Mapping Acute Stroke in the Mouse Using Structural and Diffusion MRI

Kamila U. Szulc-Lerch[1], Eugenio Graceffo[1,2], Cristiana Tisca[1], Aurea B. Martins-Bach[1], Karla L. Miller[1], Clémence Ligneul[1], Jason P. Lerch[1], Yvonne Couch[3], Tracy D. Farr[4], Heidi Johansen-Berg[1]

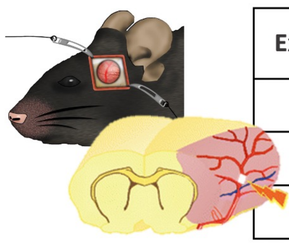
[1] Wellcome Centre for Integrative Neuroimaging, FMRIB, Nuffield Department of Clinical Neurosciences, University of Oxford , Oxford, United Kingdom, [2] Charité - Medical University of Berlin, Department of Paediatric Neurology , Berlin, Germany, [3] Radcliffe Department of Medicine, Nuffield Department of Clinical Neurosciences, University of Oxford , Oxford, United Kingdom, [4] School of Life Sciences, University of Nottingham Medical School, Nottingham, United Kingdom

Introduction & Aims

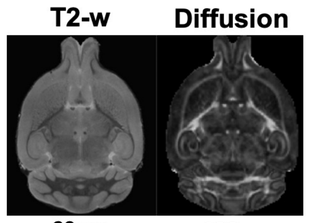
The distal middle cerebral artery occlusion (dMCAo) mouse model has been used in the field of preclinical stroke research to study brain injury and dynamics of post-stroke recovery. MRI is advantageous in that it allows excellent visualization and volume quantification of brain tissue abnormalities following brain trauma such as ischemic stroke, it is non-invasive, non-destructive, and sensitive to brain tissue changes at macroscopic, mesoscopic and microscopic levels, and it allows the whole brain coverage. In this study we performed whole brain, ex vivo, multi-modal MRI characterization of the dMCAo mouse model of stroke at 24h. We used T2-weighted and multi-shell diffusion scans for quantitative and qualitative analysis of stroke induced brain damage.

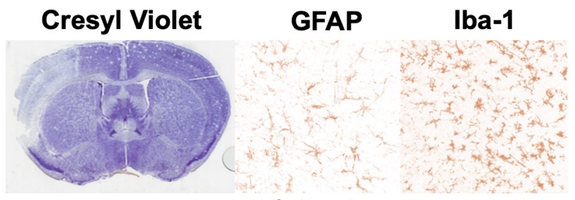
Methods

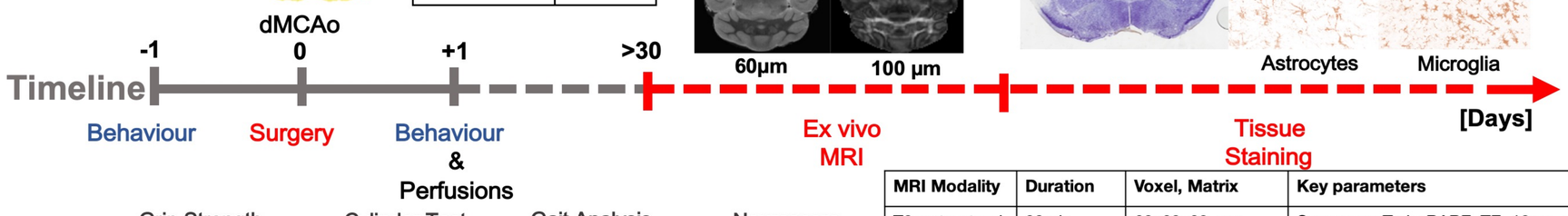
Study Design



Exp Group	N (30)
Stroke	12 (6♀)
Sham	12 (6♀)
Naïve	6 (3♀)







Animals: Thirty 6-month-old wildtype C57BL/6J mice were randomised into 3 experimental groups (naïve, sham and stroke)

Surgery: Stroke was induced in the left hemisphere, using permanent distal Middle Cerebral Artery occlusion (dMCAo) via electro-cauterisation (sham group underwent the same surgical procedure, but without dMCA occlusion, and naïve group did not undergo any surgery)

MRI Modality	Duration	Voxel, Matrix	Key parameters
T2w structural	33min	60x60x60 µm, 400x160x200	Sequence: TurboRARE, TE=12ms, echo spacing=12ms, 6 echoes, TR=350ms, BW=60kHz
dMRI	13h	100x100x100µm 240x96x120	segmented EPI, 12 segments, TE=30ms, TR=500ms, d/D =6.7/13.5ms, b=0 s/mm2 (volumes: 4+1 phase-encoding reversed), b=2,500 s/mm2 (volumes: 30), b=10,000 s/mm2 (volumes: 30), BW=250kHz, directions covering the whole shell and staggered between shells

MRI: After mice were perfused, brains were kept in skull, treated with PFA with gadolinium-based contrast agent (GdCA), and stored in PBS/GdCA/sodium azide (0.02%) at 4°C. Samples were scanned in Fluorinert.

