In vivo quantification of local transient softening in the juvenile rat brain after Cannabinoïd treatment: first indications for neuronal remodeling?

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Introduction: Recent studies suggest a significant influence of type-1 cannabinoïd receptors (CB_1R) on the puberty maturation processes [1] including neuronal remodeling and modifications of cortical mechanical properties in the postnatal brain. A preliminary study showed a decrease of the hippocampus elasticity within 15 minutes after CB_1R agonist injection, while no significant modification was observed in the thalamus [2]. The aim of the current study is to assess the significance and causes of this effect in the hippocampus of juvenile rats. CB_1R is highly expressed not only in axons but also in endothelial cells of the hippocampus [3]. The first step of this study consists in the comparison of hippocampus elasticity and cerebral blood flow (CBF) values, from MR–Elastography (MRE) and Flow-sensitive Alternating Inversion Recovery (FAIR) perfusion imaging respectively, after CB_1R activation. In a second step, the sensitivity of MRE to CBF modifications is assessed using the vasodilator nicardipine.

Material and methods:

The expression of CB_1R in the juvenile rat hippocampus was assessed using immunohistochemical labeling of a brain section (*Fig 1*). MR measurements carried out in a 7T MRI scanner, consisted of an anatomical T2–weighted MR scan, a baseline MRE acquisition (*Fig 2-A*), and a second MRE acquisition 15 minutes after intraperitoneal drug injection. The tests were conducted on 10 days-old (d.-o.) rats (n=11) injected with the cannabinoïd receptor agonist CP55,940 (0.7mg/kg). The specificity of the CB_1R effect was controlled with a specific CB_1R antagonist (AM251, 3mg/kg, n=3) 15min prior to the CB_1R agonist administration, or by replacing the CP55,940 with a vehicle (NaCl+DMSO+Tween80) injection (n=3). MRE was acquired using a spin–echo sequence with synchronous motion–encoding gradients to encode 3 orthogonal displacement maps in phase images (10 axial slices of 300µm isotropic resolution). Mechanical waves were generated using a uni–axial modal exciter (1000Hz). Elasticity and viscosity maps were then obtained from 3D shear wave images [4]. CBF maps (*Fig 2-B*) were obtained using the flow alternating inversion recovery technique (TR / TE 4000 / 19 ms, 1axial slice, 300µm×600µm×3mm, 5 inversion times, 3mm inversion slab thickness, interleaved mode). In a second step, the same protocol was applied to animals (n=5) injected with a vasodilator (nicardipine, 15mg/kg) to investigate the influence of CBF on MRE measurements.

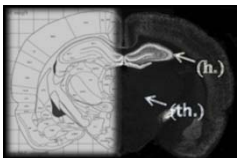

Fig 1. Immuno-labelling image (right) of CB_1R on a 10d.-o. rat transverse section.

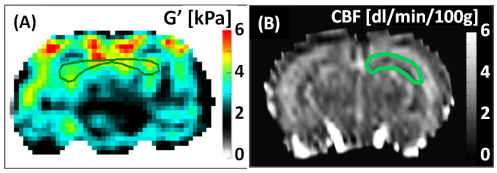

Fig 2. Examples of elasticity (A) and CBF (B) maps on transverse image with the hippocampus ROIs.

Results:

The CB_1R immuno-labelling confirmed the expression pattern of CB_1R in the hippocampus (*h.*) and thalamus (*th.*) in the rat brain (*Fig 1*). CP55,940 injection induced a significant decrease of the elastic modulus in the hippocampus (-14.1%, p<0.001), while a prior injection of AM251 inhibited this effect (*Table 1*, *Fig 3-A*). This softening was reversible to within 5% of the baseline value after one hour (p<0.01). No variation of the mechanical properties was observed after vehicle injection. All these results tend to indicate a strong involvement of CB_1R in the mechanism of decreased hippocampus elasticity mediated by cannabinoïd agonist. Brain viscosity did not appear to be sensitive to any of the investigated effects.

