PROGRESS REPORT

Original aims of the project.

The project sought to contribute to our understanding of how brain areas are connected, and how this connectivity changes as a result of brain injury, rehabilitation, and recovery. Because our understanding of the human brain and its connectivity is limited due to the use of mainly non-invasive techniques, the primary focus of our project was to explore the limitations of such techniques in a model that is well understood. For this reason, we chose to use the macaque brain as our model to explore issues related to measuring anatomical connectivity using non-invasive methods. In the proposed project, we sought to establish macaque diffusion imaging in order to examine long-term changes in post-lesion connectivity, with a particular emphasis on the brain ¶s attention network since it is a well-studied brain network in both species. We proposed to develop novel diffusion imaging techniques in the macaque and examine the limits of diffusion tractography by using fMRI-defined regions of interest and known anatomical connectivity from previous anatomical tracer studies. Further, we planned to induce focal lesions in key areas of the macaque attention network to examine changes in the connectivity profile of the network as a result of the lesion. The proposed studies would lay the groundwork to establish a highly novel methodology combining state-of-the-art diffusion imaging and an animal lesion model to study brain injury.

PROGRESS REPORT CONTINUED

Project successes.

The project aimed to establish state-of-the-art diffusion imaging in the in vivo macaque brain, and link it to known anatomical connectivity. Most current implementations of macaque diffusion imaging by other laboratories are limited to ³traditional´ diffusion tensor imaging (DTI) with a small number of diffusion directions, low signal to noise ratio (SNR), and large spatial distortions. This project began with attempts to explore the limits of macaque DTI imaging, and successfully ended with an advanced diffusion protocol that goes well beyond typical DTI in many respects. As described below, we encountered methodological and technical limits regarding what could be accomplished at our Siemens Allegra 3T MRI scanner, which led us to switching the project to our new imaging facility that houses the first US installation of a new state-of-the-art Siemens Prisma 3T MRI scanner.

In brief, our testing found that a rigid stereotaxic instrument (Figure 1) worked best as opposed to an MRI-compatible primate chair system that we had started with. While attempts to use an MRI-compatible primate chair with an integrated head holder were successful, using such a chair limited our use to only animals with cranial head-fixation devices surgically implanted. We also found that the materials used in such implants above the skull compromised the imaging quality. Alternatively we found the use of an MRI-compatible plastic stereotaxic frame provided both good head stabilization in animals without surgical implants, and also provided a system for RF coil stabilization over and around the head. Most importantly, the stereotaxic frame provided the additional advantage of placing the head in the Horsley-Clarke frame of reference so that MRI images could be used for surgical guidance in future lesion studies.

Comparisons were made between single-loop surface RF coils, volume ³birdcage´ circular-polarized coils, and multi-channel phase array coils. Much of the data collected during the first half of the project was at the Siemens Allegra scanner using a single-loop surface coil. The coil provided high SNR for the dorsal regions of the brain, but SNR suffered with increasing distance from the coil, leading to

varReportResuts continued

difficulties in tractography ventral to the corpus callosum. This difficulty is one of several reasons (see Project Challenges) why we later transitioned to the Siemens Prisma scanner where we had access to and implemented a 4-channel ³Flex Coil´ as shown in Figure 2. The coil was a remarkable success²its flexible design and small size allowed for ideal placement around the head. Unlike the single-loop surface coil, the four small channels provided uniform SNR across the entire brain that extended ventrally past the cerebellum (Figure 3). Furthermore, the multi-channel design allowed us to implement accelerated parallel imaging (i.e. integrated parallel acquisition techniques, or IPAT). This allowed us to decrease our scanning time (that is, we could collect more data in a given amount of time), increase the spatial resolution, and decrease the diffusion echo time (TE) for considerable SNR gains unobtainable at the Siemens Allegra scanner.

The transition from the Siemens Allegra scanner to the Siemens Prisma scanner allowed us to push our diffusion imaging acquisition schemed from a ³traditional´ DTI acquisition to the current cutting edge of the field: multi-shell high angular resolution imaging (HARDI) acquisitions. At the Siemens Allegra scanner, we were limited to a diffusion tensor imaging sequence of 60 gradient directions with a diffusion weighting of b = 1,000 s/mm^2. While a 60-direction DTI sequence can resolve major white matter fiber tracts fairly well, it quickly reaches its limits when attempting to track through areas with heterogeneous fiber orientations. This is an inherent limitation of the tensor model that it cannot easily resolve fibers crossing, bending, or twisting within an individual voxel. Since the goals of our project are to use diffusion imaging to examine changes in complex neural architecture after trauma, it was critical that we move beyond simple tensor models to more advanced q-space diffusion imaging techniques.

