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In Utero Localized Diffusion MRI of the Embryonic Mouse Brain Microstructure and Injury

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Abstract

Purpose—To develop an in vivo diffusion magnetic resonance imaging (dMRI) technique to study embryonic mouse brain structure and injury.

Materials and Methods—Pregnant CD-1 mice were examined on embryonic day 17 on an 11.7T scanner. Spatially selective excitation pulses were used to achieve localized imaging of individual mouse brains, in combination with a 3D fast imaging sequence to acquire dMRI at 0.16–0.2 mm isotropic resolution. Subject motions were corrected by navigator echoes and image registration. Further acceleration was achieved by simultaneous imaging of two embryos in an interleaved fashion. We applied this technique to detect embryonic brain injury in a mouse model of intrauterine inflammation.

Results—With the localized imaging technique, we achieved in utero high-resolution T_2 -weighted and dMRI of the embryonic mouse brain for the first time. Early embryonic brain structures were delineated from diffusion tensor images, and major white matter tracts were reconstructed in 3D. Comparison with ex vivo data showed significant changes in the apparent diffusion coefficient (ADC), but mostly unchanged fractional anisotropy. In the inflammation-affected embryonic brains, ADC in the cortical regions was reduced at 6 hours after the injury, potentially caused by cellular edema.

Conclusion—The feasibility of in utero dMRI of embryonic mouse brains was demonstrated. The technique is important for noninvasive monitoring of embryonic mouse brain microstructure and injury.

During embryonic and fetal development, the brain undergoes rapid growth, including formation of basic functional units and critical neural circuitry. Injuries during this critical

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period often have profound impacts on brain structures and functions at later stages. For example, intrauterine inflammation is one of the common causes of preterm birth, with adverse neurological outcome.¹ Fetal brain magnetic resonance imaging (MRI) is emerging as a promising tool to study brain development^{2,3} and to detect fetal brain injury.^{4–7} Compared to other imaging modalities, primarily ultrasound, MRI provides rich tissue contrasts for delineation of fetal brain structures and injuries. Once abnormalities are detected by ultrasound, MRI is the technique of choice in the clinic to establish the pattern of injuries.⁸

Compared to conventional T_1/T_2 MRI, diffusion MRI (dMRI), especially diffusion tensor imaging (DTI),^{9,10} is well suited for characterization of the fetal brain structures and injuries because its contrasts reflect the organization of brain microstructures and are less dependent on myelin content.^{10,11} Its unique ability in characterizing developing gray and white matter structures has been established by studies of postmortem fetal brain specimens^{12,13} as well as preterm babies and infants.^{14,15} Several groups have pioneered in utero dMRI of the human fetal brain^{16–20} to evaluate normal and abnormal fetal brain development.

To realize the full diagnostic potential of in utero fetal brain dMRI, it is important to understand the relationships between tissue microstructural changes and their manifestation in diagnostic markers. The laboratory mouse provides a convenient vehicle to examine these relationships, as it is commonly used to study the dynamics of mammalian brain development and pathogenesis. In addition, the existence of a large repertoire of genetically modified mouse lines further facilitates the investigations of the genetic mechanisms controlling brain development and responses to injuries. MRI has been used to examine the embryonic mouse brain, but mainly in postmortem specimens. Several groups demonstrated 3D T_1 - and T_2 -weighted MRI of ex vivo embryonic mouse brains with spatial resolutions up to 20 μm ,^{21,22} and ex vivo DTI of embryonic mouse brain with superb tissue contrasts and resolutions up to 50 μm .^{23,24} Despite the advances in ex vivo dMRI of the mouse brain, there is no substitute for in vivo MRI, as death and chemical fixation inevitably alter tissue microstructural properties^{25,26} and MR signatures of key pathological events, such as edema, may not be preserved in ex vivo specimens.^{27,28}

In utero MRI of the live mouse embryos is extremely challenging due to motions from both the embryo and maternal mice and limited signal-to-noise ratio (SNR). Moreover, high resolution in all three dimensions is often required to resolve structures within the miniature brains (<6 mm in any dimension). Only recently, Turnbull and colleagues^{29,30} demonstrated successful in utero embryonic mouse brain T_1 -weighted MRI using advanced motion correction techniques. Compared to conventional T_1/T_2 MRI, dMRI is known to be particularly sensitive to motion, and the diffusion-related signal attenuation further reduces SNR. Due to these technical challenges, feasibility of in utero dMRI embryonic mouse brain has not been reported.

