A pathophysiological wiring defect in epileptic animals as depicted by DTI fiber tracking

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Introduction: The Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is a well-validated model of absence epilepsy. GAERS and Non Epileptic Control (NEC) rats originate from inbred strains of Wistar rats selected on their epileptic phenotype as evidenced by the occurrence or not, respectively, of spontaneous spike-and-waves discharges on their electroencephalograms (1). Monoamines, with special emphasis on dopamine, have been shown to play a modulatory role on absence seizures (2). The fasciculus retroflexus (FR) is a white matter tract, which exerts a tonic inhibitory influence on the release of dopamine. Preliminary histological observations suggesting a defasciculation of the FR in GAERS are reminiscent of a putative dysfunction of dopamine release. To further characterize the integrity of the FR, a microscopic 3D-Diffusion Tensor Imaging sequence was developed and applied *ex vivo* on GAERS and NEC rats. Fiber-tracking was performed to quantify the volume and number of fibers in the two rat strains.

Subject and Method:

Animals: Three-month old adults GAERS (n=6) and NEC (n=6) were used. Brains were fixed by transcardiac perfusion (4% paraformaldehyde in phosphate buffered saline containing 6.25mmol/L Gd-DOTA) and the whole skulls were maintained for at least 4 days in 6.25mmol/L Gd-DOTA in order to reduce relaxation times and accelerate the MRI acquisitions. They were then preserved individually in syringes containing Flomblin-oil.

MRI acquisitions: Experiments were performed at 7T (Bruker, 600mT), 11 days after Gd-DOTA administration, a time for which T_1 and T_2 values were uniforme throughout the brain, respectively 60 and 8 ms. As the expected results are anatomical and not functional, DTI sequence was a 3D spin-echo sequence (TE/TR= 16/90ms, NA=13) rather than EPI, with diffusion gradients (δ= 3.5ms Δ=8ms) applied in six spatial directions ([1 1 0], [1 -1 0],[0 1 1],[0 1 -1],[1 0 1],[-1 0 1]) with a b-value of 1500s/mm². Field of view (FOV) and spatial resolution were set to 34x14x18 mm^3 and 100x100x100 μm^3 respectively. This sequence was applied during the week end but sufficient NMR sensitivity is also obtained in 24h. The diffusion data analysis and the fiber-tracking were computed using MedINRIA software (3).

Results*:* No significant morphological modifications of the FR (red arrow) appeared in the T2 weighted images between GAERS and NEC rats (Fig 1 a,c). The fiber reconstruction, limited to the FR, gave a less dense FR for the GAERS rat (Fig1, b-d). A significant decrease of the fiber volume (24 %) and of the fiber number (18%) were observed in GAERS as compared to NEC.

Discussion/conclusion: Based on these preliminary results, DTI fiber-tracking showed a decrease in the number of fibers detected in the FR, suggesting a contribution of this tract in the modified dopaminergic tone observed in epileptic rats. Quantitative analyses are in progress in other white matter structures of the brain to address for the specificity of such changes in FR. The 3D-DTI fiber-tracking could thus be used as a tool to target white matter deficits in animal models validated by histological observations using antibodies raised against the different populations of glial cells.

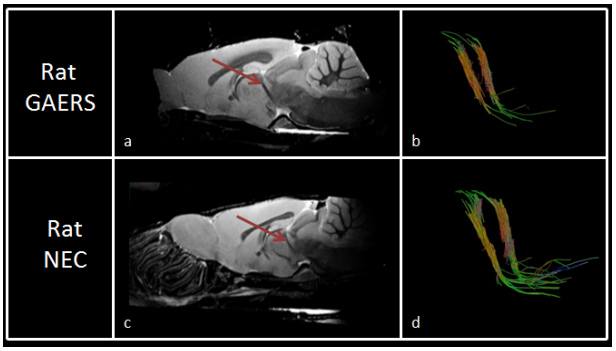

Figure 1: T2 weighted image of sagittal slice for GAERS (a) and NEC (c) rats, and tractography of the fasciculus retroflexus (b and d), indicated by a red arrow.

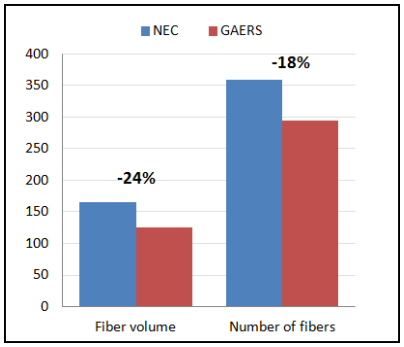

Figure 2: Histograms of the volume and the number of fibers for GAERS (n=6) and NEC (n=6) and rats (*p<0.05, Student test).

(1) Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C (1998) Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog. Neurobiol.* 55, 27-57. **(2)** Deransart C., Riban V., Lê B.T., Marescaux C, Depaulis A (2000) Dopamine in the nucleus accumbens modulates seizures in a genetic model of absence epilepsy in the rat. Neuroscience, 100: 335-44. **(3)** http://www-sop.inria.fr/asclepios/software/MedINRIA/