Longitudinal MRI assessment of brain microstructural changes induced by chronic Toxoplasma gondii infection in mice

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Introduction: *Toxoplasma gondii (T. gondii)* is a widespread intracellular parasite, with a complex life cycle in different host species, including humans. It chronically infects more than 500 million persons around the world (1). After proliferation of tachyzoites in various organs during the acute infection stage, the parasite forms cysts preferentially in the brain. A variety of brain cells could be infected, including neurons, astrocytes or microglia. Independent studies carried out in recent years suggest the ability of *T. gondii* infection to contribute to neurological and psychiatric symptoms (2). Interestingly, in rodents, the parasite is able to manipulate the animal's behavior in relation to predator-prey interactions, by reducing the natural aversion of mice to cat odor (3). Moreover, it was showed that acquired infection with *T. gondii* in adult mice results in sensorimotor deficits while the cognitive responses were not altered despite widespread brain pathology (4). These results point out possible subtle forms of neuroplasticity induced by parasite-host interaction in specific brain areas. In this context, the aim of our study was to map overtime the localization of parasite burden across the whole mouse brain and to investigate by diffusion tensor MRI (DT-MRI) and fiber tracking the possible connectivity induced changes, in order to better understand the substrate of the behavioral abnormalities observed.

Material and Methods: 15 female 8-week old C57BL/6 mice were infected by i.p injection, with 3 cysts of ME49 type II *Toxoplasma* strain. Mouse brain T2*-weighted images were acquired at different time points for monitoring the development of the pathology from acute to chronic stages (w0: before infection, w3: acute infection and w8: chronic infection) and to localize the T. gondii produced lesions. At the chronic stage (w8) in-vivo mouse brain DT-MRI was additionally performed. At each timepoint 3-4 subjects were used for histopathological investigation.

MRI: The mice were scanned under isoflurane anesthesia, using a 9.4T small bore animal scanner, a transmit/receive 1H mouse quadrature birdcage resonator and the ParaVision 5 software interface (Biospec 94/20 Bruker). After localized shimming procedure on a volume of interest (4.8 x 5.3 x 9 mm^3), inside the mouse brain, multislice T2*-weighted images (78 x 78 x 500 μm^3) were acquired with the following parameters: TR/TE=920/12 ms, field of view=1.5×1.5 cm, matrix size=256×256, slice thickness=0.5 mm, FA= 60°.

DT-MRI data was acquired with a 4-shots DT-EPI sequence. Diffusion gradients were applied in 45 non-collinear directions (b factor = 1000s/mm^2), TR/TE = 5000/30 ms, time Δ=17ms, diffusion gradient duration δ=7ms. The in-plane image resolution was 156 x 156 μm^2, for 20 slices (500µm). Respiratory gating was performed in order to avoid respiratory movement artifacts resulting in a total acquisition time of about 2h.

Diffusion data post-processing was performed using a FiberTool package developed in-house (5). Fiber tracking was done using a global optimization algorithm (6) that reconstructed all fiber bundles simultaneously, for the whole brain. High resolution fiber density maps, in which the fiber's directionality was color-coded were generated using a described procedure (6, 7).

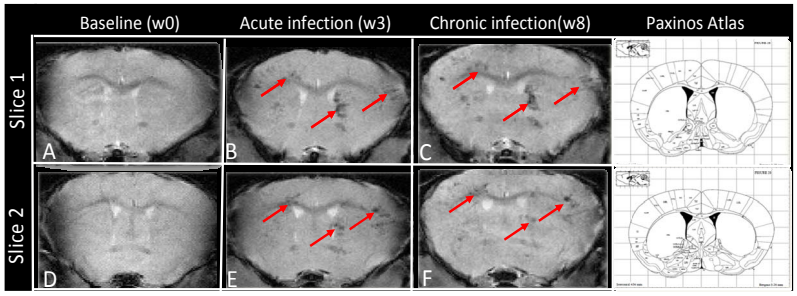

Figure 1: Example of *Toxoplasma*-induced microlesions in mouse brain across two different T2*-weighted axial MRI slices. Note the localization of the injuries (arrows) in the cortex, perventricular areas, striatum and close to the white matter structure during acute (w3 - B,E) and chronic (w8 - C, F) *T.*

