Regional differences in viscoelasticity in normal and inflamed mouse brain

Jing Guo[1], Juergen Braun[2], Dominique Berndt[3], Carmen Infante-Duarte[3,4], Ingolf Sack[1], and Jason M. Millward[3,4]

[1]*Department of Radiology, Charite - Universitaetsmedizin Berlin, Berlin, Berlin, Germany,* [2]*Department of Medical Informatics, Charite - Universitaetsmedizin Berlin, Berlin, Germany,* [3]*Department of Medical Immunology, Charite - Universitaetsmedizin Berlin, Berlin, Germany,* [4]*Experimental and Clinical Research Center, Charite-Universitaetsmedizin Berlin and the Max-Delbrueck Center for Molecular Medicine Berlin, Berlin, Germany*

Target audience: Physicians and biologists interested in the mechanical properties and the viscoelastic changes of the mouse brain in the context of neuroinflammation, using the experimental autoimmune encephalomyelitis (EAE) animal model of multiple sclerosis (MS).

Background: Magnetic resonance elastography (MRE) [1] is capable of measuring in vivo the effective viscoelastic constants of mouse brain [2,3]. Recent findings demonstrated that tissue mechanical properties can reveal information about pathological alterations of the brain associated with neuroinflammation[4]. Here we use MRE to examine the mechanical properties in different mouse brain regions, and assess how these properties are altered during disease in a variant of the EAE model – a mouse lacking the cytokine interferon-gamma – in which pathology in the cerebellum and brainstem occurs to a greater extent than in wild-type disease [5,6].

Purpose: To investigate the tissue viscoelasticity in different brain regions in healthy mice, and to study the regional alterations during the course of neuroinflammatory disease using the EAE model.

Methods: MRE was applied to ten healthy unmanipulated C57/Bl6 wild-type (WT) mice (age 6-8 weeks) to establish baseline values. In parallel, age-matched interferon-gamma knockout (GKO) mice on the same genetic background were examined by MRE. These mice were then immunized to induce EAE using standard methods [6]. MRE was repeated in these mice at day 14 post-immunization, and again at day 21, when disease was well established. MRE was performed on a 7 T scanner (Bruker Pharma Scan, Ettlingen, Germany). A FLASH sequence was applied with a sinusoidal motion sensitizing gradients (MSG) and 900 Hz frequency matched to the mechanical vibration induced by air-cooled Lorentz coils [4]. Further imaging parameters: 128x128 matrix, 25 mm FoV, 4.15 ms TE, 150 ms TR, 285 mT/m MSG strength, eight time steps over a vibration period. The complex modulus G^* was calculated by direct Helmholtz inversion of complex wave images, the real part of the complex shear modulus $G'=\mathrm{Re}(G^*)$ (the storage modulus, reflecting elasticity) was averaged over three ROIs in a midsagittal slice: entire brain, anterior, and posterior regions (Fig.1a) .

Results: Comparison of baseline values showed no statistically significant difference in brain elasticity (G') between the WT and GKO mice (p=0.1337, unpaired t-test, Fig.1b). Based on this result, the WT and GKO mice were combined into one group and the values of G' over the entire brain (4.7±1.0 kPa) were compared with those of the posterior portion (cerebellum and brain stem, 3.8±0.8 kPa) and anterior portion (cerebrum, 5.2±1.2 kPa). This comparison showed that the tissue elasticity in these regions is significantly different: the anterior region is stiffer than the posterior region (p<0.0001, ANOVA, Fig.1b). Next, we examined the alterations in G' in the different brain regions of the GKO mice during EAE. Considering the entire brain, there was a significant reduction in elasticity, over the whole disease course (p =0.020, repeated-measures ANOVA). However, the anterior and posterior regions showed different responses. Compared to baseline, there was a significant increase in the ratio of G' between the posterior and anterior part of the brain, both at d14 and d21 post-immunization (p = 0.021, repeated-measures ANOVA).

