# Investigate the mechanisms of anesthetic-induced unconsciousness in a mouse model by high-resolution manganese enhanced MRI (MEMRI) technique: A Preliminary Study

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**INTRODUCTION:** Manganese is a useful contrast agent for MRI of animals [1], which utilizes the fact that paramagnetic manganese ions ($Mn^{2+}$) enter synaptically activated neurons through voltage-gated calcium channels [2], resulting in enhancement on T1-weighted MRI images. Although much progress has been made deciphering the effects of anesthetics upon individual ion channels, we are only beginning to identify putative neural substrates upon which anesthetics act to produce their behavioral effects. Of the key components that characterize the anesthetized state, we focus on volatile anesthetic-induced unconsciousness, defined as a lack of perceptive awareness to non-noxious stimuli. Two emerging hypothalamic targets with proven ability to affect arousal state are the median preoptic nucleus, MnPO, and ventrolateral preoptic nucleus, VLPO. Depolarization of these two regions is respectively thought to underlie onset and maintenance of natural sleep. Our hypothesis is that volatile anesthetics cause unconsciousness (behavioral hypnosis) by affecting VLPO and MnPO function. In this preliminary study, we verified the hypothesis by high-resolution MEMRI technique, the results showed clear signal enhancements within MnPO of the anesthetized mice compared to the awake control.

**METHODS:** All the experiments were conducted with approval of the IACUC (*Institutional Animal Care and Use Committee*) at the University of Pennsylvania. Twenty-four hours prior to MRI scan, mice received an intraperitoneal injection of 30mM manganese chloride for a total dose of 0.4mmol/kg [3]. Based on the landmark demonstration that MEMRI could be used to map activation of auditory evoked neural activity following presentation of a defined sound in mice for 24 hours [3], we used a similar continuous "stimulus" paradigm of either enforced wakefulness or 1.0% isoflurane-induced unconsciousness to map arousal circuit activity, but reduced our "stimulus" (anesthesia or wakefulness) to 12 hours to avoid other confounds such as hypothermia, and food/water deprivation due to anesthetic administration. The control mice ($n$=4) were placed in constantly turning righting reflex chambers to promote wakefulness. Unconsciousness of anesthetized mice ($n$=3) was induced by identical treatment of isoflurane. Both awake and anesthetized mice breathed 100% oxygen and were actively warmed by radiant heat transferred from the water bath for 12 hours immediately prior to MRI scan. To achieve necessary SNR (signal to noise ratio) with high resolution scans (multiple averages resulting in long imaging time) and ensure absolute immobility, all mice were sacrificed via perfusion and scanned *ex vivo*. This requires delivery of a brief anesthetic to the "awake" control group. As MEMRI is sensitive to changes in neural activity over the preceding 1-24 hours [3-5], delivery during perfusion of a brief isoflurane anesthetic to the enforced wake group should only minimally confound our results.
All MRI scans were performed on a Varian 9.4T vertical MR system with console Inova and Vnmr interface (Varian Inc., Palo Alto, CA), equipped with a 45 mm ID gradient unit capable of generating maximum gradient amplitude 100 gauss/cm (RRI, Billerica, MA). A 25 mm loop-gap resonator constructed in house was used for RF transmitting and receiving. T1 weighted 3D gradient echo sequence was used for data acquisition, with TR=50ms, TE=2ms, and FA=65º, resulting in a volumetric image set covering the entire brain, and voxel size 80μm × 80μm × 100μm. Number of averages=14, total acquisition time was around 4.5 hours. After imaging, data were transferred to the laboratory workstation for post-processing.

**RESULTS AND DISCUSSIONS:** The brain images were realigned manually by comparing with a mouse brain atlas [6]. Images that cover the regions of MnPO and VLPO were examined slice by slice to identify the potential signal enhancement. Clear signal enhancements within MnPO were found in the anesthetized brains compared to the control, Figure 1. An atlas image (close to Figure 1 anatomically) was shown in Figure 2 to indicate the location of MnPO.

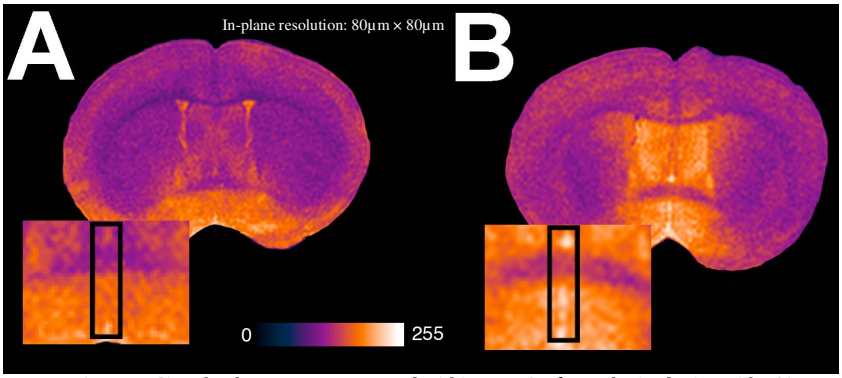


*Figure 1. Signal enhancements occurred within MnPO of anesthetized mice with 1% Isoflurane (B), compared to awake control (A).*

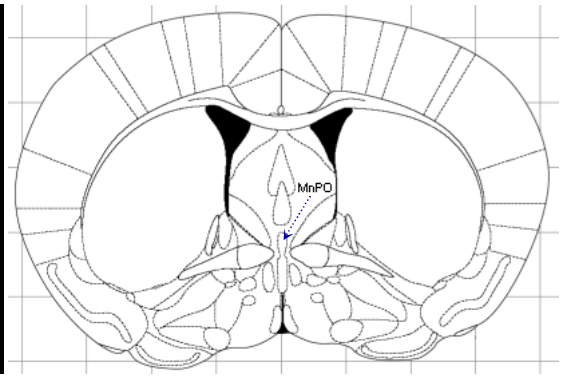


*Figure 2. An atlas image shows the MnPO region.*

In summary, signal enhancement was identified within MnPO by the MEMRI technique as expected, which validated our hypothesis and verified the feasibility of our protocol. The current results did not show signal enhancement in VLPO (a minor structure in the anterior hypothalamus), one possible reason is that the present MRI resolution is not fine enough for this specific region, which suggests use of higher resolution to investigate VLPO in the further experiments.

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