Observation of Neural Activity in Crayfish with Mn-Enhanced MRI

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Intructions

Conventional histological techniques have shown that the olfactory receptor neurons in the olfactory organs of crayfish project to a discrete neuropil in the brain, the olfactory lobe. The character of the olfactory system in crayfish attracts much interest from biologists in the study of processing modalities common to olfactory systems in other species. Because of the small size of the brain, electrophysiology is the predominant method for investigation of activity in the crayfish nervous system. While highly accurate, this method is limited to single-cell or at best multi-unit recordings. In contrast, Manganese-enhanced MRI (MEMRI) allows the measurement of synaptic activity across the entire brain, as well as the opportunity to trace functionally significant neuronal connections. Crayfish have no blood-brain barrier (BBB), and as a result systemically injected Mn^{2+} is quickly distributed throughout the brain and surrounding tissues. Mn^{2+} is an analogue of Ca^{2+}, and can travel through voltage-gated Ca channels that open when a neuron is active. In this work, activity in the crayfish brain was probed with the MEMRI technique. The time dependence of manganese uptake in the brain was investigated and manganese enhancement was observed in the crayfish brain after unilateral stimulation of the antennules.

Methods

The MR experiments were performed with a 4.7T/33cm Varian scanner with horizontal magnet and an actively shielded, 11.7cm inner-diameter gradient-insert operating at a maximum gradient of 25G/cm and a rise time of 250 microseconds. A quadrature birdcage RF coil with a 3.7cm inner diameter was employed to study the time dependence of manganese uptake in the crayfish. A surface coil with 2 cm diameter was utilized for all other studies. A multi-slice spin echo sequence was used to obtain T1 -weighted images of the brain to identify the anatomical structures of the crayfish. The body of crayfish was carefully secured in a home-built restrainer and the crayfish was placed inside the magnet in its awake state. The room temperature was maintained at 19ºC. The experimental parameters for the time dependence of Mn uptake were: TR=1500ms, TE=14ms, slice thickness = 1mm, FOV=3.5×3.5cm, Matrix = 512×128, NEX = 2. Images were acquired every 6 minutes immediately before and within one hour after the injection of $MnCl_2$ solution (10㎖, 120mM). In order to visualize the neural activity in the brain, an electronic stimulus was applied outside the magnetic bore, using a hook electrode around the lateral flagellum of the left or right antennules. The acquisition parameters were: TR=800ms, TE = 12.5 ms, NEX = 8 or 32, slice thickness = 0.2mm, data matrix size = 128×128, FOV = 1.8×1.8cm. Adult crayfish (*Procambarus clarkii*) were used and were cannulated for $MnCl_2$ injection in the pericardial sinus to the immediate left or right of the heart.

Results and Discussion

Fig 1 shows the dependence on time of image intensities in the brain and body after injection of the agent. Initially there is a selective uptake of the Mn by the brain. In Fig 2, a regional variation in the crayfish brain was observed in certain brain areas after the stimulus. The two figures are consistent with the stimulus applied to either the left or right antennule of the crayfish. It can be predicted that the major contributor to the enhancement was due to direct Mn^{2+} uptake in the neuron. The subsequent transport of manganese trans-synaptically may have contributed to labeling of areas to which the olfactory lobe projects. From this result, we conclude that MEMRI has the potential to identify specific areas of neural activity in the crayfish brain.

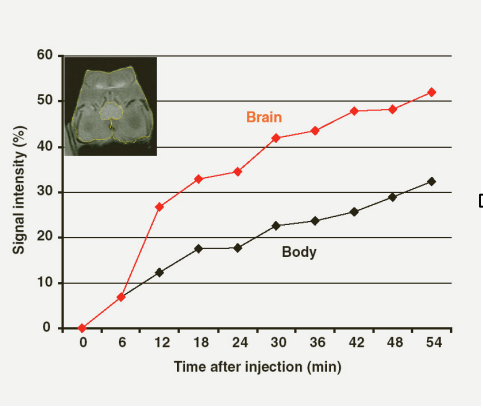
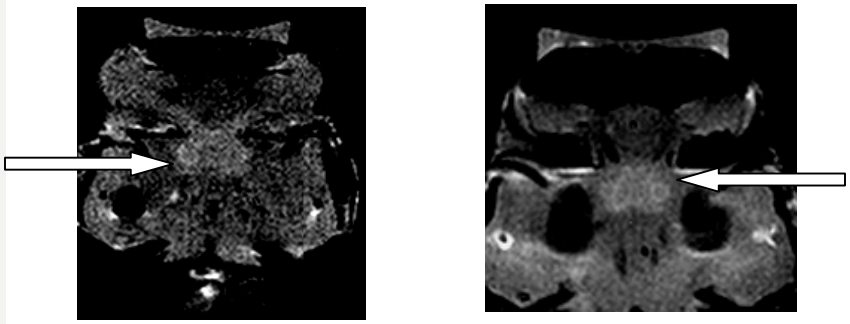


Fig. 1 Time dependence of the manganese uptake



(A) (B)

Fig. 2 Manganese Enhanced MRI of crayfish brain under stimulus in either the left (A) or right (B) antennule (Arrays point to the enhanced areas).

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Reference: Pautler RG, KoretskyAP, 2002,Neuroimage, 16:441-448