Injection	MRE 1: Reference		MRE 2: injection + 15min		MRE 3: injection + 60min	
	G' [kPa]	G'' [kPa]	G' [kPa]	G'' [kPa]	G' [kPa]	G'' [kPa]
CP55,940	3.82 ± 0.35	2.78 ± 0.26	3.28 ± 0.34	2.75 ± 0.28	3.74 ± 0.44	2.59 ± 0.23
AM251 + CP55,940	3.86 ± 0.28	2.66 ± 0.21	3.87 ± 0.36	2.60 ± 0.21	- (1)	- (1)
Vehicle	3.71 ± 0.23	2.67 ± 0.21	3.60 ± 0.23	2.69 ± 0.26	- (1)	- (1)
Nicardipine	3.58 ± 0.12	2.63 ± 0.21	3.34 ± 0.29	2.40 ± 0.17	- (1)	- (1)

Table 1. Elasticity (G') and viscosity (G'') values measured in the juvenile rat hippocampus before, 15min and 60min after injection.
(1 not acquired)

MRI perfusion results show a significant CBF decrease of 17.7% in the hippocampus 15 minutes after injection (*Fig 3-A*). However, the non–return of CBF measurements to their baseline values within one hour after injection shows that, by themselves, CBF modifications cannot explain the observed changes in mechanical properties after CB_1R agonist injection. In addition, while nicardipine injection induced a decrease of the CBF similar to the one obtained with CP55,940 (*Fig 3-B*), the decrease of elasticity mediated by nicardipine is twice lower than what is observed after CP55940 injection. This shows that perfusion changes cannot be the only cause for the mechanical properties modifications mediated by CB_1R activation.

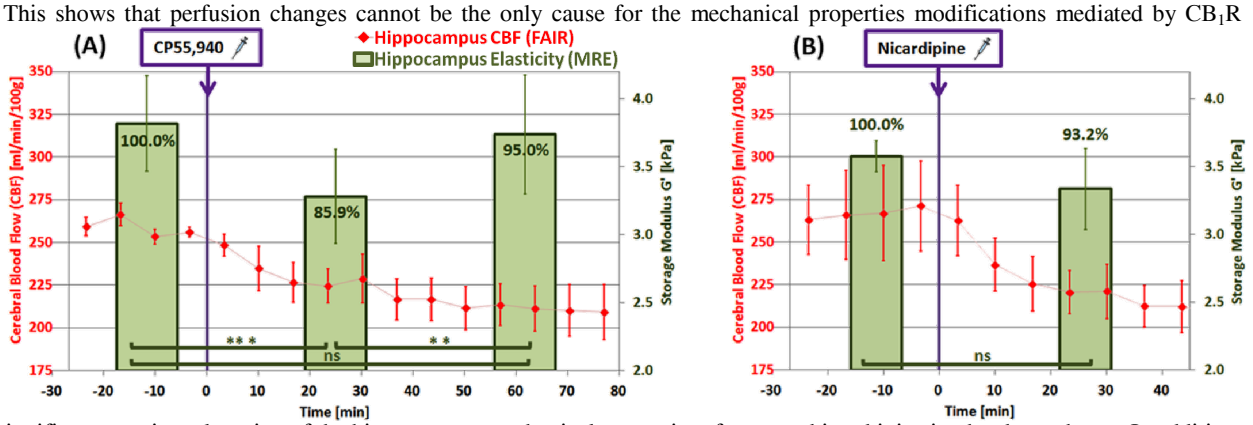

Fig 3. Time evolution of elasticity (green) and CBF (red) in the hippocampus after CB_1R agonist (A) and classical vasodilatator (B) injection.

****p<0.001*
***p<0.01*
ns: p>0.1

Conclusion:

For the first time, a significant transient alteration of the hippocampus mechanical properties after cannabinoïd injection has been shown. In addition, even though this study shows for the first time that MRE is sensitive to CBF variations in the brain (via nicardipine), the elasticity modifications after CB_1R activation cannot be explained solely by a decrease in CBF. Previous studies have described CB_1R as critical for the regulation of the neuronal cytoskeleton integrity [5]. Consequently, a neuronal remodeling is proposed as further source of effects on brain stiffness after CB_1R activation, especially in the hippocampus, which plays a major role in memory and learning functions.

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