In this study, we explored the feasibility of in utero dMRI of the embryonic mouse brain using a localized imaging approach^{31,32} with spatially selective excitation pulses, which were designed based on a linear class of large tip-angle (LCLTA) pulses.³³ The localized imaging strategy is advantageous because a pregnant mouse has multiple embryos, each

located within its own gestational sac inside the uterus and occupies only a small portion of the maternal body. Localization can significantly reduce the field-of-view (FOV), and therefore shorten the imaging time and reduce susceptibility to motion. Combined with a 3D fast imaging sequence and motion correction techniques, our goal was to use in utero dMRI to study microstructural features in normal and injured embryonic mouse brains.

Materials and Methods

Animal Preparation

All experimental procedures were approved by the Animal Use and Care Committee at the Johns Hopkins University School of Medicine. Pregnant CD-1 mice (Charles River Laboratories, Wilmington, MA) with an average litter size of 11 pups (19 days on full-term gestation) were used in this study. Ten pregnant dams of embryonic day 17 (E17) were imaged without intervention. Three pregnant dams were subjected to a model of intrauterine inflammation according to previous studies.^{34–36} Briefly, on E17 pregnant mice were placed under isoflurane anesthesia and a mini-laparotomy was performed. Lipopolysaccharide (LPS, Sigma, St. Louis, MO, Lot. 102M4017V) 50 μ g in a 100 μ L phosphate-buffered solution (PBS) was infused between two gestational sacs in the lower right uterine horn. Routine closure was applied and the dams recovered in individual cages. Two pregnant dams that underwent the same surgery procedure but were injected with 100 μ L PBS were included as sham controls.

During imaging, pregnant mice were anesthetized with isoflurane (1%), together with air and oxygen mixed at a 3:1 ratio, via a vaporizer. Respiration was monitored via a pressure sensor (SAII, Stony Brook, NY) and maintained at 30–60 breaths per minute. Among the ten normal pregnant dams, five received Gd-DTPA (Magnevist, Berlex Imaging, Wayne, NJ) at a dose of 0.4 mMol/kg via intraperitoneal (i.p.) injection at the lower abdomen at ~2 hours before MRI.

Pulse Sequences

Localized imaging targeting a selected mouse embryo was achieved using 2D spatially selective 90° excitation pulses, calculated based on the LCLTA pulses.³³ The pulses were designed to excite a rectangular field of excitation (FOE) in the x–y plane that covered the target embryonic mouse brain, with a duration of 3 msec, an amplitude of 9–10 μ T, and a 12-turn spiral-in excitation *k*-space (maximum gradient strength = 148 mT/m). In phantom experiments using 4% agarose gel in a 3-cm diameter tube, the pulses provided satisfactory excitation profiles within an 8 $\times$ 8 mm FOE (Fig. 1A,B). The coefficient of variation (CV, ratio between standard deviation and mean) of signal intensity within the FOE was 0.05 with a full-width half-maximum (FWHM) 1.06 times of the desired FOE size, and the out-volume signal was under 5% of the in-volume signal (Fig. 1A,B). A slab-selective refocusing pulse³⁷ was applied to restrict the imaging slab in the *z* direction. The selective excitation pulses were combined with a house-made 3D diffusion-weighted gradient spin-echo sequence (DW-GRASE)^{23,38} with an echo train length of 20 for fast imaging (Fig. 1C). Twin navigator echoes³⁹ were appended after the imaging echoes to correct phase errors due to motion and instrument instability. Respiratory triggering was not used.

