MRI of the Developing Mouse Brain: From Anatomical to Molecular Imaging

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The Mouse as a Model Organism for Studies of Brain Development and Disease

Genetically engineered mice have become the preferred experimental organism for studies of mammalian brain development (Fuccillo and Joyner, 2006; Sillitoe and Joyner, 2007). Critical insights into the multiple roles that defined genes play in brain development have been obtained by loss-of-gene function ("knock-out") and overexpression or misexpression ("knock-in" and transgenic) studies in mice. Furthermore, by introducing mutations in genes associated with a variety of human diseases, great progress has been made during the past decade toward generating mouse models of human neurodevelopmental disorders. These advances in mouse developmental genetics have led to the widespread use of the mouse in developmental neurobiology. Lacking in these efforts have been effective methods for three-dimensional (3D) *in vivo* imaging of the developing mouse brain, a gap that magnetic resonance imaging (MRI) has the potential to fill (Turnbull and Mori, 2007).

Mn-Enhanced MRI for Neonatal-to-Adult Mouse Brain Imaging

The lack of myelin in the early postnatal mouse brain makes it difficult to obtain contrast using conventional MRI, which relies largely on relaxation-based (T1, T2) differences that depend on regional concentration of myelin. We have found that manganese (Mn)–enhanced MRI (MEMRI) (Silva et al., 2004; Pautler, 2006) provides a straightforward and effective method for imaging the mouse brain beginning with the earliest postnatal and even fetal stages (Wadghiri et al., 2004; Deans et al., 2008). In this course chapter, MEMRI protocols will be reviewed that we have found to be most useful for longitudinal, *in vivo* MRI of the neonatal mouse brain. The power of this method will be illustrated with selected examples of MEMRI-based analyses of postnatal brain development in normal and defined mutant mouse models.

Moving beyond anatomical imaging, MEMRI also provides a method for analyzing neural activity, based on the known cellular uptake of paramagnetic Mn^{2+} ions via voltage-gated calcium channels (Lin and Koretsky, 1997). We have developed MEMRI protocols for analysis of sound-evoked activity in the central auditory system of mice, providing a noninvasive method for assessing auditory brain function at preweaning stages in mice (Yu et al., 2005). We will present our results in order to demonstrate the utility of MEMRI-based analyses of both developmental plasticity induced by unusual acoustic environments during rearing (Yu et al., 2007) as well as anatomical and functional changes in defined mouse mutant models of neurodevelopmental disease (Yu et al., 2011).

In Utero MRI of Fetal Mouse Brain Development

Because much of brain development occurs *in utero*, before birth, it is also imperative to develop MRI approaches to analyze the fetal mouse brain. In this course chapter, we will describe methods that have recently been developed to acquire high-resolution *in utero* images of mouse embryos over a wide range of developmental stages (Deans et al., 2008; Nieman et al., 2009; Berrios-Otero et al., 2012; Parasoglou et al., 2012). We will present examples of 3D *in vivo* MRI analyses of the embryonic brain anatomy and cerebral vasculature and discuss the future prospects for MRI analysis of the interdependent development of morphological and vascular patterns in mouse embryos.

Reporter Genes in Developmental Neurobiology

The same genetic-engineering approaches used to create mouse models of human neurodevelopmental diseases are widely employed to express reporter genes such as bacterial *lacZ* and jellyfish *Green Fluorescent Protein* (*GFP*). This strategy has been used successfully to detect brain region–specific and cell-specific gene expression patterns and to analyze changes in gene expression induced by defined genetic modifications. Fluorescent reporters such as *enhanced GFP* (*eGFP*) have been useful for *in vivo* imaging in lower organisms—most notably in transparent zebrafish embryos. However, the use of conventional optical reporters in the mouse brain has been limited largely to static studies in histological sections or *in vitro* imaging of acute brain slices in culture. Effective MRI reporter systems for 3D *in vivo* imaging in the mouse brain would enable unprecedented dynamic studies of gene expression patterns in the context of the developing neuroanatomical structures and functional connections.

