has been proposed as a noninvasive neuroimaging method to detect neuronal activity more directly than BOLD fMRI, yet, initial findings have proven difficult to interpret and reproduce. Here, we study the possible relationship between water diffusion and neuronal activity by simultaneous intracellular calcium fluorescence imaging and diffusion MR of organotypic rat brain cortex cultures. Although we found that diffusion MR can follow pathological changes during hyperexcitability, e.g., as those seen in epilepsy or during anoxia, it does not appear to be sensitive or specific enough to detect or follow normal neuronal activity.

Purpose

Diffusion MRI has been proposed as an alternative fMRI method to detect neuronal activity more directly and accurately than BOLD fMRI,[1,2] yet, initial findings have proven difficult to interpret and reproduce.[3,4] Given that the underlying relationship between water diffusion changes and neuronal activity remains unclear, the biophysical rationale for using diffusion MRI to monitor neuronal activity also requires further investigation. In this work, we attempted to answer one fundamental question – can neuronal activity be detected by diffusion MRI directly in the absence of hemodynamic and other confounds?

Methods

We study the correlation between water diffusion and underlying neuronal activity *in-vitro* by simultaneous calcium fluorescence (optical) imaging and diffusion magnetic resonance (MR) acquisition in a novel test bed developed for fMRI assessment.[5] We used organotypic cortex cultures from rat brains as the biological model of neuronal activity, in which spontaneous neuronal activity robustly emerges free of hemodynamic artifacts. Fluorescent calcium images of neuronal activity are then directly correlated with diffusion MR signals, which were acquired with a static-gradient spin echo sequence in a one-sided NMR system (NMR-MOUSE).[5,6] Here 5 b-values (b = 0, 600, 1200, 1800, and 2400 s/mm^2) with TR = 2 s were used. The diffusion MR signal, S, was fitted by a bi-compartment model:

$$S(b) = S_0 \left[(1-f)\exp(-bD_{fast}) + f\,\exp(-bD_{slow})\right]$$

where D_{fast} and D_{slow} are the self-diffusion coefficients of the slow and fast diffusion components, respectively, f is the slow diffusion component fraction, and S_0 is the MR signal without diffusion weighting. The direct effects of neuronal activity on the diffusion MR signals are studied by time-series analysis of the simultaneous calcium and MR signals during normal neuronal activity and in different pathological states, which include induced hyperexcitability by kainic acid (kainate), suppression of excitability by tetrodotoxin (TTX), a oxygen/glucose deprivation (OGD) protocol mimicking stroke, and cell volume modulation caused by osmolarity changes.

Results

(1) Kainate induces hyperexcitability by activating glutamate receptors, which results in a strong increase in calcium fluorescence intensity *F* (Fig. 1a). The diffusion MR signals change almost simultaneously and similarly in scale with *F*. Larger increases of the diffusion MR signal are observed at higher *b*-values and the slow diffusion component fraction *f* increasing significantly by 20.1 ± 3.4% with respect to the pre-drug levels.

(2) Fig. 2 shows that changes in the diffusion-weighted signals diminish as the depolarization level (fluorescence baseline F_0) decreases during the reduction of kainate concentration from 100 μM to 1 μM, while the number of calcium neuronal spikes in the population increase. This phenomenon suggests that increases in the diffusion-weighted MR signals were dependent only on the culture's level of depolarization, but were independent of the level of the normal neuronal population events (spike activity).

(3) Fig. 3 shows that suppression of normal spontaneous activity by TTX does not affect the diffusion MRI signal. No significant changes were found in either the diffusion MR signals or *f* during the application of 0.2 μM TTX (Fig. 3c), which demonstrates that the diffusion MR signal is not sensitive to the level of normal spontaneous neuronal activity.

(4) In the OGD (stroke) model (Fig. 4), the ADC drops by 4.99% during an acute 30-min OGD perfusion and keeps decreasing until it reaches 14.9% at the end of the 2-hr OGD perfusion, as similar to in vivo observations.[7] Together with ADC changes, an increase in the baseline of *F* with normal neuronal spikes suppressed was also observed.

(5) During normal neuronal activity (without pharmacological agents or other interventions), periods of high ("active") and low ("resting") neuronal activity were identified in the intracellular calcium signal. The MR signal was then binned into active or resting states on using the fluorescence imaging time-base (Fig. 5). Two types of hypothesis were tested and illustrated in Fig. 5a and b. Paired students *t*-Test found no evidence that each single event of normal neuronal activity correlated significantly with the highly diffusion-weighted MR signals (b = 1800 s/mm^2) within a temporal resolution of 100 ms (Fig. 5c and d).

