MEMRI for *In Vivo* Imaging of the Embryonic Mouse Nervous System

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Introduction.

The mouse is the preferred organism for studies of mammalian development and disease due to the extensive genetic methods that have been optimized in this system. Most of mammalian brain development occurs *in utero* prior to birth, and a continuing challenge in this field is the establishment of *in vivo* and longitudinal methods of analysis. Ultrasound has been the mainstay for *in vivo* embryo imaging both in mouse research (for recent review: [1,2]) and clinically. However, ultrasound is limited by depth of penetration and offers few methods for selective contrast modification. MRI has tremendous versatility of contrast in the presence and absence of exogenous contrast agents, but has been limited in previous attempts at *in vivo* embryo imaging in rodents by motion and signal to noise (SNR) challenges [3-5]. Manganese-enhanced MRI (MEMRI) methods for studies of neuroanatomy and function have demonstrated accumulation of manganese ions in neural tissue of small animals postnatally [6-9]. In this study, we use MEMRI to increase SNR in the embryonic brain and spinal cord in combination with respiratory gating to decrease motion artifacts to visualize the developing CNS in the living mouse embryo from mid to late gestation.

Methods.

Timed-pregnant ICR mice were intra-peritoneally injected with $MnCl_2$ solution (80 mg/kg) 24 hours prior to imaging at embryonic day (E) E11.5, E13.5, or E16.5 (E0.5 is noon on the day after mating). MRI of the mouse abdomen was performed under isofluorane anesthesia (5% induction, 1-1.5% maintenance) at E12.5, E14.5, or E17.5 using a custom half-cylinder shaped surface coil (length= 30mm , ID= 14mm). Images were acquired using a T1-weighted 3D gradient echo sequence (TR= 40ms, TE=5ms, Flip angle= 35˚, FOV= 24mm x 24mm x 48mm, Matrix= 240x240x480). Respiratory motion was monitored using a pneumogram transducer pillow (BioPAC systems) fixed to the abdomen of the pregnant mouse using a Velcro strap. The analog signal (Fig.1a) was converted to TTL using thresholding to identify the active part of the breathing cycle (Fig.1b). The 3D excitation pulse (RF) was applied every TR regardless of breathing motion to maintain relative steady state magnetization (Fig. 1c). The data acquisition was re-iterated with the same phase encoding until gating conditions were met, i.e. until the mouse was not actively breathing (Fig. 1d). Total imaging time per mouse was 70-90 minutes. Image segmentation was done using Amira software.

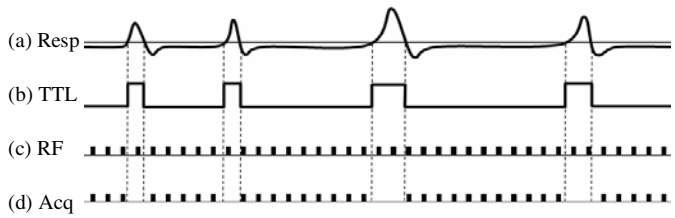

Fig.1 Prospective respiratory gating: (a) analog signal, (b) threshold conversion to TTL, (c) continuous excitation, and (d) gated acquisition.

Results.

Manganese was clearly observed to accumulate in the developing neural tissue, demonstrating enhancement in the spinal cord and ventral regions of the brain from E12.5 (Fig. 2). The SNR (mean signal over standard deviation of noise) was increased close to 3-fold compared to un-enhanced embryos (from 22-24 to 63-66), facilitating segmentation and 3D visualization of the brain and spinal cord (Fig. 3b). Particularly at later stages, substructure within the brain was easily appreciated (Fig. 3a), including the neocortex (magenta), hippocampus (orange), thalamus (blue), hypothalamus (aqua), globus pallidus (red), striatum (green), and amygdala/ rhinencenphalon (yellow).

Conclusions.

MEMRI methods show great potential for studies of neural development in the living mouse embryo. Techniques including segmentation of the CNS and brain substructures will be tremendously useful for single time-point or longitudinal analysis of living embryos during normal and altered neurodevelopment.

Acknowledgments.
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References.

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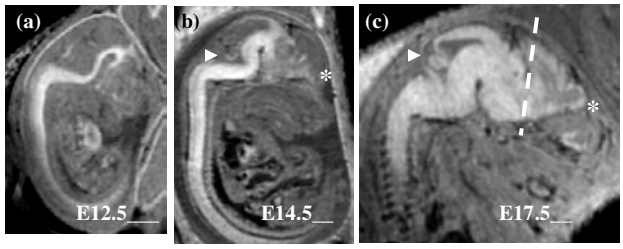

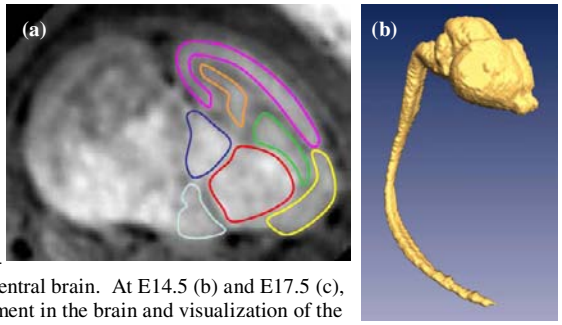

Fig.2. (left) *In vivo* MEMRI at E12.5 (a) showed enhancement in the spinal cord and ventral brain. At E14.5 (b) and E17.5 (c), there was increased enhancement in the brain and visualization of the olfactory bulb (*) and the developing cerebellum (►). Scale bar= 500µm.

Fig.3. (above right) Structures within the brain are visible at E17.5 (a) (section as indicated in Fig.2c), including the cortex, hippocampus, thalamus, hypothalamus, globus pallidus, striatum, and amygdala/ rhinencephalon. Increased SNR allows efficient segmentation of the brain and spinal cord at E17.5 (b).