Diffusion MRI and anatomical tracer tractography of association pathways in the same brain

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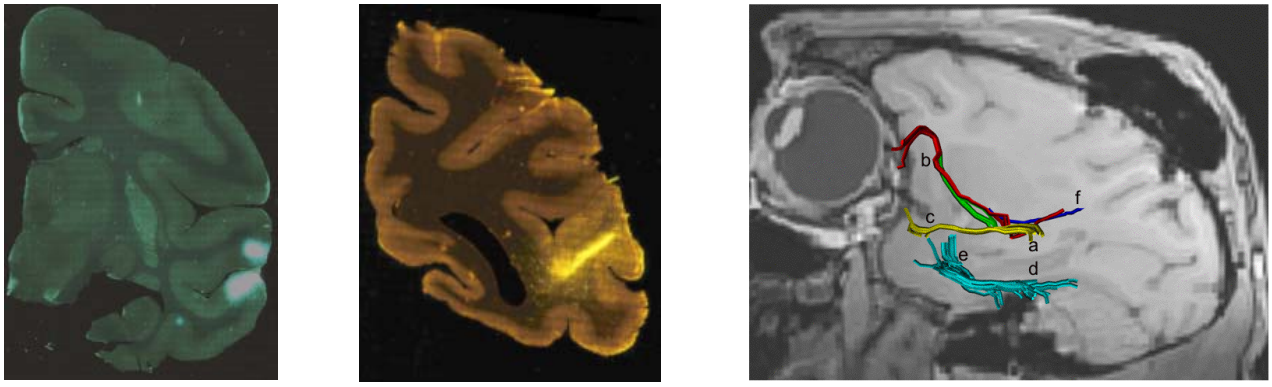
Introduction: The objective of the current study was to perform both diffusion MRI tractography and traditional tracer injection tract tracing in the association pathways of the same rhesus macaque monkey. Reliable *in vivo* diffusion MRI fibre tractography, particularly in association pathways, remains a difficult task due to a mismatch between the tract size and the image resolution achievable in a reasonable scan time. In non-human primates, it is possible to use traditional tracer injection techniques to trace pathways directly from the cortex, and this tract tracing can be used to evaluate diffusion MRI tractography methods. Other studies using tracer injection and tractography in the same animal were limited to DTI reconstruction in major projection and commissural fibre pathways [1] or were done in a different species [2]. Similar association pathways were investigated using high angular resolution diffusion MRI [3], but tracer injection was not carried out in the same animal.

Methods: Diffusion MRI tractography was used to investigate connections between two tracer injection sites and specific cortical regions of interest (ROIs) shown to be monosynaptically connected to them by tracer transport. Injections of the fluorescent anatomical tracers Fast Blue (FB) and Diamidino Yellow dihydrochloride (DY) were placed within the superior temporal sulcus (STS) of one rhesus macaque monkey. FB was placed in the dorsal bank of the STS, and DY was placed in the ventral bank of the STS. After the brain was perfused with saline, the animal was euthanized, the head severed, and the brain scanned fresh in situ, in an attempt to maintain diffusion properties similar to those in vivo and to limit non-linear warping caused by brain extraction. High spatial and angular resolution diffusion data was acquired on a Siemens TIM trio, using a multi-shot EPI sequence with TE=112ms, TR=8.2s, 55 slices, and 1 mm isotropic resolution. Diffusion encoding was applied in 90 directions with a b value of 2000 s/mm^2 for 12 runs, resulting in a total scan time of 12 hours. An MPRAGE T1 weighted anatomical scan was also acquired, with 0.6 mm isotropic resolution. Following the MR image acquisition, the brain was removed from the skull and formalin-fixed. To account for scanner drift accruing during the scanning session and potential warping of the tissue, non-linear registration was used to align all images to a target acquired near the beginning of the session. From the diffusion weighted signal profile, the fibre directions and associated uncertainties were estimated using a residual bootstrap based probabilistic deconvolution approach that allows for one to three fibre directions per voxel [4]. Diffusion MRI tractography was then performed using a two ROI approach to extract specific trajectories between the injection sites and several cortical regions that were confirmed to be connected to them with the FB and DY tracers. The tractography algorithm produces a confidence value for all tracts traced, and the "brute force" tract initiation technique was used [4]. ROIs were drawn at the injection sites by inspecting the T1 image, and then extending the ROI from the injection site in cortical grey matter into to the white matter along the expected trajectory. The extension into the white matter was done by manual drawing by an expert, staying within the white matter that was known to connect to the injection site based on previous anteriograde tracer studies performed in other rhesus monkeys using mini-ruby (MiR) tracer. The pairs of ROIs investigated were: (i) (a) dorsal STS injection (FB) and (b) Brodman area 45, (ii) (a) dorsal STS injection (FB) and (c) dorsal temporal pole, (iii) (d) ventral STS injection (DY) and (b) Brodman area 45, (iv) (d) ventral STS injection (DY) and (e) ventral temporal pole, and (v) (d) ventral STS injection (DY) and (f) intraparietal sulcus (IPS). These ROIs (a-f) are labeled below.