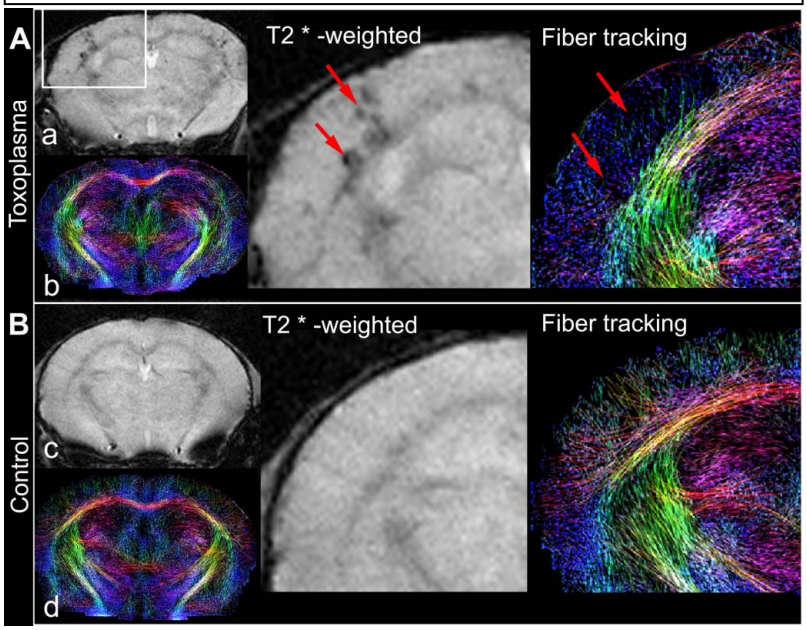

Figure 2: Comparative visualization of T2*-weighted (a) and fiber density (b) maps of a T. gondii infected (A) and a control (C) mouse brain. Magnified views show the localization of the injuries (arrows) in the somatosensory cortex and the fiber density modifications.

Results and Discussion

During acute infection with *T. gondii* cysts (w3 timepoint), T2*-weighted images revealed hypointense microlesions (Fig 1. B, E) widespread across the whole mouse brain. The lesions are size and shape different (red arrows), with a preponderant localization in the prefrontal and sensorimotor cortices, in the striatum, at the border line with the lateral ventricles and also near white matter structures. No obvious illness behavior could be assessed at this timepoint. Chronic infection was clinically manifested mainly by locomotor and autonomic disabilities. Figure 1 (C and F) shows the persistence of the brain injuries at w8. However many other brain areas were found to be affected at this chronic phase of the infections, including thalamus and amygdala. Amigdalar nuclei are very important regions of the limbic system shown to be involved in modulating for example the fear behavior. The impact of the T. gondii lesions on the general wiring scheme was also assessed. The lesions identified in T2* images served as regions of interest (ROI) for DT-MRI data analysis. Representative areas (see Fig 2) were chosen to assess the fiber density modifications. High resolution connectivity maps point out a loss of structural continuity in the sensorimotor cortex (Fig. 2), together with a general reduction of thalamocortical and amygdala-cortex projections. This impaired connectivity might explain the sensorimotor deficits observed in the infected mice.

Conclusion: Our data provide longitudinal insight into the pathological effects of the T. gondii infection on the brain microstructure. We explore and analyze for the first time the impact of the T. gondii infection on the whole brain connectivity pattern, suggesting impaired structural connectivity between important areas of the somatosensory and limbic systems. Further analysis of the data is warranted to answer the question if possible compensatory neuroplasticity patterns are developed.

References: (**1**). Tenter et al., 2000; IntJournal for Parasitology. (**2**). Torrey and Yolken, 2003; Emerg Infect Dis. . (**3**). Vyas et al. 2007; Proc Natl Acad Sci U S A. (**4**). Gulinello et al. 2010; Microbes Infect. ; (5) Kreher BW et al, MRM, 2008; (6) Reisert M et al., Neuroimage 2010; (7) Calamante F et al., Neuroimage 2011.