Results

Quantitative MRI Data Analysis

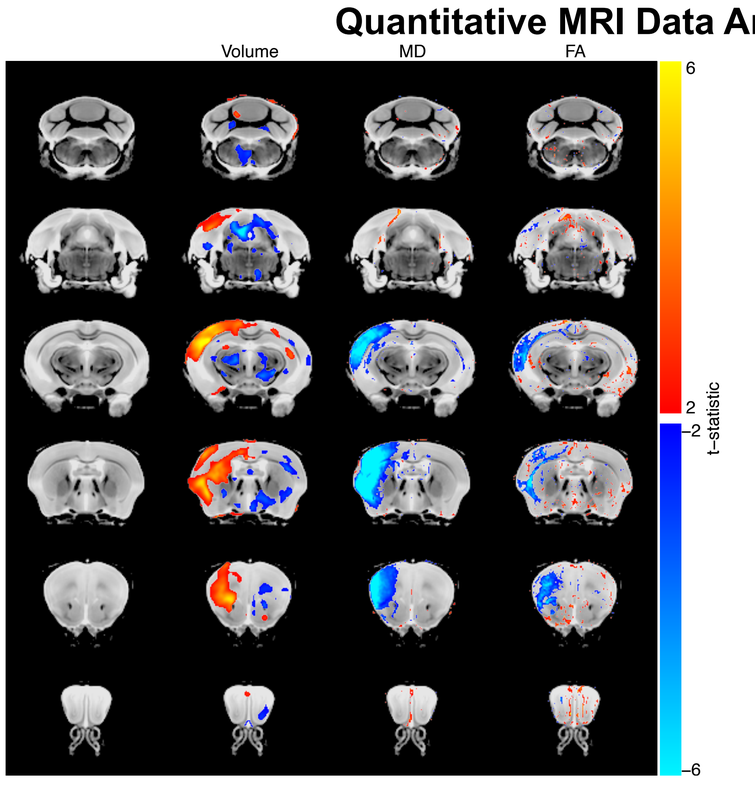
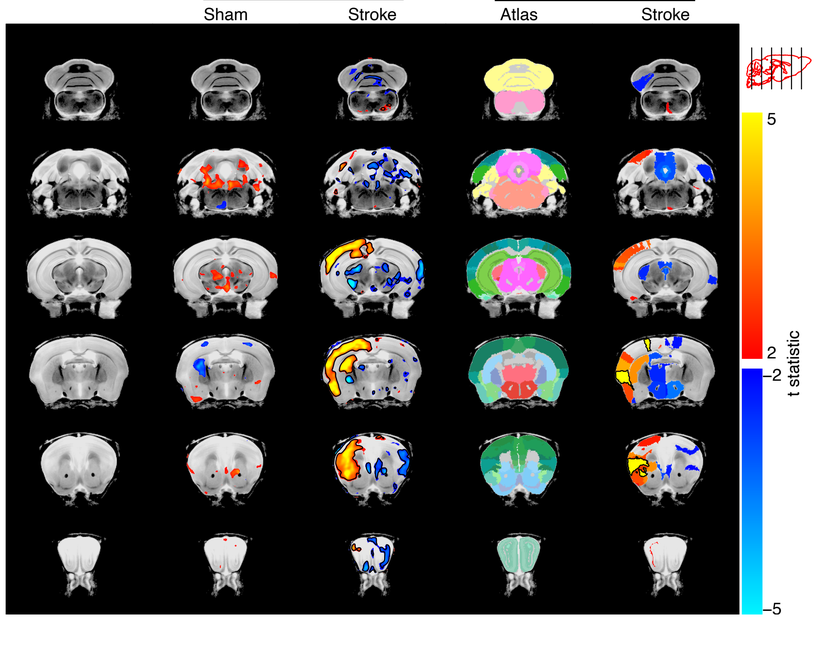


Fig.1 Results of diffusion data analysis. Note regions in which volume, mean diffusivity (MD) and fractional anisotropy (FA) were increased (red) and decreased (blue) in dMCAo mice, compared to sham mice. At 24h-post ischemic stroke induction, diffusion MRI showed clear delineation of the lesion on mean diffusivity (MD) maps and more localized underlying white matter changes in fractional anisotropy (FA).

Volumetric analysis revealed interesting structural changes at brain regions distant from the stroke lesion: claustrum and insula showed volume increase, and thalamus showed volume decrease, in ipsilateral hemispheres.



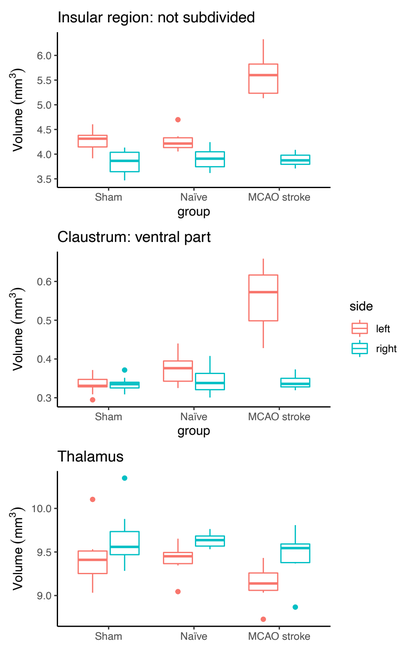


Fig.2 Results of analysis of anatomical T2-w data. Note volume increases (red) and decreases (blue) in sham and stroke mice compared to controls. Significant (10% FDR) changes are outlined in black. Three of the most significant regions are plotted.

