In vivo brain connectivity: optimization of manganese enhanced MRI for neuronal tract tracing

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One of the main problems in systems biology is to obtain information between interconnected groups of neurons in highly distributed networks. The recently introduced technique of manganese (Mn^{2+}) enhanced MRI (MEMRI) to study neuronal connectivity in vivo opens the possibility to these studies. However, several drawbacks exist that challenge the applicability of this technique. High Mn^{2+} concentrations produce cytotoxic effects that can perturb the circuits under study. On the other hand, the MR signal is proportional to the Mn^{2+} concentration in tissue and thus, significant amounts of Mn^{2+} are required to produce detectable contrast and reliable connectivity maps.

Here we attempt to optimize the MEMRI technique by preventing toxicity and improving the quality and extension of the obtained connectivity maps. The somatosensory cortex of male SD rats was stereotaxically injected with different Mn^{2+}-containing solutions. Total amount of injected Mn^{2+} ranged between 1 and 16 nmol and the injected volumes between 10 and 80 nL. Osmolarity and pH effects were investigated injecting pH buffered solutions of Mn^{2+} (pH 7.3 in Tris-HCl buffer vs. 5.5 in H_2O) at different concentration (0.05, 0.1 and 0.8 M $MnCl_2$). Same amounts of Mn^{2+} (8nmol) delivered to the tissue at different infusion rates were also compared. Following the injection, T_1-weighted MR imaging (250 mm isotropic resolution) was performed in a 7T scanner at different time points. Fifteen days after the injection animals were sacrificed and brains processed for histology. Nissl staining as well as GFAP and NeuN immunohistochemistry (selective staining for astrocytes and neurons, respectively) were performed in the brain sections to examine cellular toxicity. All injections produced connectivity maps consistent with the known anterograde projections of SI cortex based on classical neuronal tract-tracing techniques. Our results show that pH buffered solution improve the effectiveness of MEMRI, increasing T_1 contrast in the projection sites. In addition, injections of pH buffered and isotonic solutions of 50 and 100 mM $MnCl_2$ yielded extensive connectivity maps. In particular, ipsi- and contra-lateral corticocortical connections were evident in all animal injected with those solutions but not with the classical MEMRI protocol (0.8M $MnCl_2$ in H_2O). Hypertonic and non-buffered solutions containing 8nmol Mn^{2+} resulted in neuronal death and astrogliosis in extensive areas around the injection point. In contrast, no neuronal toxicity was observed with injections containing up to 8nmol of Mn^{2+} in isotonic solutions of up to 100 mM $MnCl_2$ and pH 7.3. Slow infusion rates demonstrated also to be advantageous and permitted application of larger amounts of Mn^{2+} without toxic effects, resulting in better T_1 contrast in the low density projection fields. No sign of toxicity was observed in any condition in the projection fields.
We conclude that refined protocols for MEMRI improve the quality and extension of connectivity maps and preserves tissue viability, assuring the application of this technique in longitudinal experiments.