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Transcranial manganese delivery for neuronal tract tracing using MEMRI

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ABSTRACT

There has been a growing interest in the use of manganese-enhanced MRI (MEMRI) for neuronal tract tracing in mammals, especially in rodents. For this MEMRI application, manganese solutions are usually directly injected into specific brain regions. Recently it was reported that manganese ions can diffuse through intact rat skull. Here the local manganese concentrations in the brain tissue after transcranial manganese application were quantified and the effectiveness of tracing from the area under the skull where delivery occurred was determined. It was established that transcranially applied manganese yields brain tissue enhancement dependent on the location of application on the skull and that manganese that enters the brain transcranially can trace to deeper brain areas.

Introduction

Manganese is an essential element that has fundamental roles in cellular processes in living organisms, especially in brain function (Aschner et al., 2006). Mn^{2+} has similar ionic radius as Ca^{2+} and behaves as a Ca^{2+} analog (Merritt et al., 1989; Narita et al., 1990). Thus, Mn^{2+} can enter excitable cells such as neurons via L-type voltage-gated Ca^{2+} channels (Narita et al., 1990; Pautler, 2006) and can activate N-methyl-D-aspartate glutamate receptors (Gobbo et al., 2012). Once inside neurons, Mn^{2+} is transported along the axons in a microtubule dependent manner and gets released into the synaptic cleft, where it gets taken up by the next neuron in the neural circuit (Sloot and Gramsbergen, 1994; Pautler et al., 1998, 2003; Takeda et al., 1998; Pautler and Koretsky, 2002). Moreover, Mn^{2+} is a paramagnetic ion and acts as an excellent contrast agent in magnetic resonance imaging (MRI) (Mendonca-Dias et al., 1983; Geraldes et al., 1986; Cory et al., 1987). Accumulation of Mn^{2+} in a tissue leads to shortening of the spin-lattice relaxation time, T_1, and the spin-spin relaxation time, T_2, of water molecules in that tissue. T_1 relaxivity is typically more sensitive than T_2 relaxivity for MRI at higher magnetic fields. Shortening of T_1 of the tissue in which Mn^{2+} accumulates results in a positive contrast enhancement in T_1-weighted MRI of that tissue. These combined properties of Mn^{2+} have led to use of manganese-enhanced MRI (MEMRI) in the past couple of decades in a large variety of studies. Presently, there are three major uses of MEMRI in the brain: for enhancement of brain cytoarchitecture in anatomical studies (Natt et al., 2002; Watanabe et al., 2002, 2004; Aoki et al., 2004b), for mapping neuronal activity (Lin and Koretsky, 1997; Aoki et al., 2002, 2004a; Hsu et al., 2007; Yu et al., 2008) and for *in vivo* tracing of neuronal connections (Pautler et al., 1998, 2003; Van der Linden et al., 2002). The use of MEMRI for anatomy and function extends to other tissues as well, such as heart (Hu et al., 2001), pancreas (Gimi et al., 2006) and tumors (Banerjee et al., 2007; Hasegawa et al., 2011).

The first study to use MEMRI for neuronal tract tracing focused on tracing of olfactory and visual pathways in mouse brain (Pautler et al., 1998). Since then, many MEMRI tract tracing studies have been successfully performed on different neural pathways and species, such as the song control pathway in songbirds (Van der Linden et al., 2002, 2004), olfactory pathway in rats (Cross et al., 2004; Chuang and Koretsky, 2006), visual pathway in rats and nonhuman primates (Watanabe et al., 2001; Thuen et al., 2005; Murayama et al., 2006), somatosensory pathway in rats (Allegrini and Wiessner, 2003; Leergaard et al., 2003), basal ganglia pathway in macaques, mice and rats (Saleem et al., 2002; Pautler et al., 2003; Pelled et al., 2007), auditory pathway in guinea pigs (Lee et al., 2007) and corticospinal pathway in rats and marmosets (Bilgen et al., 2006; Demain et al., 2015). For neuronal tract tracing, Mn^{2+} solutions are usually directly injected into specific brain or peripheral areas, or delivered nasally when tracing olfactory pathways.