Comparison of Lesion Appearance between Histology and MRI

Fig.3 Coronal brain slices of representative examples of sham (top) and dMCAo stroke (bottom) mice. First set of slices are brain slices stained with cresyl violet, second set are T2w images, and third set are images derived from diffusion MRI. Note the differences in delineation of the lesion between T2w and MD images.

Infarct Volume Analysis

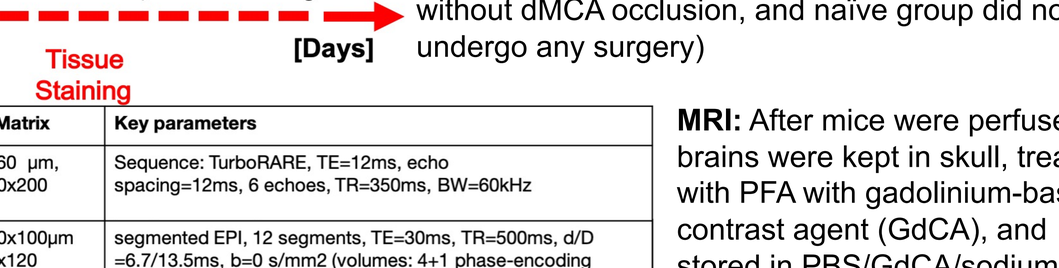
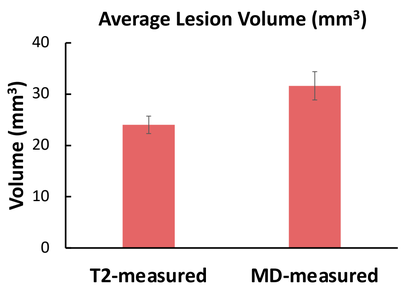


Fig.4 Axial (a, a'), sagittal (b, b') and coronal (c, c') slices of one of the dMCAo stroke model mice from **T2-weighted (a-c)** and **mean diffusivity (MD) MRI images (a'-c')**. Red colour indicates stroke-induced infarct. Infarct was segmented on every slice of 3-dimensional images on which lesion was visible. Bar chart shows the the average volume (mm3) of infarct lesion calculated from T2-w and MD images.



Behavioural Analysis

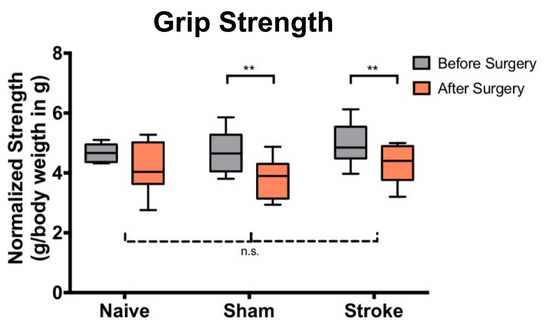


Fig.5 Graph showing grip strength, normalised over body weight of naïve, sham and stroke mice 24h pre- (grey) and 24h post-surgery (orange).

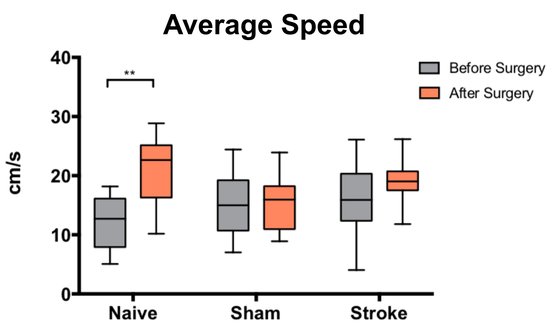


Fig.6 Graph showing the average speed of naïve, sham and stroke mice 24h pre- (grey) and 24h post-surgery (orange).

Conclusions

- Whole brain, 3-dimensional MRI used in this study has allowed us to visualize, identify and quantify not only the stroke lesion but also stroke impact on the rest of the brain. Tissue staining completed so far has allowed us to better understand the cellular nature of these MRI findings. Additional tissue staining analyses are ongoing.
- Portrayal and detailed characterization of brain injury 24h post-stroke in the dMCAo model using whole brain multi-modal MRI and tissue staining, as done in the current study, will be of value to the field.

Contact

Email: kamila.szulc-lerch@ndcn.ox.ac.uk

References

1. Dorr A et al. (2008). Neuroimage 42:60-69.
2. Szulc KU et al. (2013). Magnetic resonance in medicine 70:1707-1717.
3. Ullmann JF et al. (2013). Neuroimage 78:196-203.
4. van Eede MC et al. (2013). Neuroimage 82:226-236.

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