# Ex vivo visualization of cortical lesions in non-human primates with MS using inversion recovery experiments

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## Introduction

Cortical pathology is an important feature in the development of MS. The visualization with MRI is however hampered although FLAIR and Double Inversion Recovery (DIR) experiments showed increased sensitivity [Geurts 2005]. Cortical lesions are histologically described in the common marmoset MS model (Pomeroy 2005), recognized as a valid experimental model for MS. We explored with this model if the detection of cortical lesions could be increased by using various inversion recovery experiments. Single inversion recovery (IR) experiments were included in which either CSF (FLAIR), grey (Grey matter Attenuated IR: GAIR) or white matter (White matter Attenuated IR: WAIR) was suppressed. A DIR experiment was included in which both white and grey mater was suppressed. Data were compared with conventional MR techniques.

## Material and Methods

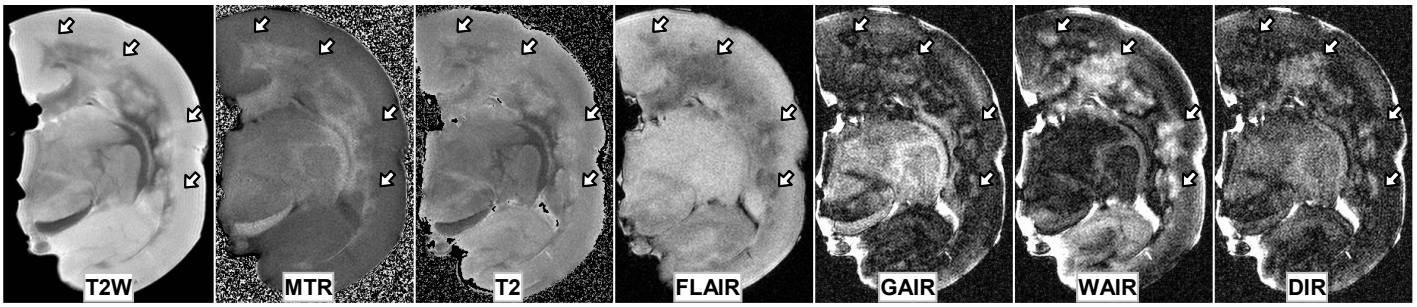
Experimental Autoimmune Encephalomyelitis in marmosets (n=6) was induced by immunization with recombinant human MOG in CFA [Brok 2000]. Animals were untreated controls used for therapeutic intervention studies either sacrificed at the end of the study or after reaching a disability score 3 [Brok 2000]. Brains were harvested and prepared for histology (fixed in 4% buffered formaldehyde, stored in PBS with sodium-azide). Only right hemispheres were examined

Ex vivo MRI was performed using a 9.4T horizontal bore NMR spectrometer (Varian, USA) with a quadrature volume coil. The following images (FOV=25x25mm, 41x0.75mm slices, matrix=256x256, NEX=2) were collected: *1)* single IR experiments (FSE; TR=4000ms, 4 echo's , echo spacing =10ms, $TE_{eff}$=20ms) with suppression of CSF (FLAIR; TI=725 ms), grey matter (GAIR; TI=500ms) or white matter (WAIR; TI=425ms). *2)* DIR experiments (FSE, TR=4000ms, 8 echo's with a spacing of 12.5ms, $TE_{eff}$=25ms). For DIR imaging, a subset of combinations of TR's, TI1's and TI2's were tested for the ability to generate images with the highest contrast ratios of cortical lesion resulting in TR=4000ms, TI1=550ms and TI2=10 ms. *3)* Magnetization Transfer Ration images (MTR; SE, TR=1675ms; MT-pulse: 10 ms gaussian shaped pulse, nominal flip angle $1000^0$, offset – 9.7 kHz). *4)* $T_2$ relaxation time images (MEMS, TR=2600ms, TE=6*10ms, mono-exponential fit).

All cortical lesions were outlined on a combined $T_2$-weighted ($T_2$W, TE=20 ms, for white-grey matter boundary) and WAIR image (qualitatively best contrast of cortical lesions). A surrounding control area adjacent to the lesion was also outlined in the cortex. A squared box control area was placed outside the brain for noise calculations. Area size, signal intensities (SI) and standard deviation (SD) were determined. Signal to Noise Ratio's of lesions ($SNR_{Lesion}$) surrounding areas ($SNR_{Surrounding}$), Contrast ratio's ($CR_{Lesion\text{-}Surrounding}$: $|(SI_{Lesion}\text{-}SI_{Surrounding}|)$ and contrast to noise ratio's ($CNR_{Lesion\text{-}Surrounding}$: $|(SI_{Lesion}\text{-}SI_{Surrounding})|/SD_{Noise}$) were determined. A maximum of 10 lesions per animal were included in the calculations to prevent that lesions of a single animal dominated the outcome. Data are presented as mean±sd.

## Results

Five out of 6 animals showed grey matter abnormalities on the combined $T_2$W/WAIR images. On average 13±23 lesions (ranging from 0-62) were detected. The 24 included lesions had an averaged size of $0.54\pm0.50mm^3$. An example of an animal with cortical lesions in which some, although not all, lesions are depicted (white arrows) is shown below. Qualitatively, lesions were best appreciated on the WAIR images.




The table shows calculated characteristics of all included lesions. Highest SNR values for lesions were found in the $T_2$W and FLAIR images, CNR were the highest for $T_2$W, FLAIR and WAIR images. High CR values were found in the GAIR and DIR images but particularly in the WAIR images.

| | $T_2$W | MTR | $T_2$ | FLAIR | GAIR | WAIR | DIR* |
|---|---|---|---|---|---|---|---|
| $SNR_{Lesion}$ | 43.61±3.62 | 1.08±0.19 | 4.30±0.84 | 12.69±3.22 | 2.42±0.68 | 4.81±1.40 | 1.51±0.42* |
| $SNR_{Surrounding}$ | 45.55±2.86 | 1.02±0.14 | 4.65±0.98 | 10.52±2.89 | 3.23±1.01 | 7.77±1.51 | 2.09±0.69* |
| $CR_{Lesion\text{-}Surrounding}$ | 1.05±0.05 | 0.95±0.05 | 1.08±0.06 | 0.83±0.07 | 1.45±0.63 | 1.70±0.39 | 1.49±0.64 |
| $CRN_{Lesion\text{-}Surrounding}$ | 3.93±3.11 | 0.05±0.05 | 0.19±0.08 | 4.10±1.79 | 2.51±1.75 | 5.53±2.25 | 1.52±1.19* |

* Displayed DIR values are corrected for NEX=4. All other experiments were collected with NEX=2.

## Discussion

All inversion recovery experiments showed improved cortical lesions detection compared to the more classical used MR experiments. Regarding the DIR experiments, best results were obtained using a sequence in which grey and white matter was suppressed. This is different from other ex vivo studies performed in humans in which CSF and white matter was suppressed (Geurts 2005). Overall, best results were obtained in the single inversion recovery experiment with suppression of white matter.

The data awaits further histological analyses to explore contrast ratios for different subtypes of cortical lesions. Furthermore the examined sequences should also be tested in vivo. Nevertheless, results may be interesting for clinical applications, especially as we have chosen for sequences which are easily implementable.

**References:** Geurts et al., Radiology 2005,236:254-260. Brok et al., J Immunol 2000,165:1093-1101; Pomeroy et al., Brain 2005,128:2713-2721.