Morphology and Development: MRI of the Developing Mouse Brain

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

The mouse is the preferred model organism for studies of mammalian development, offering large letter sizes (6-12 pups per litter), a short gestation time (~3 weeks *in utero*) and a wide variety of tools for manipulation of the mouse genome[1,2]. In the area of brain development, critical insights into the multiple roles of defined genes have been obtained by loss-of-gene function ("knock-out") and over- or mis-expression ("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 over the past decade in 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.

Challenges of MRI Analysis of Mouse Brain Development

Magnetic resonance imaging (MRI) can play a major role in studies of the developing mouse brain, providing effective methods for three-(3D) and four-dimensional (4D), high-resolution, *in vivo* analyses[3-5]. The small size and cellular features of the developing 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[3,4]. 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 presentation, I will describe methods that have been developed to enable acquisition of high-resolution *in utero* images of mouse embryos over a wide range of pre-natal stages[6-9]. Examples of 3D *in vivo* MRI analyses of the embryonic brain anatomy and cerebral vasculature will be presented, including comparisons of *in utero* MRI[9] and ultrasound[10] images.

MEMRI for Anatomical and Functional Analyses of the Developing Brain

The lack of myelin in the early postnatal mouse brain makes it difficult to obtain contrast with conventional MRI, which relies largely on relaxation-based (T1, T2) differences that depend on regional concentration of myelin and other features of mature neural cells. We have found that manganese (Mn)-enhanced MRI (MEMRI)[11] provides a straightforward and effective method for imaging the mouse brain from early postnatal[12-15] and even fetal stages[6]. In this presentation, MEMRI protocols will be reviewed that we have found to be most useful for longitudinal, *in vivo* MRI of the neonatal mouse brain, and the power of this method will be illustrated with selected examples of MEMRI-based analyses of brain development in normal and defined mutant mouse models[14-15]. 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[16]. In this presentation, MEMRI protocols for analysis of sound-evoked activity in the central auditory system of mice will be discussed briefly[17-19], providing an example of how MEMRI can be applied to assess brain function at pre-weaning stages in mice[18]. Results will be presented to demonstrate the utility of MEMRI-based analyses of developmental plasticity in both normal animals and in defined mouse mutant models of neurodevelopmental disease[18-19].

MEMRI-based Molecular Imaging of Mouse Brain Development

Ultimately, MRI assessment of dynamic gene expression changes, occurring simultaneously with morphological changes, would provide a powerful new approach for *in vivo* studies of mouse brain development. Toward this end, we have investigated several proteins that internalize or bind Mn ions in cells as candidate reporter systems[20-21]. Progress in this area will be discussed, including prospects for direct visualization of developing circuitry in the developing mouse brain using molecular-MEMRI.

Conclusions

In conclusion, *in vivo* MRI-based imaging tools are now available for analyzing anatomical, functional and molecular parameters in the normal and mutant mouse brain, over a wide range of developmental stages from embryo to adult.

Acknowledgements

I thank all the past and current members of my laboratory who have worked on the projects described in this presentation. This work was supported by grants from the National Institutes of Health (R01NS038461, R01HL078665).

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