A recent study of traumatic brain injury (TBI) has shown that small

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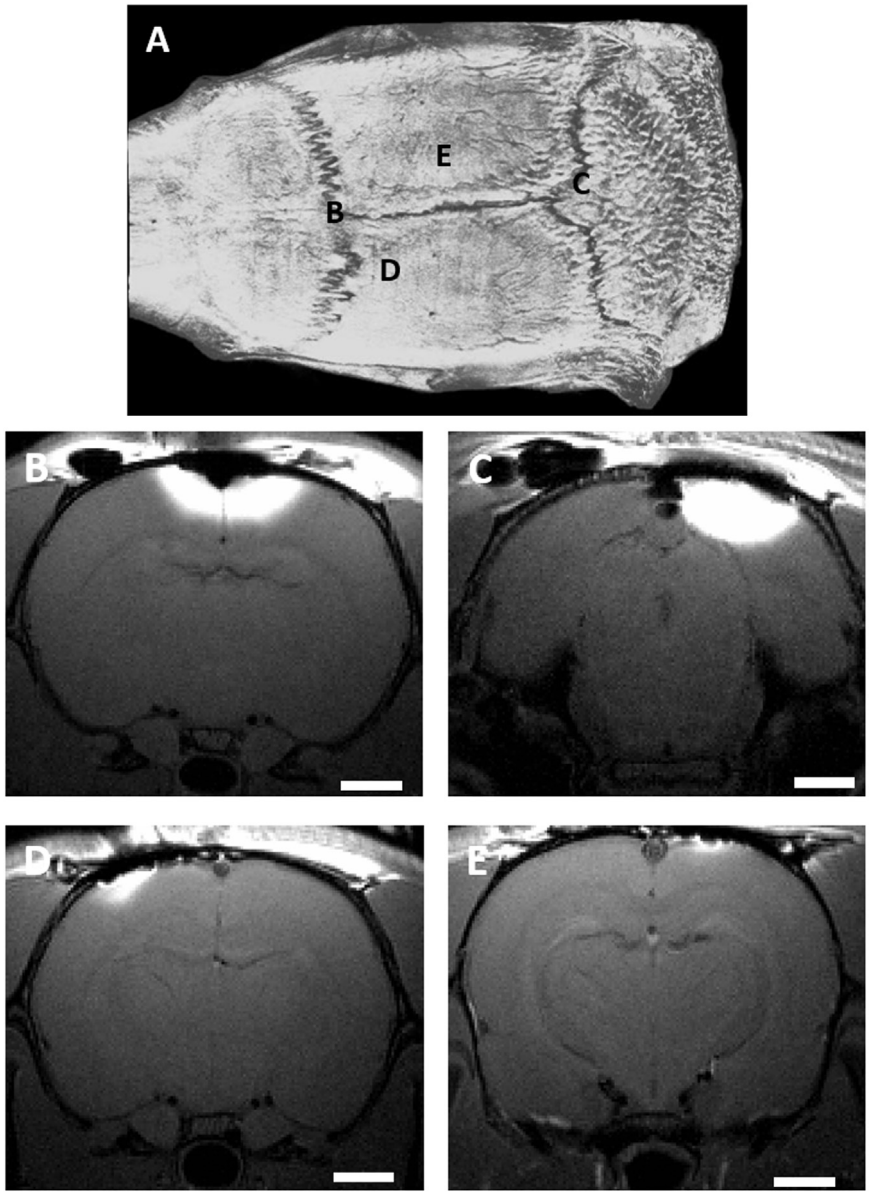

Fig. 1. Location dependence of transcranial manganese delivery efficiency: CT image of a rat skull (A) and T_1 - weighted MR images of rats receiving 500 mM solution of $MnCl_2$ on the bregma (B), on the lambda (C), left side close to S1 (D), approximately −1.4 mm posterior from bregma and lateral −2 mm, and right side away from the bregma (E), approximately −5 mm posterior from bregma and lateral +3 mm. Scale bar, 2 mm. Images are representative of three rats per group.

molecular weight fluorescent molecules as well as dextrans of various sizes can diffuse through intact murine skull into the meningeal space (Roth et al., 2014). Meningeal concentrations of these molecules were found to be dependent on the size of the applied molecules and length of the application to the thinned skull. This method of substance delivery to the brain is called transcranial application. In addition to fluorescent probes, several purinergic receptors and manganese solutions were demonstrated to reach the brain with transcranial application. In the case of $MnCl_2$, MRI enhancement was detected below the area of the brain where the $MnCl_2$ was applied. In that study, the Mn^{2+} passage through the intact rat skull was demonstrated but not well characterized. The purpose of this study was to determine the factors that affect transcranially applied Mn^{2+} and determine whether the Mn^{2+} that enters the brain can be used to trace neural systems.

Materials and methods

Animal preparation

Adult male Sprague-Dawley rats (body weights 200–300 g), obtained from Harlan Laboratories, were used for this study. The animals were provided Open Formula Rat and Mouse Diet (NIH-07). All animal work was performed according to the guidelines of the Animal Care and Use Committee of National Institute of Neurological Disorders and Stroke, National Institutes of Health (Bethesda, MD). The rats were initially anesthetized with 5% isoflurane, and then switched to 1–2% isoflurane for maintenance throughout the procedure. Body temperature was monitored and maintained by a heated water bath. As previously described (Roth et al., 2014), animals were placed in a stereotaxic apparatus and a single midline incision with a sterile scalpel