Diffusion parameter changes in white matter induced by direct intracortical stimulation in rats

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Introduction: While existing MR techniques can reliably detect stimuli-induced activation in gray matter (GM), very little is known about the impact of the stimuli on connective white matter (WM) regions. A recent study has looked into this aspect using functional DTI (fDTI)[1] and reported subtle increases in fractional anisotropy (FA) in specific WM pathways in response to motor or visual stimulation in human subjects. In the current study, we aimed to validate these findings with a direct cortical stimulation paradigm in rats.[2] Our fDTI study based on this very effective stimulation paradigm enabled us to perform voxel- and ROI-based analysis, which allowed specific assessment of changes in diffusion parameters (FA, mean diffusivity (MD) and T_2 signal without diffusion weighting (S0)) in selective WM and GM regions. There are strong indications suggesting that diffusion related changes do take place in WM due to stimulation. There are also indications that the vascular origins of these changes cannot be neglected, as reported by other researchers.[3]

Methods: All experiments were performed on a 4.7T animal MR system after obtaining approval from the animal ethics committee. Adult male Sprague-Dawley rats (n=3) were anesthetized by mechanical ventilation with 1-2% isoflurane in air/O_2 (2:1). MR-compatible custom-made bipolar insulated gold electrodes with a 200-µm diameter exposed tip were carefully positioned in two burr holes of the skull at 2.5 mm lateral and 0.5 and 3 mm anterior to bregma. Tips of the electrodes were placed in the left primary motor cortex at a depth of 1.7 mm in order to stimulate layer III of the cortex. During the experiments, anesthesia was maintained at 1% isoflurane and rats were infused with a muscle relaxant (pancoronium bromide, 1.0mg/kg/h, i.v.). Details and a schematic of the fDTI acquisition are given in Fig.1. A total of 576 3D diffusion-weighted datasets were acquired, as 8 batches of 72 contiguous sets. Each batch had a specific b-weighting in a single direction (2 datasets with b=0 + 6 directions with b=1645s/mm^2, Δ/δ=10/16.2ms). The effective temporal resolution was 4s. During the acquisition of each batch, the rat was intracortically stimulated for 12s (between sets 24 and 26). The stimulation consisted of pulses of 0.3ms duration at 300Hz in 50ms trains repeated five times per second. The time between two stimuli was 4.5 min. Each scan session comprised of 3 fDTI runs with stimulation amplitudes of 0 (baseline), 0.5 and 1.0mA, respectively. In addition, we acquired 94 fDTI datasets in combination with a carbogen challenge, which involved 9.6 min ventilation with carbogen (5% CO_2/95% O_2) preceded and followed by fDTI batches under i) 67% air/33% O_2 and ii) 100% O_2 ventilation. This experiment enabled us to measure possible variation in diffusion parameters induced by heamodynamic changes.

BOLD fMRI analysis[4] was performed on the b=0 diffusion datasets (acquired at the beginning and half way through the fDTI scans) to check for activation responses. To study the temporal behavior of diffusion parameters in WM, parts of the corpus callosum were manually outlined as regions-of–interest (ROIs) (Fig.2). The 8 batches of diffusion weighted sets were then grouped and treated as a single stimulation run with diffusion-weighting in all 8 directions. Spatial smoothing was omitted, to avoid partial volume effects in the relatively small WM ROIs. To improve SNR, we applied a temporal sliding (rectangular) window analysis. A window size of 20 sets (80s) was chosen, and averaging was done at the complex image level. Thus, after discarding the first set in each direction to mitigate possible effects due to gradient transitions, the remaining 71 3D datasets were temporally smoothed to yield 51 valid time-points. To make sure that rearrangement of the data (as sets of diffusion-weighted data in different directions acquired simultaneously) and temporal smoothing still preserved the effect of stimulation, Hotelling's T^2 statistics were computed on intensity vectors at each voxel location using mean and covariance estimates from baseline data. This 4D dataset (4th dimension representing the T^2 statistic in time) was subjected to fMRI analysis[4], with a single box-car to model the 'effective stimulus' (stimulation window after smoothing). Those sets which showed significant activation were further analyzed for changes in WM regions. For each chosen ROI, the average temporal time-series was computed (by spatial averaging). Diffusion parameters were estimated[5] for each time point and Z-scores were calculated using the mean of parameters obtained from datasets acquired before stimulation and variance estimates obtained from baseline. Such direct use of baseline statistics was facilitated by the near absence of motion during the scans. Shapiro-Wilk test on FA, S0 and MD in WM voxels indicated that about 75% of voxels passed the test for normality at p=0.05, indicating that Z-scores could effectively signify the changes. We report only those cases in which the magnitude of Z-scores crossed 4, since, the same processing technique when applied to baseline showed very few Z-scores above 3.

