MEMRI of the Projections of Periaqueductal Gray Matter to Pontine Reticular Nucleus in Mice

X. Zhang[1], and R. Jacobs[1]
[1]Biological Imaging Center, California Institute of Technology, Pasadena, California, United States

Introduction: The midbrain periaqueductal grey (PAG) plays a crucial role in endogenous pain attenuation mechanisms in the central nervous system [1]. It is known that when microinjected into PAG, neurotensin and morphine regulate pain transmission by modulating PAG-pontine reticular nucleus (PAG-PRN) antinociceptive neuronal circuitry [2]. Efforts to understand these effects in small animal models have been limited by available techniques, *i.e.* conventional histology. Cellular Ca^{2+} fluorescence measurement indicated that neurotensin rapidly opens nonselective cation ion channels and induces Ca^{2+} release from intracellular stores in PAG-PRN neurons. Manganese (Mn^{2+}), an MRI detectable neuronal tract tracer, enters neurons *in vivo* via voltage and ligand gated calcium channels and is an indicator of neuronal activity [3]. In this study we injected nanoliter volumes of Mn^{2+} into the PAG of mice and followed the time course of Mn^{2+} uptake, transport, and accumulation over 48 hr by sequential high resolution MRI. The aim of this study was to map Mn^{2+}projection from PAG to PRN and provide an alternative methodology for antinociception study.

Material and Method: Adult female mice (C57BL/6, n =3) were anesthetized with isoflurane and placed in a stereotaxic frame. A volume of 5 nL Mn^{2+} (500 mM was injected into right ventrolateral PAG (0.4 mm ML, -4.2 mm AP and -2.4 mm DV) using a glass pipette (inner diameter=15 micron). Imaging was performed in a vertical-bore 11.7-T (500 MHz) Bruker AVANCE imaging spectrometer with a microimaging gradient insert and 20mm birdcage radiofrequency coil. T1 weighted 3D fast low angle shot (FLASH) (pulse repetition time/echo time, 60 ms/3.5 ms; flip angle $=45^0$) with the image matrixes (128x110x70) and field of view (16x13.7x8.7 mm^3) for a voxel size of 125^3 $micron^3$. MR images were acquired 30 min, 2.5, 24 and 48 hr after injection. Signal intensities in specific regions of interest were measured with imageJ. Areas with enhanced contrast were identified using http://www.brain-map.org in combination with the mouse brain in stereotaxic coordinates. A two-tailed Student t-test was used for statistical analyses. Statistical significance was accepted at a P value less than 0.05.

Results and discussion: Mn^{2+} trans-synaptic tract tracings were visualized by MEMRI originating at the site of the injection in the PAG tracing to both the anterior and posterior neuronal connections. After 30 min and 2.5 hr injection, diffusion of Mn^{2+} ions was seen proximal to the injectioin site of PAG. At 24 hr, the interpeduncular nucleus (IPN), the thalamus (TH) and the red nucleus (RN) exhibited hyperintensity (Fig. 1). Furthermore, direct tracings to the hypothalamus (HY), the dentate gyrus of the hippocampus (DG) and the superior colliculus (SC) were observed. Measurements of signal intensities in HY, IPN and PRN are summarized in Fig 2. Remarkably, there is a significant signal enhancement in the contralateral PRN. We hypothesize that the PAG may exert an inhibitory effect on spinal nociceptive functions through activation of descending serotonergic and noradrenergic neurons in the PRN [4]. The results of MEMRM support the notion that the pontobulbar reticular formation receives direct inputs from the PAG. After 48 hr, visualization of active transport is impaired by diffusion of Mn^{2+} ions throughout the midbrain and pons.

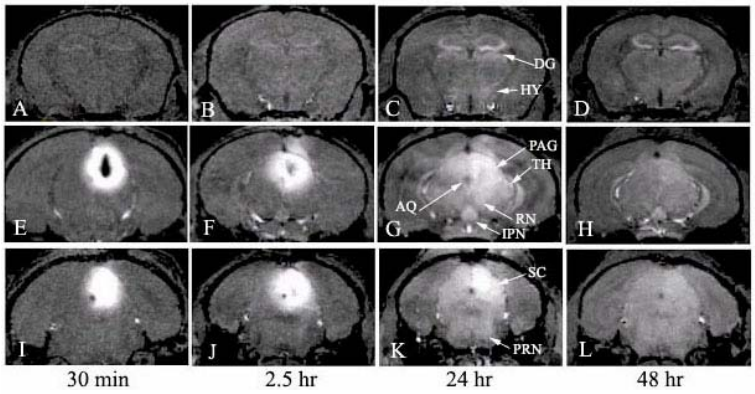

Fig. 1. Example in vivo mouse brain images acquired after Mn^{2+} injection. Top row: 2.5 mm from the injection site; middle: at the injection site; and bottom: -0.7 mm. Note the midbrain nuclei (RN, TH and IPN) and contralateral PRN are hyperintense at 24 hr post-injection.

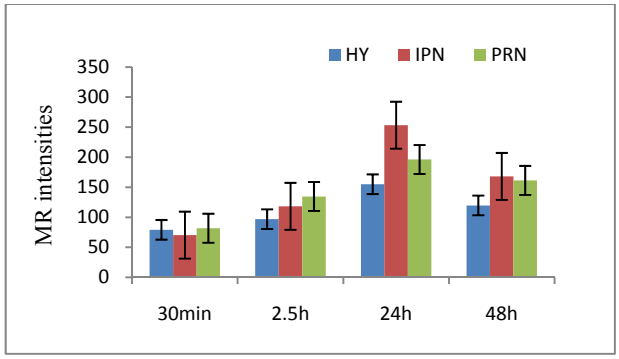

Fig. 2. MR signal intensities from 3D FLASH images in selected brain regions at four time points after injection. Compared to the PRN at 30 min and 24 hr after injection, the signal intensities are significantly increased ($P < 0.05$). N=3.

Conclusion: Using MEMRM plus a stereotaxic injection methodology, efferent neural projections from the PAG to PRN were delineated in vivo. Since changes in the pattern of Mn^{2+} contrast enhancement in MRI reflect changes in activity (increased or decreased neuronal firing), MEMRM may be used for studies of analgesic effects in neuronal responses to the inhibition or stimulation produced by the opioid peptides and morphine.

Reference

[1] Annu. Rev. Neurosci 1991(14), 219;
[2] Curr. Opinion. Neurobiol.1999(9), 436;
[3] MRM 2003 (50), 33;
[4] Neuroscience 1992(47), 863;