Manganese enhanced MRI reveals stimulus-evoked neuronal activation in Aplysia californica

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Target audience: Neuroscience and neurobiology communities.

Purpose: Manganese Enhanced MRI (MEMRI) has become an established technique in neuroimaging with numerous applications including the detection of neuronal activity and neuronal tract tracing[1]. Recently, MEMRI has been used to highlight networks of identified neurons in excised ganglia of *Aplysia californica*[2]. In addition, it has been shown that an injection of $MnCl_2$ solution, *in vivo,* leads to Mn^{2+} accumulation into the neurons of all ganglia in less than two hours[3]. The purpose of the present study was to evaluate whether the MEMRI technique can reveal stimulus-evoked neuronal activation processes as they take place in the living *Aplysia.* We show an increase in MEMRI signal enhancements in ganglia coming from animals subject to specific stimulation protocols vs naïve animals.

Methods: Adult *Aplysia* (100 – 250g) were injected with 100mM $MnCl_2$ solution (500µl/100g) in artificial sea water (ASW). After 1½ hours the animals were anesthetized by an injection of an $MgCl_2$ solution (360 mM). Either the pleural/pedal, cerebral or buccal ganglia were resected, inserted into a 2mm diameter capillary filled with ASW and imaged. MRI was performed in a 17.2T magnet (Bruker BioSpin, Ettlingen, Germany) using a home-built solenoid. 3D, 25 µm isotropic, FLASH (TR/TE = 150/2.44ms) and RARE (TR/TE = 3500/20ms) images were acquired. Baseline experiments: Two ganglia of each type were imaged to estimate the baseline Mn^{2+} distribution. Stimulus-evoked experiments: Six other ganglia (two cerebral, two buccal and two pleural/pedal) were imaged following specific stimulation protocols applied during the 1½ hours between the $MnCl_2$ injection and animal sacrifice. For the cerebral and buccal ganglia the stimulation protocol consisted in feeding. The pleural/pedal ganglia were activated by applying a mild tactile stimulus to the skin. Control experiments: Three additional ganglia (one of each type), from an animal not injected with $MnCl_2$ solution, were imaged as controls. Data analysis: The data were processed using in house written Matlab scripts (MathWorks, Nattick, Massachusetts). For each FLASH image, the ganglia were manually segmented and the segmented voxels were normalized to the ASW signal. Voxels with signal intensity ratios between 1.4 and 2.5 were grouped into 21 bins, depending on their normalized signal level R ($1.45 < R \leq 1.5$; $1.5 < R \leq 1.55$; etc).

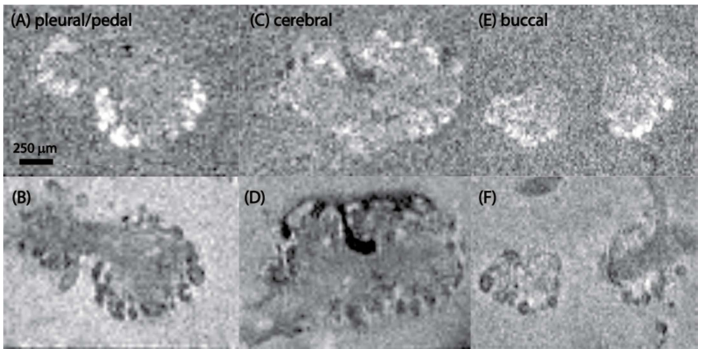

Fig. 1. Selected slices of the pleural/pedal (A, B), cerebral (C, D) and buccal (E, F) ganglia coming from animals injected with $MnCl_2$. (A), (C), (E) T1w images. The hyperintense regions are neurons which have accumulated Mn^{2+}. (B), (D), (F) T2w images in which the cells bodies show up hypointense.

