Introduction
Retrospective studies reveal that up to 50% of epilepsy patients with mesial temporal sclerosis have a history of febrile seizures (FS) during childhood, suggesting that there may be a causal relationship [1]. As longitudinal clinical studies are complicated, experimental FS 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 FS. It has been shown that from these animals about 35% develop spontaneous seizures (PN90-180) [3]. The present study combines this FS model with a quantitative MRI examination, and serially assessed (24 hours and 8 weeks after experimental FS) the hippocampal volume and metabolites, and cerebral T2 relaxation and DTI. Histological analysis, comprising cellular size and density and fiber density and anisotropy in the hippocampus and amygdala, was performed 9 weeks after FS.

Material and Methods
Animals
Sprague-Dawley rats (Harlan, The Netherlands) were born and housed under standard conditions. Hyperthermia (HT) was induced on PN9. Rat pups were placed in a cylinder, their core temperature raised with an adjustable stream of heated air to 41–42.5°C for 30 minutes. Core temperatures were measured every 2.5 min during the HT treatment. The behavioral seizures were stereotyped as previously shown to correlate with hippocampal EEG discharges. Sixty-seven percent of the rats showed FS behavior after HT (HT+ rats). Littermates were used as normothermia (NT) controls, which were exposed to the same conditions, though the air had room temperature. MRI experiments were performed on a 6.3 Tesla magnet (Oxford Instruments, England) interfaced to a Bruker Biospec console (Bruker, Ettlingen, Germany), using a linear transmit volume coil and a butterfly surface receive coil (Rapid Biomed, Rimpar, Germany). MR was performed at PN10 on 9 NT and 10 HT+ rats, and at PN66 on 9 NT and 9 HT+ rats. Anesthesia was induced with a mixture of 2-4% isoflurane and medicinal air, and maintained with 1-2.5% isoflurane. For anatomical reference, a proton density and T2-weighted multi slice multi spin-echo pulse sequence (MSME) was used, acquiring 15 coronal slices (1 mm with a repetition time (TR) of 4937 ms and echo times (TE) of 12.2 and 128.3 ms (256 x 192 matrix, field of view 4 x 4 cm2, 1 average). Quantitative T2 imaging was performed using an MSME sequence with a TR of 5 s and TE: 17.2, 43.0, 77.3, 111.7, 146.1, and 180.4 ms (15 coronal slices, 1 mm, 128 x 128 matrix, field of view 4 x 4 cm2, 2 averages). T2 relaxation times were calculated on a pixel-by-pixel basis using a nonlinear monoexponential fit. For DTI, an echo planar imaging sequence was used, with 30 directions (TR = 3 s, TE = 34 ms, b = 0 and 1000 s/mm2, diffusion gradient duration 4 ms, diffusion gradient strength 239.1 mT/m, 15 coronal slices, 1 mm, 128x128 matrix, field of view 4 x 4 cm2, 2 averages). The apparent diffusion coefficient (ADC, unit 10-6 mm²/s) and fractional anisotropy (FA, unit %) maps were calculated on a voxel-by-voxel basis. Single-voxel proton spectroscopy was applied to a 5x4x2 mm3 (0.04 ml) voxel mainly covering the hippocampus. (PRESS, TE 14 ms, TR 10 s, 256 averages, spectral bandwidth 4006 Hz, and number of points 1977, water suppression CHESS) Metabolic concentrations were expressed in mmol/l, using the water reference signal (16 averages) [4]. Hippocampal volumetry was performed using the TE = 128.3 ms MSME image. Bilateral regions of interest (ROI) were manually drawn on a T2-weighted image in the thalamus, hippocampus, retrosplenial cortex, amygdala, piriform cortex, and corpus callosum. The mean T2, diffusion ADC and FA values were calculated for the selected structures. Multiple end point testing was controlled for by first combining T2, ADC, and FA MRI outcomes (all effected by water changes) per region. For each region, differences between the two patient groups for the combined MRI measures were tested using the ordinary least squares test of O-Brien and Läuter [5]. Statistical significance was calculated with two-tailed Student’s t tests with Hochberg correction for multiple comparisons. Histology At PN73 each rat (NT, n=9; HT+, n=9) was perfused with 4% paraformaldehyde under pentobarbital anesthesia. For staining were used, Nissl, Black-Gold II and glial fibrillary acidic protein (GFAP). Hippocampal volume, amygdalar cell and hippocampal granule cell volume and density were studied using the Nissl stained sections, and astrocyte density in the amygdala using the GFAP stained sections. Black-Gold stained sections were used for analysis of fiber density and directionality within the hippocampal granule cell layer (GCL).

Results
MRI
The ordinary least squares test revealed significant HT-induced MR alterations at PN10 for the retrosplenial cortex, corpus callosum, amygdala, and piriform cortex and at PN66 for the hippocampus. There were no significant statistical differences in hippocampal volumes on T2-weighted images between the two groups at both time points. At PN10, HT+ rats had elevated T2 relaxation values in the hippocampus (+5%, p<0.05) (Fig 1AB). At PN66, no differences were found in T2 relaxation times. ADC values were significantly decreased at PN10 (-10%) in the amygdala and piriform cortex (p<0.05). At PN66 ADC values were decreased in the amygdala (p<0.05) (Fig 1CD). At PN66 FA values were higher (+30%) in the thalamus (p<0.05). No changes observed in the concentrations of the neurotransmitters glutamate and GABA. A significantly higher concentration of tCr (+ 7%) was observed at PN66 for the HT+ rats (p<0.05). Histology Relative volume occupied by the cells in the amygdala was decreased after FS (p<0.05). In HT+ animals, a higher percentage of the GCL was occupied by stained fibers (+50%, p<0.05). Also, the directionality was significantly higher in the GCL (+23%, p<0.05) (Fig 2).

Discussion
Elevated T2 relaxation times concur with earlier findings by Dubé et al [6], who reported hyperintense T2-weighted images at 24 hours and 8 days after FS. However, no differences in hippocampal volume were found in the present study. Dubé et al also showed that HT-induced FS can result in transient cell damage without evolving into cell loss. This might explain that acute T2 signal changes resolve over time without causing persisting neuronal damage. To interpret the cellular changes underlying the long-term MR results (PN66), histology was performed. Analyses of cell volume and density revealed no changes. Microscopic fiber density and anisotropy in the GCL was increased, suggesting mossy fiber sprouting as previously observed by Bender et al [7]. Although histology provided evidence of microstructural changes that are linked to the long-term MR diffusion abnormalities detected, correct interpretation of diffusion results remains difficult.

Conclusion
Using non-invasive MRI, it was shown that early-life HT-induced FS give rise to both transient and foremost chronic micro-structural changes to the limbic system (hippocampus and amygdala). The long-term findings support the concept that early-life FS are related to epilepsy. Whether the detected MRI and histological abnormalities are specific for epileptogenesis or are the consequence of epileptiform activity remains to be elucidated.

References