MRI Reporter Genes

MRI reporters are conceptually and functionally different than fluorescent reporters used for optical imaging. Rather than generating a signal directly, as does *GFP*, MRI reporter strategies have relied on proteins and peptides that indirectly affect the nuclear magnetic resonance (NMR) signal. To date,

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most investigations have focused on two classes of MRI reporter systems:

(1) Proteins that interact with paramagnetic metals, with the iron storage molecule, Ferritin (FT) being the archetypical example (Genove et al., 2005; Cohen et al., 2007); and

(2) Protein or peptide sequences that induce chemical shifts in the NMR signal (Gilad et al., 2007a,b).

These systems will be discussed, focusing on their potential for studies of brain development and/or disease in the mouse brain, using transgenic approaches to express the reporters in cells of interest. An alternative strategy has also been described, based on advances in targeted imaging of cell-surface receptors with receptor-specific contrast agents. In this latter approach, specific receptors, such as *Transferrin Receptor*, are chosen based on the ability to effectively target MRI contrast agents to the receptor (Moore et al., 2001). More recently, biotin acceptor peptides have also been used to biotinylate the surface of transfected tumor cells for increased targeting efficiency, based on the high binding affinity of avidin-based contrast agents to cell-surface biotin (Tannous et al., 2006).

In our investigations of potential MRI reporter systems, we have tried to build on the past experiences with these systems. We have sought to increase the potential for gene-expression imaging in the developing mouse brain—from embryonic to adult stages.

Two Novel Reporter Systems for MRI

Two novel reporter systems will be described and results shown to demonstrate their potential for studies of mouse brain development.

First we present a cell-surface biotinylation transgene that can be used in combination with avidinated MRI contrast agents (Bartelle et al., 2012). In this "Biotag" system, a bacterial biotinylation enzyme is coexpressed with a surface display protein that has been modified to include multiple biotin recognition sites, resulting in optimal biotin tagging on the surface of expressing cells. This system is similar to previous reports of tumor cell biotinylation via expression of a biotin acceptor peptide (Tannous et al., 2006), but the new Biotag transgene is completely self-contained and can be used to report on a wide variety of genetic processes in the vasculature. Moreover, the ability to include multiple biotinylation sites provides the potential for significant signal amplification compared with previous reports. Transgenic mice have been generated that express the *Biotag* transgene in vascular endothelial cells. This expression has enabled direct, endothelial-targeted imaging of cerebral vasculature in the developing mouse brain, as well as several mouse tumor models.

A second reporter system has been developed recently for MEMRI. In this system, we have employed the divalent metal transporter DMT1 to label cells *in vitro* and *in vivo* in the mouse brain (Bartelle et al., 2012) after systemic (intraperitoneal) injection of $MnCl_2$ at doses used in many previous MEMRI reports. Our initial results indicate significant advantages of DMT1 over previously reported MRI reporters, providing positive cellular enhancement with greater relaxation effects and faster switching times than *FT*. The potential of the *DMT1* reporter system for gene-expression imaging and circuit tracing in the developing mouse brain will be discussed.

Conclusions

In conclusion, MRI methods are now available for imaging anatomical and functional parameters in the developing mouse brain, from embryonic to adult stages. In our studies, MEMRI has provided a critical new tool for these *in vivo* analyses. We have also developed reporter systems that can be used for *in vivo* MRI in the developing mouse brain. While further optimization will undoubtedly be possible in future, our current results already show great potential of these MRI reporters for cell-specific and gene-expression imaging in the mouse brain. In future, the power of mouse genetic-engineering approaches, already in wide use by developmental neurobiologists, can be used to enhance the functionality of these fledgling MRI reporters, enabling them to be turned on and off with precision in cells of interest and at defined developmental stages.

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