Results: The uptake of the Mn^{2+} into the cells was visible on FLASH images acquired on ganglia coming from animals injected with $MnCl_2$ solution regardless whether they were stimulated or not. Fig. 1 shows FLASH and RARE images obtained on pleural/pedal (A, B), cerebral (C, D) and buccal (E, F) ganglia. Co-registration between T1w and T2w images ensured that the hyperintense voxels seen on FLASH corresponded to cellular regions and were not artifactual. In the control FLASH experiments (images not shown) the contrast did not allow cell visualization. Fig. 2 shows histograms of voxel counts in each bin (expressed as fractions (%) of retained voxels) corresponding to the three ganglia types and the two conditions. Each represented data set is the average of two independent experiments.

Discussion and Conclusion: The comparison of histograms obtained for stimulated vs non stimulated ganglia revealed an increase in the populations of bins with R higher that 1.85, 1.75 and 1.75 for pleural/pedal, cerebral and buccal ganglia, respectively. This increase reflects an increase in intracellular Mn^{2+} accumulation triggered by the sensory stimuli which are expected to activate the central neuronal networks. Is it worth noting that the animals were not anesthetized or restrained after the injection of $MnCl_2$. It is possible that more physically active and, in particular, non-stimulated animals experienced higher overall neuronal activity leading to an increase in non-localized Mn^{2+} uptake, which would underestimate the effects of stimulation. Studies on a larger number of animals are necessary to investigate this possibility.

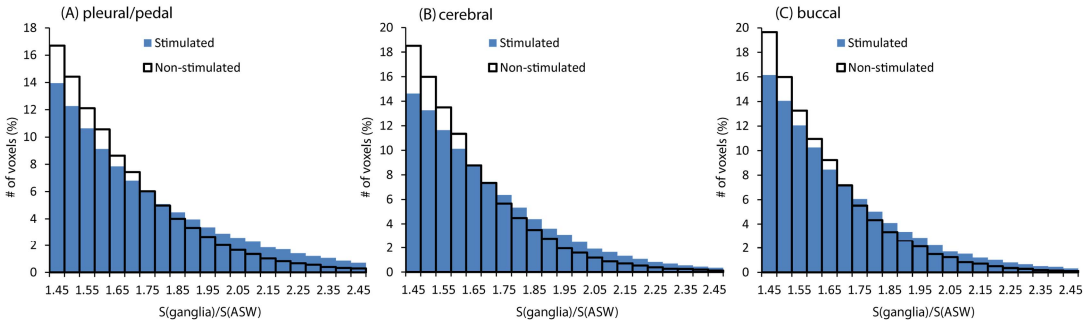

Fig. 2. Distributions of voxel intensities (as % of total voxels with $1.4 < R < 2.5$) for stimulated and non-stimulated conditions in pleural/pedal (A), cerebral (B) and buccal ganglia (C).

Although not quantitatively evaluated in the present study, T1w images showed non-uniform signal intensity distribution, perhaps corresponding to distinct patterns of neuronal activation. Given the high spatial resolution achieved (cellular), one expects that the identification of spontaneous and/or sensory-induced activity in neuronal circuits responsible for such patterns will be possible and therefore the MEMRI approach presented here could provide a basis for combining circuit-based anatomical analyses with electrophysiological recordings in *Aplysia.*

In conclusion, we demonstrate, for the first time, the feasibility of using MEMRI to label activity-dependent Mn^{2+} uptake into the nervous system of a widespread model system in neuroscience, the *Aplysia californica*. Such studies have the potential to provide insight into the cellular plasticity which gives rise to long-lasting modifications of neuronal circuitries as observed in crucial neurological processes including learning, aging and behavioral disorders.

References: 1. Pautler, R.G. et al.. *In vivo* neuronal tract tracing using manganese enhanced magnetic resonance imaging. Magn. Res. Med. 1998; 40:740-748. 2. Jelescu I. O. et al. Highlighting manganese transport in the nervous system with MEMRI in Aplysia californica, Proceedings of the 20th Annual Meeting of the ISMRM 2012, Melbourne, Australia. 3. Radecki G. et al. Toward in vivo functional neuroimaging of Aplysia using manganese enhanced MRI, Proceedings ESMRMB 2012, Lisbon, Portugal.