Evaluation of Mouse Brain Development with MRI

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Introduction

Extensive genetic information and the expanding number of techniques available to manipulate the genome of the mouse have led to its widespread use in studies of brain development and to model human neurodevelopmental diseases. Traditionally, the results of gene manipulation experiments in mice have been analyzed using histological methods which are static and two-dimensional, making it difficult to understand the underlying developmental and disease processes which are three-dimensional (3D) and dynamic, evolving over variable time periods from days to weeks.

Magnetic resonance imaging (MRI) is an inherently 3D imaging method that has been applied and refined over the past decade for *in vivo* phenotype analyses of a wide variety of neurological disease models in adult mice (e.g., reviewed in Anderson 2007; Nieman 2007). The small neonatal and embryonic mouse brain presents additional challenges to provide sufficient resolution as well as contrast, since many cells and tissues are undifferentiated or immature, resulting in minimal differences in the MR relaxation properties that are usually exploited for image contrast in the adult animal (reviewed in Turnbull, 2007). Furthermore, many critical events in mammalian brain development occur inside the maternal uterus where physiological motion presents significant challenges for effective acquisition of artifact-free MR data. In this short overview, progress in the area of MRI for *in vivo* analysis of normal and abnormal mouse brain development is reviewed.

Neonatal Brain MRI

Neonatal mouse imaging provides the most feasible entry point for MRI studies of brain development, since motion artifacts can generally be minimized or eliminated using a well-designed head holder. Many important developmental events, including maturation and functional development of most brain subregions, occur at early postnatal stages. Importantly, it has been established that freely inhaled isoflurane anesthesia can be applied safely in mouse neonates, similar to most current *in vivo* MRI studies of adult mice. MRI has been used to assess gross disease including hemorrhages in the cerebral ventricles (Xue, 2003), and hypoxic ischemia in the neonatal mouse brain (Aden, 2002). For finer detail, the pre-myelinated neonatal mouse brain presents a challenge for conventional MRI, using relaxation-based (T_1, T_2) contrast that is generally dominated by regional myelin concentration. Diffusion tensor imaging (DTI) provides a potential solution to this problem, producing contrast based on the underlying cellular and tissue architecture in the immature mouse brain (Mori, 2001; Zhang, 2005). At this point *in vivo* DTI has not been demonstrated in neonatal mice, but recent reports in weaning stage mice provides optimism that *in vivo* studies will be soon be feasible at earlier developmental stages (Lope-Piedrafita, 2008). Manganese-enhanced MRI (MEMRI) has been found to be useful for analyzing the neonatal mouse brain *in vivo*, enhancing several prominent brain regions after systemic administration of $MnCl_2$, including olfactory bulb, hippocampus and cerebellum, and providing high resolution data (100-μm isotropic) in scanning times (~2h) acceptable for *in vivo* imaging (Wadghiri, 2004). MEMRI has also been reported for longitudinal analysis of variable cerebellum patterning defects in neonatal *Gbx2* mutant mice (Szulc, 2008).

In Utero Micro-MRI

The use of MRI to image mouse embryos dates back to the pioneering studies of Smith, Johnson and co-workers at Duke University in the mid-1990s (Smith, 1994). The general approach developed in those first studies, preparing fixed embryo samples and acquiring high resolution (20-50 µm) *ex vivo* 3D data over long (6-24 h) imaging times, continues to be utilized for both relaxation-based (Dhenain, 2001) and DTI-based MRI studies (Zhang, 2003). These studies have emphasized the advantages of 3D MRI as a complement to histology, rather than for dynamic and longitudinal studies.

In vivo MRI of mouse embryos inside the maternal uterus has been demonstrated (Hogers, 2000; Chapon, 2002), using fast imaging methods to identify and monitor intra-embryonic structures over time, but resolution in these studies was limited to the whole organ/brain level. One recent study imaged very early stage embryos, using MRI to identify implantation sites and to analyze early vascular changes during implantation (Plaks, 2006). Although gross brain structures were identified in these reports (brain tissue versus fluid-containing ventricles), neural development was not emphasized, in part because of a lack of significant contrast within developing brain tissues.