Results: fMRI analysis on T^2 statistic showed significant cortical activation responses in all 3 fDTI datasets acquired at 1.0mA stimulation but not at 0.5mA. The primary region of observed activation was ipsilateral sensorimotor cortex (also contralaterally in one set). Fig.2 shows Z-statistics (|Z|>5) overlaid on FA map, along with ROIs used for the analysis in one dataset. Though this analysis also showed stimulus induced changes in corpus callosum, they were weak in comparison to GM. However, these changes were picked up by the Z-scoring procedure that followed. Fig.3 shows the temporal evolution of FA and MD at ROIs marked as 2 and 1, respectively, in Fig.2, in a single rat (in units of 4s). The black bar on top indicates the effective stimulation window. To facilitate the quantification of these change, in Fig.4, we plot the Z-scores of these and one another time evolution from an ROI in a different animal. As we can observe, the temporal evolution at these ROIs differed. While FA increased and then returned to baseline in one ROI (Green), S0 and MD did not change significantly. Similarly, ROI 1 (Red) shows relatively large changes in MD without significant changes in FA or S0. A significant decrease in FA was also observed in one animal (blue). These and few other recurrent patterns of change we observed, though varied, were significant, and thus strongly hint that changes in diffusion parameters in WM do occur as a result of stimulation. This leads to an obvious quest in search of the physiological underpinnings of these changes. Preliminary results from our ongoing carbogen challenge studies indicate that vascular effects cannot be neglected. But, most changes in this case seem to stem from or accompany significant changes in S0, which does not seem to be the case with intra-cortical stimulation, as can be see in Fig.4. This perhaps indicates that other factors may also be influencing these changes. Besides, several interesting patterns could also be observed in different regions of GM[6] with the fDTI acquisitions. These could also aid in elucidating these change mechanisms. Though no conclusive answers could be reached regarding the origins of these changes in this preliminary study, we hope this experimental setup will eventually yield good insights in this regard.

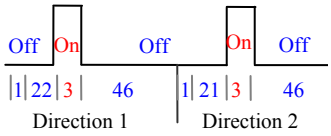

Fig.1 fDTI Acquisition scheme
Scan parameters:
4 Shot Multi-slice EPI. TR: 1000ms, TE: 38ms, FOV: 32x32x17mm
k-matrix: 64x64x17, Averages: 1
Images:128x128x7 (zero-pad recon)

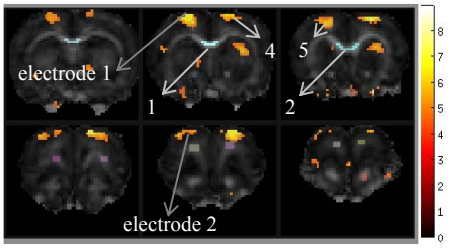

Fig.2 Activation (Z-scores) and ROIs overlaid on FA.

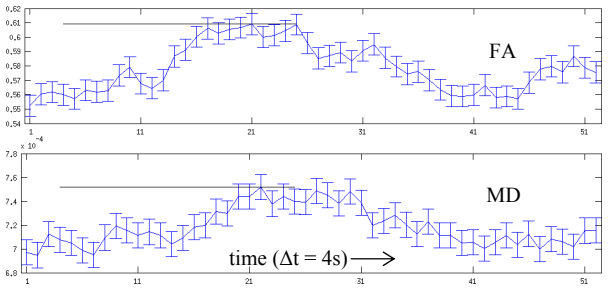

Fig. 3 Evolution FA and MD at ROI 2 and ROI 1, respectively.

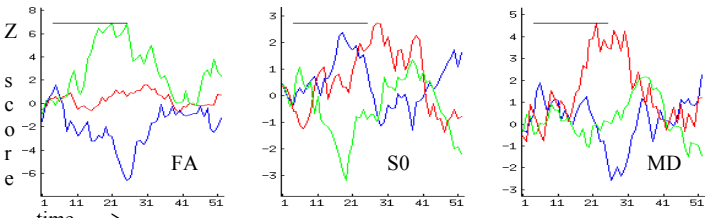

Fig. 4 Evolution of FA/S0/MD in WM regions. Red: ROI 1, Green: ROI 2, Blue: similar to ROI 1 in another animal.

References: 1) Rene C. W. Mandl et al., Plos One, 3 (11), e3631, 2008. 2) M. P. van Meer et al., Abstract # 3283, Proc.Intl.Soc.Mag.Reson.Med. 17, 2009 3) Karla L. Miller et al., PNAS 104 (52), pp20967-20972, 2007. 4) FEAT (FSL Tools) 5) FDT (FMRIB's Diffusion Toolbox) 6) Denis Le Bihan et al., PNAS 103 (21), pp8263-8268, 2006.