Prospective brain T2 relaxometry and diffusion tensor imaging in a rat model of early-life febrile convulsions

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Introduction

Retrospective studies reveal that up to 50% of epilepsy patients with mesial temporal sclerosis have a history of febrile convulsions (FC) during childhood, suggesting that there may be a causal relationship [1]. During FC, children between 3 months and 5 years of age endure seizure activity accompanied with high fever. Longitudinal clinical studies are complicated, as there is a silent period between the FC (< 5 months) and the first epileptic seizures (> 15 y). Therefore experimental FC rat models have been developed. In the hyperthermia rat model of epileptogenesis [2], young rats (post natal day, PN = 9) are subjected to a hyperthermia treatment that induces FC. It has been shown that from these animals about 35% develop spontaneous seizures (PN90-180), as demonstrated by cortical EEG and chronic video monitoring [3]. In this study we examined rat brain using T2 relaxometry and DTI at two time points after experimental hyperthermia induced FC, to determine microstructural changes in tissue characteristics possibly related to pathological cellular processes that promote epileptogenesis.

Material and Methods

30 male Sprague–Dawley rats (Harlan, The Netherlands) were divided into the following three groups: (a) normothermia (NT) (n = 10); (b) hyperthermia with typical FC behavior (HT+; n = 10), and (c) hyperthermia without typical FC behavior (HT-, n = 10). PN9 rat pups were placed in heated air (50–52◦C) to raise their body temperature from 35◦C to 41–42.5◦C [4]. When the temperature reached 41◦C, the core temperature was maintained at 41–42.5◦C for 30 min. The behavioral seizures were stereotyped and previously shown to correlate with EEG discharges in the hippocampus [2]. Based on occurrence of FC behavior (body flexion), pups were assigned to either to HT- or HT+. After the HT treatment, rats were returned to their mother. NT controls from the same litter as the HT rats were exposed to the same conditions, except that the air temperature was only 35◦C. The MR experiments were performed at PN10 (9 NT, 10 HT+, & 3 HT-) and PN66 (9 NT, 9 HT+, & 8 HT-) on a 6.3 Tesla horizontal bore magnet (Oxford Instruments, England) interfaced to a Bruker Biospec (Bruker, Ettlingen, Germany) MR Imaging console. A dedicated Helmholtz volume and a butterfly surface coil (Rapid Biomed, Germany) were used for pulse transmission and signal detection, respectively. Rats were anesthetized with isoflurane (1-2%) and placed in a home built cradle, equipped with a mask for anesthesia gas supply and a warm water pad. Respiration and rectal temperature were monitored. T2 imaging was performed using a multi slice multi spin-echo sequence with a TR of 5 s and TEs of 17.2, 43.0, 77.3, 111.7, 146.1, and 180.4 ms (15 coronal slices, 1 mm thickness, 128 x 128 matrix, field of view 4 x 4 cm, 2 averages). The T2 map was calculated using a nonlinear monoexponential fit. For diffusion, an echo planar imaging sequence was used, with 30 directions (TR = 3 s, TE=34 ms, b values= 0 and 1000 s/mm^2, diffusion gradient duration 4 ms, diffusion gradient strength 239.1 mT/m, 15 coronal slices, 1 mm thickness,128x128 matrix, field of view 4 x 4 cm, 2 averages). The apparent diffusion coefficient (ADC, 10^{-6} mm^2/s) and fractional anisotropy (FA, %) maps were calculated using the diffusion software available on the MRI scanner. Bilateral regions of interest (ROI) were manually drawn on a T2-weighted image in the thalamus (TH), Hippocampus (HC), retrosplenial cortex (RC), amygdala (A), piriformic cortex (PC), and corpus callosum (CC), on the basis of the Paxinos brain atlas [5] (Figure 1A). The mean T2, diffusion ADC and FA values were calculated for the selected structures.