Discussion: Comparison of baseline results of WT and GKO mice verified that the deletion of the interferon-gamma gene did not influence brain elasticity. Our data provide the direct comparison between different regions of the mouse brain. Unexpectedly, we show that the posterior region (comprising the cerebellum and brainstem) is softer than the anterior region. This likely reflects underlying anatomical differences between these regions, and is likely related to the white matter to grey mater ratio, and the direction of the fiber tracks with respect to the path of the propagating waves – a subject that must be pursued in future investigations. The reduction in G' of the GKO mice during the course of disease, reflecting a decrease in the elasticity of the entire brain, correlating with reduced tissue mechanical cohesion during disease is consistent with previous report in SJL mice[4]. Interestingly, in the GKO mice where more cerebellum involvement is expected during EAE, we observed a significant increase in the ratio of G' between the posterior and anterior part of the brain. Considering that the baseline posterior region is softer than the anterior region, this suggests a dramatic change in the mechanical structure in the tissue. One speculation is that this may relate to the infiltration of immune cells at this stage of the disease, as revealed by the presence of gadolinium-enhancing lesions in the cerebellum, indicating disruption of the blood brain barrier.

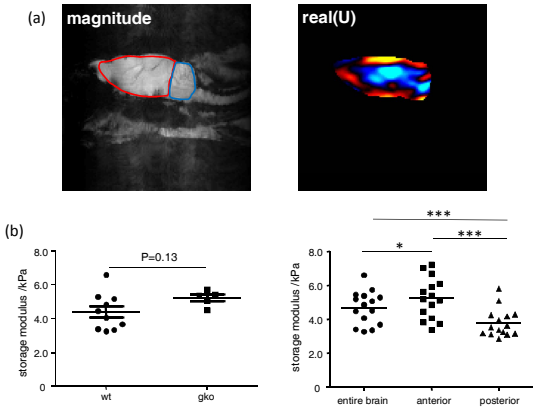

Fig.1: (a)MRE magnitude and the real part of the complex wave images. ROIs are outlined on the magnitude image: anterior (red) and posterior (blue). (b)Scatter plots of the storage modulus of entire brain in WT and GKO, and in different regions . (*p<0.05; **p<0.01; ***p<0.001)

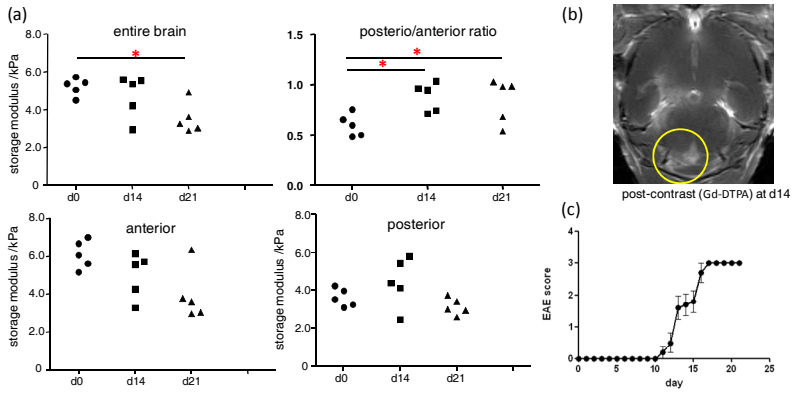

Fig.2: (a)Scatter plots showing the alteration of the storage modulus from baseline (d0) to d21 post immunization, plotted for three ROIs and the posterior/anterior ratio. (b) T1-weighted scan showing Gd-enhancing lesions at d14 for one mouse, outlined by yellow circle. (c) EAE clinical score over the disease course.

Conclusion: We applied MRE to WT and GKO mice and established baseline reference values for different brain regions. We also observed in the GKO group an increased ratio of G' between the posterior and anterior part of the brain, implying a local sensitivity at a relatively early time point during the neuroinflammation process, which could potentially serve as an early marker for tissue structure alternations.

Literature: [1] Muthupillai et al. *Science* 1995;269;1854-1857.[2] Clayton et al. *Phys Med Biol*. 2011;56; 2391-406.[3] Pepin et al. *Magnetic Resonance in Medicine* 2013, doi: 10.1002/mrm.24825.[4] Riek et al. *NeuroImage: Clinical* 2012; 1;81-90 [5] Pierson et al. *Immunol Rev* 2012; 248;205-15.[6] Krakowski et al. *Eur J Immunol* 1996, 26;1641-6.