The sequence was extended to more than one localized imaging modules, targeting different embryos, for simultaneous acquisition of multiple embryonic mouse brains in an interleaved fashion. As illustrated in Fig. 1C, two different selective excitation pulses and signal acquisitions were evenly spaced in each repetition time (TR). To avoid interference, it was necessary to keep the two FOEs from overlapping in the x–y plane and along the z direction, as shown in Fig. 2A. With this approach, the idle time in TR can be utilized to improve the efficiency, and a longer TR can be afforded in 3D acquisition to enhance the SNR as well as contrast in T_2 -weighted images.

Image Acquisition

In vivo imaging was performed on a horizontal 11.7T MR scanner (Bruker Biospin, Billerica, MA) with an integrated shim and active shielded triple-axis gradient (B-GA 9S, Bruker Biospin; inner diameter = 90 mm, maximum gradient strength = 740 mT/m). Radio frequency (RF) pulses were transmitted through a 72-mm diameter quadrature volume coil. All MRI experiments are summarized in Table 1 and described as follows.

Diffusion-weighted imaging (DWI) data were acquired from E17 embryos (nine LPS-treated embryos from three pregnant dams, and four sham-treated embryos from two pregnant dams) using an 8-channel phased array receive-only rat body coil (Bruker Biospin), which covered the entire abdomen. To locate the target embryos and define the corresponding FOEs, coronal and sagittal multislice T_2 -weighted images of the entire abdomen were acquired (echo time [TE]/TR = 50/3000 msec, in-plane resolution = 0.16×0.16 mm, slice thickness = 1 mm, and scan time ~8 min) as the reference images. FOEs in the size of $10 \times 10 \times 8$ mm (yellow and blue boxes in Fig. 2A), each containing the head of a selected embryo, were defined based on the reference T_2 -weighted images. 3D GRASE with the selective excitation pulse was performed with the following parameters: TE/TR = 21/1000 msec; two signal averages; spectral width = 120 kHz; two nondiffusion-weighted (b_0) images and six diffusion-weighted images ($b = 800$ s/mm², gradient directions: [1 1 0], [1 0 1], [0 1 1], [−1 1 0], [1 0 −1], [0 −1 1]); FOV = $12.8 \times 12.8 \times 8$ mm; and a native spatial resolution of $0.2 \times 0.2 \times 0.2$ mm in 34 minutes. The same setup and imaging parameters were used for multiple FOE imaging (two embryos from one normal pregnant dam).

DTI of the normal E17 mice (ten normal embryos from ten pregnant dams) was performed using a 15-mm diameter planar surface receive-only coil (Bruker Biospin), which provided limited coverage but higher sensitivity than the body coil. The surface coil was placed directly under the mouse abdomen. 3D DTI data were acquired using the same sequence with the following parameters: TE/TR = 21/500 msec; two signal averages; spectral width = 120 kHz; four b_0 images and 30 diffusion directions⁴⁰; b-value = 1000 s/mm²; FOV = $12.8 \times 12.8 \times 8$ mm; and spatial resolution = $0.2 \times 0.2 \times 0.2$ mm in 72 minutes (2 min per diffusion-weighted image) or $0.16 \times 0.16 \times 0.16$ mm in 113 minutes. High-resolution 3D T_2 -weighted images were acquired using the same setup but without diffusion weighting: TE/TR = 24/1000 msec and resolution = $0.13 \times 0.13 \times 0.13$ mm in 10 minutes.

Ex vivo DTI was performed on the dissected embryonic mouse brains ($n = 5$) from one normal pregnant dam (not the same mice used for in utero imaging). The brain specimens were immersion-fixed in 4% paraformaldehyde (PFA), and later transferred to PBS with 2

mM Gd-DTPA. Specimens were scanned using a vertical 11.7T NMR spectrometer (Bruker Biospin) with a birdcage volume coil (10 mm inner diameter) and a precise temperature control system. We used the same imaging parameters as those in the in vivo DTI except that the selective excitation pulse was replaced with a conventional sinc pulse and the b-value was increased to 1500 s/mm² in order to compensate for the ADC reduction in the ex vivo samples.^{25,26} Temperature was maintained at 37° during the scan.