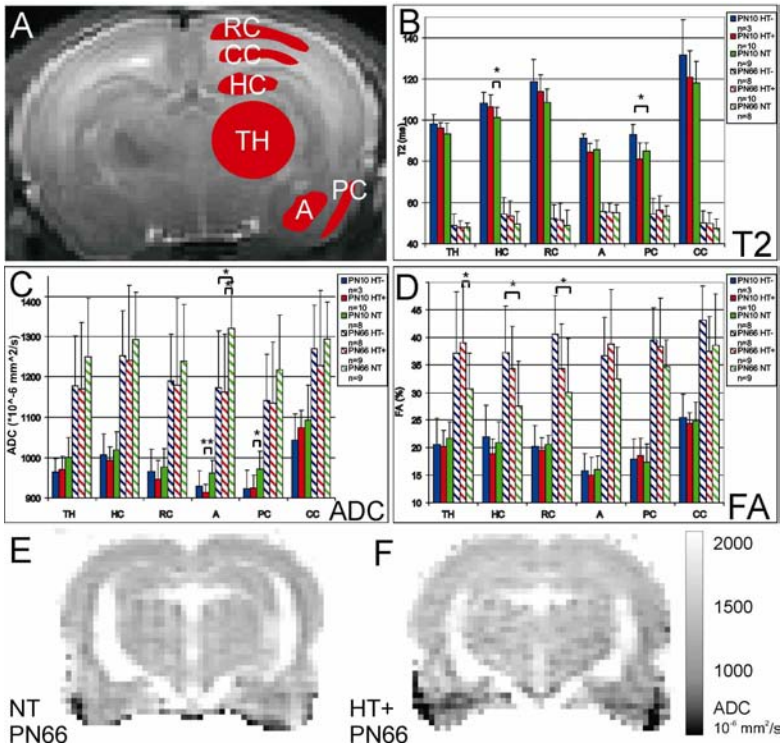

Figure 1 (A) T2-weighted image of a HT+ rat at PN10, indicating the employed regions of interests. **(B,C,D)** Bar diagrams displaying the mean±SD T2, trace and FA values, respectively, for the HT- (blue), HT+ (red), and NT (green) rat pups, at PN10 (solid) and PN66 (dashed). * and ** denote p<0.05 and p<0.01, respectively. **(E,F)** ADC images at PN66 of a typical NT rat (E) and a HT+ rat (F). Notice the pronounced ventral signal decrease in the HT+ rat.

Results (Figure 1B,C,D)

At PN10, both HT+ and HT- rats had elevated T2 values in most dorsal regions compared to NT rats. This effect was significant in the HC (HT+ vs NT, p<0.05) and PC (HT- vs NT, p<0.05). At PN66, T2 values were comparable. ADC values were significantly decreased ventrally at PN10 for HT+ rats in the amygdala (HT+ vs NT, p < 0.01) and PC (HT+ vs NT, p < 0.05). Also at PN66 ADC values were decreased in the amygdala (HT+ vs NT, p<0.05 & HT- vs NT, p<0.05) (Figure 1EF). For FA, no differences were observed at PN10, however at PN66 FA values were higher in TH (HT+ vs NT, p<0.05), and HC and RC (HT- vs NT, p<0.05).

Discussion

Maturation of rat brain explains the generally decreased T2, and increased ADC and FA values at PN66 compared with PN10. For HT- and HT+, the elevated T2 values at PN10 for most dorsal regions (TH, HC, RC, and CC) are indicative of HT-induced edema (temporarily, as values were normal at PN66). This was confirmed by hyperintense T2-weigthed images. Whether this edema is caused by the FC or merely due to fever cannot be distinguished. Although the HT- and HT+ group were separated based on visual observation of HT-induced FC behavior, only an additional EEG examination could have validated that the HT- group truly didn't have seizure activity, therefore HT- and HT+ are not necessarily different (due to FC behavior). The decrease of ADC values in the amygdala and PC, might be indicative of excitotoxicity, similar to an observation made shortly after status epilepticus [6]. It is a chronic effect, as it remains present ventrally at PN66 for both HT+ and HT-. The dorsal increase of FA values at PN66 within the TH (HT+) and HC and RC (HT-), might be indicative of a chronic pathological process, initiated to resolve the edema, but continued after the edema was eliminated. To conclude, HT induced acute dorsal edema (visualized by T2) and chronic micro-structural dorsal and ventral changes (visualized by DTI, which appears to be more sensitive than T2). The mechanisms underlying these structural changes remain to be elucidated; careful histological examination might prove useful, which is underway.

References

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