Conclusion and Discussion

A simultaneous increase of diffusion-weighted MR signals was observed together with the prolonged depolarization of neurons caused by pharmacological manipulations, in which cell swelling was demonstrated to play an important role (results not shown). However, no evidence was found that diffusion MR signals were directly correlated to normal spontaneous neuronal activity. These results suggest that while current diffusion MR methods can monitor pathological conditions of hyperexcitability, e.g., as those seen in epilepsy or anoxia, they do not appear to be sensitive or specific enough to detect or follow normal neuronal activity.

Acknowledgements

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References

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Figures

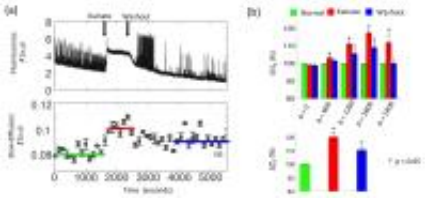

Figure 1. Prolonged depolarization with kainate. (a) The intracellular calcium fluorescence signal *F* integrated over the entire neuronal population (top) and the slow diffusion component *f* (bottom). (b) The averaged diffusion MR signals *S* and slow diffusion component *f* during three phases: pre-kainate, kainate perfusion, and artificial CSF (ACSF) washout.

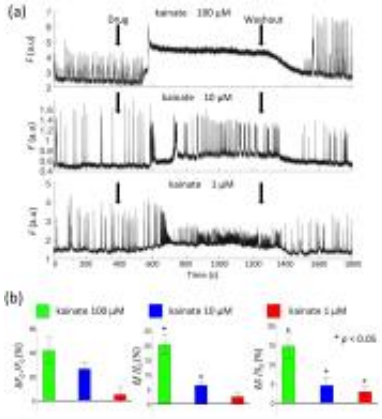

Figure 2. (a) The calcium signal *F* in response to 10-min kainate perfusion at three concentrations. (b) The changes in the fluorescence baseline F_0 (left), slow diffusion *f* (middle) and diffusion MR signals (the average at *b* = 1800 and 2400 s/mm^2, right) under kainate perfusion with various kainate concentrations.

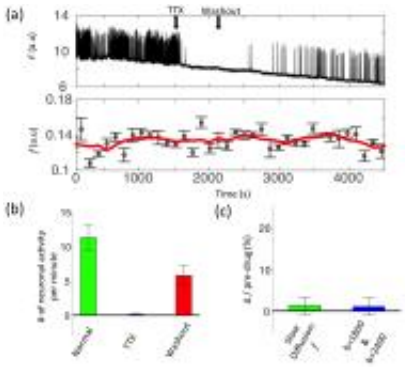

Figure 3. (a) Example of the fluorescence signal *F* (top) and the slow diffusion *f* (bottom, data average every two minutes) in response to a 10-min perfusion of 0.2 μM TTX. (b,c) The change in neuronal firing rate (b) and the diffusion MR signals (c) under perfusion of TTX.

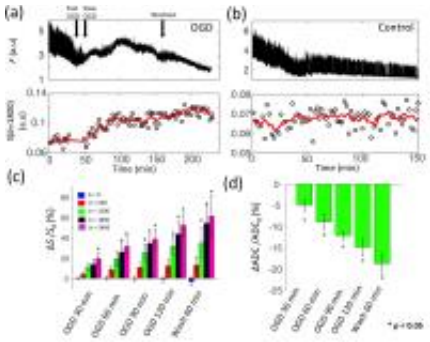

Figure 4. (a,b) The calcium signal *F* (top) and the diffusion MR signal *S* (bottom) in the response of OGD (a) and normal ACSF (b, control). (c, d) The changes in *S* at various *b*-values (c) and ADC (d, calculated at b = 1800 s/mm^2) at various OGD time.

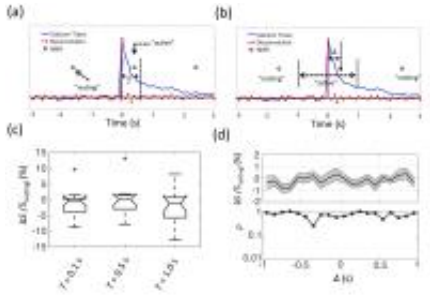

Figure 5. Two hypothesis: each neuronal activity event would only affect the diffusion MR signal (active) recorded immediately after it within a time window, *T* (a, c), or recorded within 1 s before and following it (b, d). (c, d) are statistical results showing the changes in the active state.