Image Processing

The 3D *k*-space data were apodized with a tapered cosine window, zero-padded to twice of the original size, and reconstructed in MatLab (MathWorks, Natick, MA). The twin-navigator echoes were Fourier-transformed along the readout direction, which were then used to correct the phases of odd- and even-numbered echoes from each repetition.²³ The 30 direction DWIs were aligned to the mean DWI using 3D rigid transformation to correct the interimage motion. The amount of translational motion in each diffusion direction was estimated based on the image registration results. Images with motion above 0.6 mm (3 voxels) were excluded from the following analysis.

Diffusion tensor fitting was performed in DtiStudio (www.mristudio.org) using log-linear fitting, and fiber tracts were obtained with a fractional anisotropy (FA) threshold of 0.15 and maximum angle of 60°. Spherical deconvolution of the 30 direction dMRI data was performed in MRtrix⁴¹ with a harmonic order of six to generate the fiber orientation distribution (FOD) map.

Quantitative analysis of the FA and ADC was performed in ROEditor (www.mristudio.org). FA and ADC maps from all DTI experiments were first rigidly aligned to a selected normal E17 mouse embryo image using manually placed landmarks in Diffeomap (www.mristudio.org). The regions of interest (ROIs) of the major white and gray matter structures were defined based on FA images (Supplementary Fig. S1), according to the Paxinos et al.⁴² developing mouse brain atlas. Two raters made independent ROI delineation, and each repeated twice. The Dice coefficient⁴³ was used to evaluate the rater subjectivity. The intra-rater Dice coefficients were higher than 0.8 for the cortical ROIs and above 0.75 for the other structures. The inter-rater Dice coefficients were higher than 0.7 for all ROIs.

The SNR was calculated as the ratio of the mean of a single *b*₀ image to the standard deviation of the subtraction image between two *b*₀ images in a cortical ROI that was close to the coil. The contrast-to-noise ratio (CNR) was calculated as the difference in SNR between two ROIs.

Statistical tests between the ex vivo and in vivo results and between the injured and sham mice were performed using Student's *t*-test with two-sample unequal variance and two-tailed distribution in MatLab.

Histopathological Examination

Following MRI acquisition, embryos were taken out and whole heads were fixed in 4% PFA at 4°C overnight. For the LPS-injured embryos, individual embryos were identified via their

positions in the uterus and matched to corresponding MRI results. The next day, specimens were washed with PBS extensively and immersed in 30% sucrose until saturation, followed by cryosection at 20 μ m thickness and histochemical staining. Routine Nissl and hematoxylin and eosin (H&E) stainings were performed to evaluate the morphological change of the injured fetal brains. All photographs used for quantification were taken with Zeiss AxioPlan 2 Microscope System (Jena, Germany) attached to a Canon EOS Rebel Camera (Tokyo, Japan) with a 4 $\times$ objective.

Results

On E17, a large portion of the abdominal space of a CD-1 pregnant mouse was occupied by its embryos, each located within its own gestational sac in the maternal uterus (Fig. 2A). With the localized DW-GRASE sequence, a single 3D DWI of a selected embryonic mouse brain could be acquired at 0.2 mm isotropic resolution in 2 minutes. Motion during the 2-minute period could be corrected using the twin navigator echoes, which reduced the motion-induced smearing in both nondiffusion-weighted (Fig. 3A) and diffusion-weighted (Fig. 3B) images. Motions between images, from both the mother and embryos, were corrected by image registration (Fig. 3C). Based on the rigid registration results, the overall movements of the embryonic mouse brains were estimated and plotted over time (Fig. 3D). The average translational motion during a 1-hour period was 0.18 ± 0.09 mm (the maximum motion was 0.60 ± 0.46 mm, $n = 5$). To further improve imaging throughput, we tested simultaneous imaging of two embryonic mouse brains with two FOEs (as defined in Fig. 2). Figure 4 shows the mean DWI and ADC maps from two mouse embryos acquired simultaneously from these two FOEs.