In utero MEMRI, in combination with maternal respiratory gating has been shown to provide sufficient resolution (100 µm isotropic) and contrast to perform volumetric measurements of brain subregions, including *in vivo* analysis of ventral forebrain defects in *Nkx2.1* mutant embryos (Deans, 2008). Although Mn-toxicity was shown to be minimal at later fetal and neonatal stages, earlier staged embryos were susceptible to Mn-induced lethality at doses providing optimal contrast, making MEMRI unsuitable for longitudinal studies over early embryonic stages of brain development. In addition, maternal respiration is not the only source of motion during embryonic imaging, making it necessary to implement a combination of motion-gated acquisition and image coregistration for the best elimination of *in utero* motion artifacts (Nieman, 2008; 2009).

Functional Neuroimaging During Brain Development

An important approach used in many human neuroimaging studies is functional MRI (fMRI) to analyze regional activity resulting from defined stimuli. FMRI based on BOLD contrast is challenging to implement even in adult mice (e.g., Ahrens, 2002), requiring high resolution and multiple cannulae for maintaining and monitoring animal physiology during imaging, and has not yet been adapted to pre-weaning stage mice. An important application of MEMRI is for direct imaging of neural activity, based on the uptake of Mn ions in cells through calcium channels (Lin, 1997). MEMRI has recently been applied for imaging accumulated sound-evoked activity in pre-weaning stage mice after systemic administration of Mn (Yu, 2005), which enabled unprecedented functional studies of developmental plasticity in neonatal mice from the onset of hearing (Yu, 2007).

Cellular and Molecular Imaging

Ultimately, the widest set of applications would become available if MRI were extended beyond anatomical and functional imaging, to include methods for labeling and monitoring defined neural cells and gene expression patterns over time in living mice. In the area of cellular imaging, *ex vivo* MRI of fixed mouse embryos has been used to demonstrate that micron-sized iron oxide particles (MPIO), internalized in cells of mouse blastocyst-stage embryos, could be detected more than a week later in 11.5-day mouse embryos (Shapiro, 2004). More recently, the same MPIO particles were shown to allow *in situ* labeling and *in vivo* tracking of neural progenitor cells in the adult rat brain (Shapiro, 2006). Similar methods have been demonstrated in principle for labeling progenitor cells in the embryonic mouse brain, and to image their subsequent migration patterns with *ex vivo* MRI (Deans, 2006). With the recent development of effective methods for *in utero* MRI (Deans, 2008; Nieman, 2009), it should soon be possible to

track these critical neural stem cell populations *in vivo* in the developing mouse brain. Promising results in the area of molecular imaging have also been reported, achieving *in vivo* MRI contrast by cellular iron internalization following expression of ferritin (Genove, 2005; Cohen, 2007). It remains to be seen whether this approach will provide a robust method for *in vivo* gene expression imaging in developing mouse embryos.

Summary

In summary, micro-MRI has already found a number of applications for anatomical and functional analysis in developing brains of mouse embryos and neonates. Ultimately, the full capability of MRI lies in its potential for *in vivo* imaging of both mouse embryos, inside the maternal uterus, and postnatal mice from the earliest neonatal to adult stages. The ability to image the mouse over this wide range of developmental stages will enable longitudinal studies of anatomical and functional changes in individual animals. Similar to the recent developments in adult mice, MRI may also serve as a valuable tool for high-throughput screening, especially using multiple-mouse imaging to pre-select animals of interest for analysis with other imaging and non-imaging methods (Bock, 2005; reviewed in Nieman, 2007). The main challenges for the future are to further develop robust methods for *in utero* MRI, and to develop cell-specific contrast-enhancement approaches to allow a wide variety of functional studies, including *in vivo* cell tracking and gene expression imaging in the developing mouse brain.

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