We explored several options for sampling q-space, including measurements on a single sphere with HARDI (Tuch et al. 2002), sampling multiple spheres (multi-shell) (Assaf & Basser 2005; Wu & Alexander 2007), or sampling on a Cartesian grid (diffusion spectrum imaging, DSI) (Wedeen et al. 2005).

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Currently, the analytical options available for DSI data are limited to streamline tractography techniques. Probabilistic diffusion tractography, a powerful tool for connectivity analyses, is not yet possible with DSI data. For this reason, and because we can closely approximate Cartesian sampling by a multiple-sphere scheme (Wu & Alexander 2007), our final diffusion imaging protocol is a high resolution spherical sampling scheme with 270 diffusion directions distributed optimally across three spherical shells (Caruyer et al. 2011) with a maximum shell value of b = 2,500 s/mm^2. Our comparisons of these methods have found that multi-shell schemes have greater sensitivity in detecting fiber crossings (particularly three-way crossings) than single-shell schemes. Also, for human diffusion imaging at 2 mm spatial resolution, the best trade-offs between fiber angular contrast and SNR occurs when shells are below b = 3,500 s/mm^2. Given the higher spatial resolution possible with the Siemens Prisma scanner, we were able to push the spatial resolution to 0.8 mm at a maximum b = 2,500 s/mm^2 (Figure 4). An analytical advantage to multi-shell diffusion is the data can be processed using both a model-based method or model-free methods. Currently we have implemented a model-based analysis method using a modified ball and stick model based on a continuous gamma distribution of diffusivities (Jbabdi et al. 2012). Recent work suggests such a model performs well with multi-shell data without over fitting problems, and allows for straightforward comparisons to single-shell data (Figure 5). As shown in Figure 5, the high resolution multi-shell diffusion scheme allowed for improved estimation of crossing fibers in areas of known complex fiber architecture. The white matter directly underneath the cerebrum and superior to the corpus callosum contains a complex matrix of projection, commissural, and association fibers. Such a region is particularly challenging for diffusion tractography. With respect to this project, projections between key nodes in the attention network, namely the frontal eye fields (FEF) and the lateral intraparietal area (LIP) traverse through the centrum semiovale. As described in Project Challenges, despite the imaging advancements we have made over the course of this project, we are still wrestling with this challenge and will likely have to push the spatial resolution and scanning protocols further.

varReportResuts continued

In summary, we proposed to develop novel diffusion imaging techniques in the macaque and examine the limits of diffusion tractography by using fMRI-defined regions of interest and known anatomical connectivity from previous anatomical tracer studies. To this end, we have successfully moved beyond traditional DTI imaging and established a high resolution multi-shell HARDI imaging acquisition scheme that has provided us with unprecedented detail in the macaque white matter architecture (within the limits of 3T MR technology). Further, we planned to induce focal lesions in key areas of the macaque attention network to examine changes in the connectivity profile of the network as a result of the lesion. Given the permanent nature of these lesion studies, we are continuing to advance our imaging capabilities with longer acquisition protocols, higher resolutions, and implanted RF coils so that the tractography can accurately map white matter through complex regions before the lesions are performed.

PROGRESS REPORT CONTINUED

Project challenges.

One challenge we encountered concerned the severe geometric distortions in the macaque data (more pronounced than human data due to the size and shape of the macaque brain and surrounding anatomy). Typically, diffusion images are plagued by distortions caused by sensitivity to inhomogeneities of the magnetic field. Such inhomogeneities are caused by the object that is being scanned (susceptibility-induced distortions) and by the rapid switching of the diffusion gradients (eddy current-induced distortions). In consultation with Dr. Jesper Andersson of the Karolinska Institute we addressed this problem with a model-based approach that simultaneously considers and corrects for all types of distortions. The correction is based on the idea of manipulating the acquisitions so that a given field inhomogeneity manifests itself differently in different images (Andersson et al. 2003). Specifically, images acquired with reversed phase-encoding (PE) directions carry complementary information, and by combining the pairs of images, a susceptibility induced off-resonance field can be estimated and successfully applied to obtain a corrected image (Figure 6). Our testing found unexpected asymmetries in the fiber orientations with PE directions that are often used in human imaging (i.e., anterior/posterior). In the end we found that right/left PE directions showed the least amount of asymmetry to provide an unbiased estimation of the fiber orientations in the corrected image.