Further improvement in image resolution and contrast was achieved using high-sensitivity planar surface coil and injection of Gd-DTPA. At 2 hours after i.p. injection of Gd-DTPA, signal enhancement could be observed in the mouse embryos and persist for more than 2 hours. With Gd-DTPA, the SNR measured in the brain parenchyma increased 1.7 times compared to that without Gd-DTPA (26.1 ± 4.3 [$n = 5$] versus 15.3 ± 4.4 [$n = 3$]), and the CNR between the CSF and brain parenchyma increased 1.9 times (7.07 ± 1.31 [$n = 5$] versus 3.66 ± 0.63 [$n = 3$]). Taking advantage of the high SNR, T_2 -weighted image of the embryonic mouse brain could be acquired at 0.13 mm isotropic resolution within 10 minutes, which was sufficient to define the overall brain morphology. For example, the ventricular system in the embryonic mouse brain can be outlined in 3D (Fig. 5A).

Diffusion tensor results at 0.2 mm isotropic resolution are shown in Fig. 5B. In the FA and direction-encoded colormaps, major gray matter and white matter structures in the E17 mouse brain could be delineated. The cortical plate and intermediate zone could be separated by their unique tissue orientations. Several white matter tracts could also be identified based on their high FA values and orientations. The in vivo DTI results were compared with ex vivo data acquired from a different pregnant dam at the same age, resolution, and with similar parameters (Fig. 5C). Changes in overall brain morphology were observed between the in vivo and ex vivo embryonic brains, eg, flatter brain shape and shrunken ventricles in the ex vivo results. In vivo ADC measured in both gray and white matter structures were significantly higher than ex vivo measurements. In comparison, in

vivo and ex vivo FA values in most regions, except the intermediate zone, did not show significant differences (Table 2). The results are consistent with the previous findings in the adult mouse brain.^{25,26}

Figure 6 demonstrates diffusion tensor data of an E17 mouse brain acquired at 0.16 mm isotropic resolution in 2 hours. Compared to the images at 0.2 mm isotropic resolution, several white matter structures, such as the fimbria and cerebral peduncle, could be more easily resolved. The microstructural organization of embryonic mouse cortex could be visualized using the FOD map reconstructed from the 30 direction diffusion data (Fig. 6B). The cortical plate showed dominant radial orientation, probably due to the presence of radial glial fibers, and the intermediate zone beneath it showed two fiber groups crossing each other. Major white matter tracts, eg, the cerebral peduncle (cp), optic tract (opt), and stria terminalis (st), could be reconstructed in 3D and visualized together with gray matter structures, such as the cortical plate (CP), hippocampus (Hi), and thalamus (Th) (Fig. 6C).

The localized in vivo dMRI technique was used to detect embryonic brain injury after intrauterine injection of LPS. As this is a model of inflammation in outbred CD1 mice, an expected range of cortical and subcortical injuries were found. Three representative embryonic mouse brains are demonstrated in Fig. 7. Reductions in T_2 -weighted signal and ADC value were detected in the cortical regions (yellow arrows, $ADC = 0.26 \pm 0.08 \times 10^{-3} \text{ mm}^2/\text{s}$, $n = 7$) in almost all the embryos, and several mice showed subcortical injury (blue arrows, $ADC = 0.20 \pm 0.04 \times 10^{-3} \text{ mm}^2/\text{s}$, $n = 4$). The ADC in these affected tissues was reduced to about one-third of the normal cortical ADC in the sham mice ($0.62 \pm 0.07 \times 10^{-3} \text{ mm}^2/\text{s}$, $P = 2 \times 10^{-5}$, $n = 4$), indicating acute injury, eg, cellular edema, may have developed within 6 hours after the LPS challenge. Nissl and H&E-stained sections (Fig. 7B) at similar levels showed reduced cortical thickness ($0.457 \pm 0.026 \text{ mm}$ in the LPS-treated embryos vs. $0.575 \pm 0.021 \text{ mm}$ in the controls, $P < 0.05$). At high magnification (Fig. 7B), shrunken neurons with enlarged intercellular space and many unstained regions in cortical area were observed. In the LPS group, the alignment of neurons was not as clear as the control group, and the development of neuronal processes (axon/dendrites) was disrupted and organelles became pyknotic (insets).

