Cannabinoïd treatment induces temporal biomechanical alterations in the juvenile rat hippocampus

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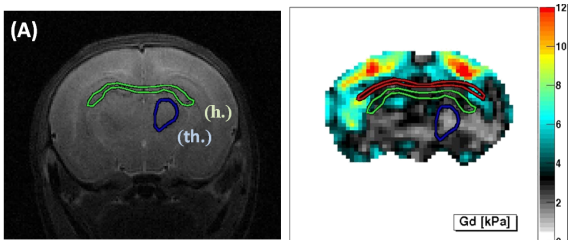
Introduction:

Recent studies suggest a significant influence of type-1 cannabinoïd receptors (CB_1R) on puberty maturation processes [1]. It could result in neuronal remodeling in the postnatal brain and also lead to local modifications of cortical mechanical properties. The objective of this study was to use MR Elastography to identify local temporal alterations of non-mature brain mechanical properties induced by cannabinoïd injection.

Material and methods:

The experimental protocol consisted successively in an anatomical T2-weighted MR scan, a baseline MR Elastography (MRE) acquisition, intra-peritoneal drug injection followed by a delay of 15 min and a second MRE acquisition to investigate drug effects. Imaging experiments were performed on a small-animal 7-Tesla MR scanner. The tests were conducted on 9 to 11 day-old healthy Sprague-Dawley rats (n=5). Four of these rats were injected with 0.7 mg/kg of synthetic cannabinoïd CB_1R agonist (CP55940). To assess the specific involvement of the CB_1R, a "negative control" has been investigated by injecting the fifth animal with a CB_1R antagonist (AM251) 15 min prior to the CB_1R agonist administration.

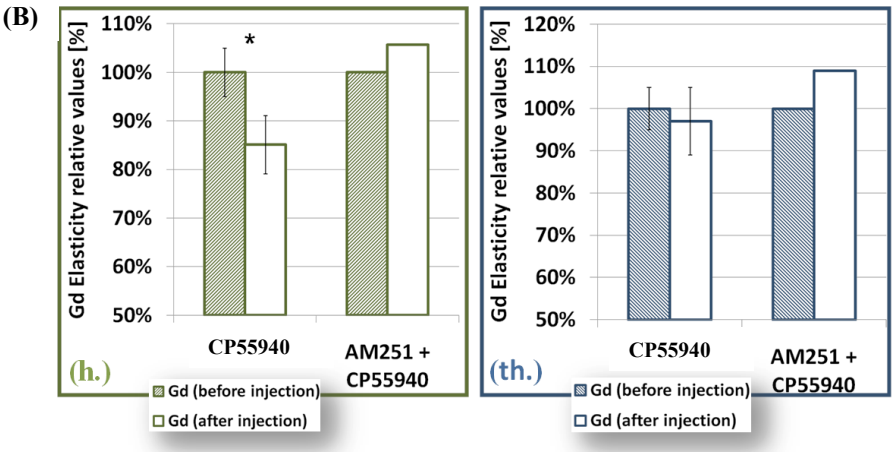


A: anatomical T2 weighted and elasticity coronal image exhibiting the 2 ROI of the current study;

MRE images were acquired using a spin-echo sequence including synchronous motion-encoding bipolar gradients with EPI readout to encode 3 orthogonal displacement maps in phase images. The MRE imaging parameters were: TR/TE=901/19.5 ms, 10 coronal slices of 300 µm in thickness, FOV = 19.2 × 19.2 mm^2, data matrix = 64 × 64, image resolution = 300 × 300 µm². Mechanical acoustic waves were generated using an uni-axial modal exciter (1000 Hz) with controlled harmonic displacement, driven by a wave generator. The animals were placed in a plaster holding helmet, which incorporated an indenting piston and an anaesthesia flexible nose-cone (isoflurane anaesthesia). Two specific regions of interest (ROI) were determined on the morphological images (figure A) and reported on the maps of mechanical properties (h.: hippocampus, th.: thalamus). Average mechanical shear parameters G_d and G_l values, corresponding to elasticity and viscosity measurement respectively, were then calculated from the 3D displacement fields of shear waves [2] in these ROI (figure A). Mean values are obtained in the ROI with their Standard Error of the Mean.

Results:

Elasticity values were normalized with respect to the baseline Gd values measured before CB_1R injection (4.03 ± 0.31 kPa and 2.62 ± 0.25 kPa for h. and th., respectively), and expressed as percent of G_d. The cannabinoïd agonist injection induced a decrease of the elasticity modulus by 14.9 ± 3.8 % in the hippocampus (* p = 0.029), while no significant mechanical modification has been observed in the thalamus (figure B) (p = 0.189). These results are in agreement with the expression pattern of CB_1R in the hippocampus and thalamus in the rat brain [3], as confirmed in this study by histology (figure C). Moreover, the additional pre-injection of the CB_1R antagonist completely suppressed the agonist-induced decrease of G_d.

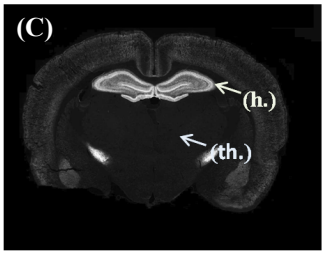


An absence of blood pressure influence on the elasticity variations is suggested by the similitude between hippocampus and thalamus vascularisation and perfusion. For all the animals, no variation was observed regarding brain viscosity ($\overline{Gl}$ = 1.13 ± 0.13 kPa and 0.86 ± 0.13 kPa for h. and th. respectively).

Conclusion:

This study shows for the first time a significant temporal alteration of young rat brain mechanical properties after cannabinoïd injection. This effect is relevant in hippocampus, a high-expressing CB_1R region. It would confirm that CB_1R receptors may play an important role in the neuronal remodeling in the postnatal brain with significant effects on brain stiffness, especially in the hippocampus, which plays a major role in memory and learning functions.

B: histograms of the relative Gd decrease and SEM values for CB_1R agonist and antagonist + agonist injections in hippocampus (h., * p < 0.05) and thalmamus (th., p > 0.05)



C: Immuno labelling of CB_1R on a 11 days-old rat coronal section.

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