One unexpected challenge surrounded the limitations imposed by the Siemens Allegra MRI scanner. The software did not allow diffusion acquisition schemes beyond 60 directions, and did not allow the end user to input custom gradient vector tables to implement multi-shell schemes with unique directions on each shell. As described earlier, being limited to a DTI acquisition scheme with limited number of diffusion directions prevented detailed white matter tractography in areas of complex architecture. Fortunately access to the newly installed Siemens Prisma scanner removed many of the barriers imposed by the Allegra, allowing us to push the limits of macaque diffusion imaging to a high spatial and high angular resolution (0.8mm^3) acquisition scheme.

varReportProblems continued

One of the goals of the project is to map the intact attention network in the macaque using diffusion imaging and compare it to known anatomical tracing results. While we ¶ve been able to successfully map ventral portions of the network (i.e. visual areas V4 and TEO to the ventral) (Saalmann e t al. 2012), there are dorsal portions of the network that have thus far proven difficult to track. Key attentional control areas in the fronto-parietal cortex, namely the frontal eye fields (FEF) and the lateral intraparietal area (LIP) have well established connections to each other and to the dorsal pulvinar. As shown in Figure 7, even with the high resolution diffusion sequence we have implemented, the probable fiber tracks between these areas are disappointingly weak. We believe the issue is related to complex fiber crossings beyond the spatial resolution of our images in white matter between these brain areas (Figure 5). Our current efforts are now aimed at pushing our imaging resolution to 0.5mm^3. Because SNR decreases with increased spatial resolution, diffusion imaging at 0.5mm voxel size will require additional measures to help increase the signal. One way to increase SNR is to collect additional data for subsequent averaging, and given that our current 0.8mm protocol requires 3 hours of scanning, we are currently working with our veterinarians to implement a 12-hour general anesthesia protocol to allow for a 4-fold increase in data. Further, the increased voxel resolution will require a decrease in the maximum diffusion weighting, which will result in reduced angular resolution. To counteract these losses, we plan to next use surgically implanted multi-channel RF coils where the coils are placed directly on the skull surface to provide an over 3-fold increase in SNR over external multi-channel coils (Janssens et al. 2012). Given the permanent nature of the planned cortical lesion studies, where we plan to examine recovery over the course of several months, it is critical that the diffusion tractography accurately follows the known anatomy before the lesions are performed in order to examine the changes in network connectivity that reflect neural reality and not biases in the measurement technique.

PROGRESS REPORT CONTINUED

Implications for future research and/or clinical treatment.

Studies in brain plasticity in animals have shown that lesions can trigger modifications in structure, wiring, and representations. When a lesion is introduced, it often induces both anterograde and retrograde degeneration (Payne and Peters 2001). Recovery is rarely full, but partial recovery does occur, more so for younger animals. Recovery of a system requires rebuilding of the damaged pathway by various means, including migration of new neurons to an alternate pathway, sprouting or rerouting of axons, the unmasking and stabilization of ephemeral axonal projections, and the expansion of dendritic arborizations (Payne and Lomber 2001). While diffusion imaging does not provide us with the spatial resolution necessary to view these cellular changes, changes in the probability of connections between areas are suggestive that such mechanisms are in use.

Recent human DTI studies have shown correlations between DTI measures and brain injury. Fractional anisotropy (FA) measures in patients with brain injury are lower in areas involved with the injury as compared to the same areas in uninjured control subjects (e.g. Lee et al 2006; Rutgers et al. 2008), marking axonal injury in those areas. Furthermore, recent DTI studies have attempted to perform tractography in brain-injured patients (Sugiyama et al. 2007; Cherubini et al. 2007). While these studies have shown the usefulness of tractography for evaluating brain injuries, our studies sought to push the limits of diffusion imaging in an animal model in order to examine how the connectivity of a large-scale network changes over time. Knowledge of the time course of recovery will undoubtedly assist in the development of environmental, behavioral, and pharmacological treatments to improve lesion-induced deficits.

PROGRESS REPORT CONTINUED

Plans to continue the research, including applications submitted to other sources for ongoing support.

We plan to continue advancing the diffusion imaging technique in the macaque model as described earlier with increases in spatial resolution and coil technologies to more accurately map known networks in macaque brain. Once completed, these advances will be used for future proposals to examine network plasticity after injury.

PROGRESS REPORT CONTINUED

Explain how you have leveraged NJCBIR funding to obtain additional federal or other support for brain injury research and list the appropriate funding organizations.

We are currently preparing an NIH proposal that will include the techniques developed thus far for diffusion imaging in the macaque to study network dynamics during visual attention. While not directly related to brain injury, the proposal aims to increase our understanding of network communication and ultimately network dysfunction.

PROGRESS REPORT CONTINUED

All papers, presentations, chapters, and abstracts should mention that the research was supported by a grant from New Jersey Commission on Brain Injury Research. Copies must be sent to the NJCBIR office, even if you inadvertently forgot to cite NJCBIR support. List and include a copy of all publications emerging from this research, including those in preparation.

Saalmann YB, Pinsk MA, Wang L, Li X, Kastner S (2012). The pulvinar regulates information transmission between cortical areas according to attention demands. Science 337: 753-756.

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PROGRESS REPORT CONTINUED

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