Discussion

In vivo dMRI of the embryonic mouse brain advances our ability to monitor the embryonic brain under physiological conditions and in a longitudinal scale. It offers a powerful tool for spatial-temporal mapping of normal embryonic brain development, detection of abnormality in prenatal injury models,³⁵ and phenotype screening in genetically modified models.⁴⁴ Knowledge gained from studies of the embryonic mouse brain may benefit in utero monitoring of fetal brain in clinical practice.

The key innovation of this study is the application of spatially selective excitation³³ to perform localized imaging of individual mouse embryo. Localized imaging is ideal for imaging the live embryonic mouse brain. As each embryo occupies a small fraction of the abdomen, reducing the FOV without aliasing provides immense benefit in imaging speed, which can be translated to higher imaging resolution and reduced sensitivity to motion. The

reduced FOV technique has been used to image several internal organs, including the heart, kidney, and spinal cord.^{31,32} Using this approach, a 3D DWI from an embryonic mouse brain can be acquired in less than 2 minutes. With navigator-based motion correction, artifacts due to intra-image motions were reduced, and inter-image motions could be mostly remedied by 3D image registration. With the current setup, we had an 80% success rate in acquiring satisfactory diffusion MRI data. Two out of the ten DTI data contained large interimage motions, due to large displacements of the surrounding organs, such as release of the bladder during imaging, which could not be fully corrected. One limitation of the current setup is the use of 2D selective excitation pulses, which restricts the selection of embryos for multiple FOE imaging (no overlap in both x–y and z). With the advances in 3D selective excitation pulses and parallel transmission hardware,³¹ the multi-FOE approach will become more flexible and lead to even higher efficiency (with three or more FOEs).

High-throughput acquisition of T_2 -weighted images and ADC maps of single or twin embryonic mouse brains was achieved using a body coil. The large coverage provided by the body coil allowed quick survey of the embryos and tracking of individual embryos based on their relative positions, which is important for longitudinal studies and comparisons of MR and histological data. These images were sufficient for monitoring brain morphology, including the sizes of the brain and ventricles, as well as certain pathology, as in the case of the LPS-induced inflammation model. We demonstrated in a mouse model of intrauterine inflammatory injury that cortical injury can be detected from the ADC maps. The reduction of ADC, as shown in vivo and the shrinkage of neurons after fixation and dehydration, suggested severe edema may have developed within 6 hours of exposure to inflammation. This information could be lost or confounded by chemical fixation in ex vivo MRI. In vivo detection of such abnormalities at acute stages may provide critical diagnostic value for evaluation of potential long-term neurological and immune deficits.³⁶ Longitudinal monitoring in the perinatal stages can further trace the disease progression and guide therapeutic interventions.

In this study, DTI of the embryonic mouse brain, which requires high SNR, was achieved using a high-sensitivity planar surface coil and after injection of Gd-DTPA. Compared to the body array coil, the planar surface coil provided higher sensitivity but only allowed a limited imaging area, which often covered less than three mouse embryos and was not suitable for multi-FOE imaging. The loss of global information also hindered the ability to identify and track individual embryos over time. The injection of Gd-DTPA significantly increased SNR due to shortening of tissue T_1 . Previous studies⁴⁵ have shown that Gd-DTPA can penetrate the placenta and embryonic mouse brain due to the undeveloped blood–brain barrier (BBB). Gd-DTPA tends to stay in the embryos for a prolonged time because it is excreted by the embryos in their surrounding fluid and then taken up by the embryo again, which provides ample time for lengthy DTI acquisition. While initial studies found no adverse effect of MRI and Gd-DTPA on embryonic mouse development in terms of weight and extremity morphology, etc.,³² more studies on the safety of Gd-DTPA and its dynamics are necessary in the future.

