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Synopsis

We performed extracellular potential recording on the dissected snail ganglia inside a 3.0 Tesla MRI scanner to investigate the effect of neuronal activity on the MR image intensity. To increase neuronal activity we applied a nitric oxide donor, sodiumnitrosocystein, to the ganglia. We observed the MR image intensity change in the ganglia region is correlated with the neuronal activity and the intensity change is as big as 5.49 ± 1.94 %. Since the snail has non-magnetic hemocyanins as oxygen carrying protein, it seems that the image intensity change is due to the local magnetic fields produced by the neuronal currents.

Introduction

Poor temporal resolution of fMRI imposes big limitations on dynamic studies of brain functions. To complement this limitation, neuronal electric or magnetic signal of the brain, i.e., electroencephalogram (EEG) or magnetoencephalogram (MEG), has been used in combination with fMRI. Recently, direct observation of neuronal activities with MRI has been tried in the human brain. Based on the fact that the magnetic field produced by neuronal currents has effects on the magnetic resonance (MR) image intensity, Xiong *et al* proposed magnetic source MRI (msMRI) with which neuronal activities can be observed in a direct way other than through the blood-oxygenation-level-dependent (BOLD) effect [1]. Based on their experimental observation, they reported that the neuronal magnetic field effect on the MR image intensity in the human brain studies was as big as half the BOLD effect (~1% at 1.5 T). As an attempt to investigate the neuronal current effect on MRI with live neuronal tissues, we performed extracellular potential recording (ECR) on dissected ganglia of *Achatina fulica* (an African agate snail) inside an MRI scanner, and we observed correlation between the ECR and the MRI signal.

Methods

Five African agate snails weighing 30-40g were anesthetized by injection of isotonic MgCl₂ (337mM, 50% of the body weight) and the whole ganglia were dissected out. The connective tissues surrounding the ganglia were carefully removed to prevent possible tissue contractions during the MRI studies. The cleared ganglia were pinned with ~5mm long copper wires on a silicone-base made of Sylgard (Dupont, USA), and they were placed inside a dish that had ~5 ml of saline (composition [in mM]: 53.7 NaCl, 3.4 KCl, 12.9 MgCl₂·6H₂O, 10.7 CaCl₂·2H₂O, 4.4 Na₂SO₄, 3.3 NaHCO₃ and pH = 7.6). Fig. 1 shows photographic and MRI images of the dissected snail ganglia. While performing MRI scans, we measured ECR from the ganglia in an interleaved MRI/ECR scheme using an MRI-compatible ECR measurement system. To increase neuronal activity in the ganglia, we applied 2 mM sodium-nitrosocystein (S-NC) containing saline which can generate nitric oxide (NO). For the MRI scan, we used a 3.0 Tesla whole body MRI scanner equipped with a gradient system capable of 35 mT/m. We used a spin echo pulse sequence with TR/TE of 500/16 ms. The TR and TE were so chosen that the ganglia region has discernible T₁-contrast. Any bipolar neuronal magnetic fields, which are parallel to the main magnetic field of MRI, will have effects on the phase of nuclear spins if the 180° RF pulse is in the time span of the neuronal magnetic fields. The maximum phase angle of the nuclear spins at the axonal surface was calculated to be about 0.05 degrees. Since there are a number of neurons inside an MRI voxel and the neurons are likely to be random directional, it seems that the neuronal magnetic fields will disperse the spin phase, thereby, decreasing the MR image intensity.

Results

In all the cases of five snail studies, we observed that the neuronal activities decreased monotonically with the time constant of about 7min after the initial increase of neuronal activity by S-NC. Fig. 2b shows the mean values of MR image intensity changes at the visceral ganglia region of the snails after the application of S-NC. It was observed that the MR image intensity change had been kept below 1.5 % peak-to-peak in the ganglia region when no S-NC was applied. Even though there were no noticeable motion artifacts in the snail MR images, we applied the rigid body image registration to the MR images to compensate the invisible motion effects. We observe that the maximum MR image intensity change in the ganglia region is as big as 5.49 ± 1.94 %. The correlation coefficient between the mean MR image intensity and the mean neuronal activity was found to be - 0.6367 (P=0.0006, degree of freedom=23). Considering the maximum phase angle caused by a single axonal current of the snail is only 0.05° , the cumulative phase dispersion should come from a number of neuronal currents.

Conclusions

We have observed that the MR image intensity in the snail ganglia region is correlated with the neuronal activities and the maximum image intensity change is as big as 5.49 ± 1.94 % when the ganglia is strongly activated by S-NC. Since the snail blood has non-magnetic hemocyanins as oxygen carrying protein, we presume that the image intensity changes are mainly due to the local neuronal magnetic fields.

References

[1] Xiong J, et al., Hum Brain Mapp 2003; 20: 41-49.



Fig. 1. (a) Photographic images of the dissected snail ganglia.(b) Spin echo MR images of the snail ganglia shown in Fig. 1a



Fig. 2. The normalized number of extra-cellular potential peaks per 20 s at the axon bundle connected with the visceral ganglia (left) and the normalized MR image intensity in the ganglia region (right).