Results:



Left: FB (dorsal) and DY (ventral) retrograde fluorescent tracer injection sites in STS. Connections were seen to all of the ROIs that were subsequently probed in the diffusion MRI tractography (Brodman area 45, temporal pole, IPS). *Centre*: MiR anteriograde fibre tracing in another animal, used as prior for injection site ROI delineation, and for evaluation of MRI-based tract trajectories. *Right*: Diffusion MRI tractography result in the same animal as at left. Connections from the dorsal STS (a) and ventral STS (d) to dorsal (c) and ventral (e) temporal pole coursed in the lateral temporal lobe, within dorsal and ventral gyri, respectively. The dorsal route is shown in yellow and the ventral route in cyan. The connections to Brodman area 45 (b) were achieved in both cases through the middle longitudinal fasciculus (MLF) and the extreme capsule (red - dorsal ROI (a) and green - ventral ROI (d)). The connection from ventral STS (d) to IPS (f) was achieved through the MLF (blue). Note that for the temporal polar connections, the diffusion tract tracing did extend to the grey-white matter boundary, whereas for the association pathways with frontal and parietal lobe, it did not. The tracts shown as lines here are those produced by following the most likely fibre direction in the fibre direction probability profile at each voxel. The trajectories overlap with those found in anteriograde tracer injection studies in other animals, as determined by expert inspection.

Discussion: Diffusion MRI tractography produced tracts connecting regions of interest shown to be connected in the same animal via retrograde fluorescent tracer injection. The evaluation showed that these two tract tracing techniques can be complimentary. Tracer injection provided additional information in that diffusion MRI tractography could not always map connectivity to the cortical edge: in some cases, the tracts reached the grey-white matter boundary, while in others, manual extension of the tract delineating ROI into the subcortical white matter based on prior knowledge was necessary. Conversely, in addition to being an *in vivo* technique, diffusion MRI has the potential to give complimentary visual information about fibre trajectories, because of our ability to delineate pathways with multiple regions of interest, instead of seeing *all* connections to and from the injection site at once, as is the case for injected tracers. The technical difficulty in reaching the cortical edge could be due to insufficient voxel volume fraction of the fibres of interest. For example, the fibre tract in question could share space with another, larger pathway, or with many other small pathways, or with grey matter. This problem can occur despite the use of high angular resolution multi-fibre diffusion MRI post-processing techniques. One potential solution is to use significantly higher spatial resolution, however, this is a considerable technical challenge at this time, noting that the protocol employed here was 12 hours long. Diffusion MRI tractography of association fibres is expected to be more difficult in monkeys than in humans, because of the challenge of achieving sufficient spatial resolution in a smaller brain, and because of the relatively larger size of the projection and commissural pathways that lie near them [5]. Validation of diffusion MRI tract tracing in these challenging configurations, using traditional tracer injection, has given us insight into its feasibility for mapping subtle connectivity in the human brain.

References & Acknowledgements: [1] Dauguet *et al*. NeuroImage 37 (2007) 530-538. [2] Dyrby *et al*. NeuroImage 37 (2007) 1267-1277. [3] Schamann *et al*. Brain (2007) 630-653. [4] MamayyezSiahkal *et al*. MICCAI (2009). [5] Behrens *et al*., ISMRM (2006).

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