At 0.2–0.16 mm isotropic resolution, we demonstrated in vivo DTI of the embryonic mouse brain for the first time. The data revealed important microstructural information in the

developing mouse brain under normal physiological conditions. In the E17 embryonic mouse brain, we found high anisotropy in the developing cortical structures (eg, CP and IZ), which had unique orientations in the colormap and FOD map (Fig. 6). These structural features agree well with previous ex vivo studies.^{23,24} Moreover, the high resolution allowed us to delineate several white matter tracts and reconstruct their 3D trajectories (Fig. 5D), which extended our ability to trace early white matter development and abnormalities in vivo. Continuing development in this area, especially improving imaging resolution, will further enhance our ability to study the brain microstructure at early developmental stages. It is necessary to note that the in vivo and ex vivo data were acquired using different instruments due to the need to dissect out individual embryos for chemical fixation to preserve tissue microstructures. The vertical-bore 11.7T scanner is optimized for imaging small samples with precise temperature control and specially designed coils. The differences between the two instruments should have minor effects on the DTI indices, as long as the SNR is sufficient.⁴⁶ The differences between in vivo and ex vivo ADC and FA values are in line with previous reports.^{25,26}

In conclusion, we demonstrate the feasibility of in utero dMRI of the embryonic mouse brain using a localized imaging approach. The technique, while still in an early stage, can potentially be used for noninvasive examination of key developmental processes, eg, formation of early white matter tracts, and injury progression in live embryonic mouse brains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Use of selective excitation pulse and a 3D diffusion-weighted gradient spin-echo sequence (DW-GRASE) sequence for localized diffusion MRI. **(A)** Performance of the spatially selective excitation pulse was tested in an agarose gel phantom. The red box in the image of the phantom represents an 8×8 mm field of excitation. The yellow and blue curves in **(B)** show the excitation profile along the x-axis and y-axis, respectively. **(C)** A diagram of the 3D DW-GRASE sequence with spatially selective excitation pulse. The sequence can be extended to include two fields of excitation and acquisition. The diagram shows two localized imaging modules that target two separate embryos in an interleaved fashion. The localized imaging module is expanded to show the timing of the 2D selective excitation pulse together with the spiral gradient in the x–y plane, diffusion sensitization, the GRASE readout, and the twin-navigator echoes. Each GRASE readout acquires five double-sampled gradient and spin echoes and is repeated four times to achieve an acceleration factor of 20 compared to the conventional spin echo sequence. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2.

Mouse embryos in the abdomen of a pregnant CD-1 mouse on gestational day 17 localized with selective excitation pulses based on user-defined FOEs. **(A)** 3D rendering of 11 mouse embryos in the uterus, reconstructed based on multislice T_2 -weighted images. The yellow and blue boxes indicate the FOEs for two embryonic mouse brains used in twin-FOE imaging. **(B)** A selected embryonic mouse brain defined in coronal and sagittal multislice T_2 -weighted images of the mouse abdomen (corresponding to FOE1 in (A)). 3D T_2 -weighted images of this embryonic mouse brain acquired using the GRASE sequence with selective excitation show minimal aliasing. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 3.

Motion correction based on twin-navigator echo phase correction and retrospective image registration. Navigator echo phase correction improves image quality by removing motion artifacts, eg, smearing of structural boundaries (indicated by the arrowheads), in both nondiffusion-weighted images (**A**) and diffusion-weighted images (**B**). Rigid image registration corrects motion-induced misalignment between images. For example, mismatch between Image #1 and Image #2 can be corrected after registration (**C**). (**D**) Translational motions of five embryonic mouse brains were estimated based on rigid transformations and plotted over a 1-hour period. Horizontal axis denotes scan time in minutes, and vertical axis denotes the translational movements in mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 4. DWI and ADC maps acquired simultaneously from two embryonic mouse brains, as defined in Fig. 2A, using the multiple FOE acquisition technique.

FIGURE 5.

(A) T_2 -weighted images of an embryonic mouse brain acquired at 0.13 mm isotropic resolution. The ventricles were reconstructed in 3D based on the high-contrast T_2 -weighted images. (B) In vivo DTI of an E17 embryonic mouse brain at 0.2 mm isotropic resolution, in comparison with ex vivo DTI result (C) at the same resolution. Several gray and white matter structures in the E17 mouse brain can be delineated in the FA maps (top rows) and direction-encoded colormaps (bottom rows), eg, the cortical plate (CP), intermediate zone (IZ), cerebral peduncle (cp), internal capsule (ic), optic tract (opt), and fimbria (fi).

FIGURE 6.

(A) In vivo DTI colormaps of an embryonic mouse brain acquired at 0.16 mm isotropic resolution (top row), compared with ex vivo DTI acquired at 0.16 mm resolution (bottom row). (B) FOD map showing microstructural organization in the cortical plate and intermediate zone, overlapped on a zoomed-in region from (A). (C) Early white matter tracts reconstructed from the in vivo dMRI data at 0.16 mm isotropic resolution. The 3D trajectories of the cerebral peduncle (cp), optic tract (opt), and stria terminalis (st) are rendered relative to the cortical plate (CP), hippocampus (Hi), and thalamus (Th). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 7.

(A) In vivo T_2 -weighted images (T_2w), DWI, and ADC maps of three E17 embryonic mouse brains at 6 hours after intrauterine injection of LPS. The images were acquired at 0.2 mm resolution using the localized DW-GRASE sequence. Yellow and blue arrows indicate cortical and subcortical injury (reduced T_2 intensity and ADC) in these mice, respectively. (B) Nissl and H&E-stained sections at similar levels as the MR images, from a control embryo and an LPS-treated embryo. The LPS-treated embryo showed reduced cortical thickness (red arrows, including the cortex plate, subplate, intermediate zone, and ventricular zone) and shrunken neurons (insets), compared to the control embryo. The black arrowheads point to the normal and shrunken cytoplasm in the cortical neurons. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Imaging Parameters Used for T₂-Weighted Imaging, DWI, and DTI of the E17 Embryonic Mouse Brain

TABLE 1

MRI Protocol	Receiver coil used	Resolution (mm)	TE/TR (ms)	Diffusion encoding	Scan time (min)	SNR
<i>In utero</i> DWI	Body coil	0.20 isotropic	21/1000	2 b_0 + 6 DWI, b = 800 s/mm ²	34	23.7 ± 3.6
<i>In utero</i> T ₂ -weighted	Surface coil	0.13 isotropic	24/1000	N/A	10	28.9
<i>In utero</i> DTI	Surface coil	0.20 isotropic	21/500	4 b_0 + 30 DWI, b = 1000 s/mm ²	72	26.2 ± 3.8
		0.16 isotropic	23/500		113	23.3
<i>Ex vivo</i> DTI	Volume coil	0.20 isotropic	21/500	4 b_0 + 30 DWI, b = 1000 s/mm ²	72	197.5 ± 11.1
		0.16 isotropic	23/500		113	173.6

TABLE 2

Apparent Diffusion Coefficient (ADC) and Fractional Anisotropy (FA) of Several Gray And White Structures Measured From the In Vivo and Ex Vivo E17 Embryonic Mouse Brains ($n = 5$)

Structures	ADC ($\times 10^{-3} \text{ mm}^2/\text{s}$)		FA	
	In vivo	Ex vivo	In vivo	Ex vivo
Frontal CP	0.65 ± 0.09	$0.47 \pm 0.02^*$	0.41 ± 0.11	0.42 ± 0.02
Temporal CP	0.59 ± 0.05	$0.45 \pm 0.03^{**}$	0.39 ± 0.06	0.33 ± 0.03
IZ	0.64 ± 0.10	$0.39 \pm 0.03^{**}$	0.44 ± 0.07	$0.24 \pm 0.02^{**}$
Cerebral peduncle	0.67 ± 0.07	$0.31 \pm 0.03^{**}$	0.34 ± 0.05	0.30 ± 0.07
Optic tract	0.77 ± 0.10	$0.33 \pm 0.03^{**}$	0.31 ± 0.07	0.29 ± 0.03
Fimbria	0.57 ± 0.10	$0.40 \pm 0.02^*$	0.35 ± 0.07	0.35 ± 0.04
Internal capsule	0.54 ± 0.08	$0.38 \pm 0.04^*$	0.37 ± 0.05	0.33 ± 0.07

Data are presented as mean $\pm$ standard deviation across subjects.

* and ** denote that a two-tailed t-test between the in vivo and ex vivo measurements produced a P-value less than 0.01 and 0.001, respectively. The ROI definitions can be found in Supplementary Fig. S1. CP, cortical plate; IZ, intermediate zone.