Neuronal Tract Tracing By Manganese Enhanced MRI (MEMRI) In Kainate Model of Epilepsy

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Introduction

Mn^{2+} is a paramagnetic ion shortening the proton spin-lattice relaxation (T_1) time. Mn^{2+} mimics Ca^{2+} ion in regard ion channels and it is taken up by living neurons and it is transported both anterogradely and retrogradely by axons as well as across synapses [1]. Thus, MRI signal enhancement by Mn^{2+} reflects diffusion in the vicinity of the injection site as well as functional properties of anatomical neuronal network connections. In the present study we have used MEMRI to assess the neuronal connections in epilepsy model. Mechanisms of epileptogenesis are still poorly understood [2] and it is anticipated that MEMRI would allow exploring the possible role of changed connections in hippocampus and perforant pathway as a disease promoting factor after status epilepticus (STE).

Methods

We used kainate model of epilepsy. Briefly, STE was induced to 5 adult male Wistars with an intraperitoneal injection of kainic acid (11mg/kg). Control rats (n=6) received saline injections. Rats were monitored 4 hours to ascertain that they reached STE criteria. Two weeks later rats (weight = 290±26g) received intracerebral injections of 30-50nl of 1M manganese chloride into entorhinal cortex. Coordinates of injection site (according to the Paxinos atlas of rat brain [3]) were: -8.3mm from bregma, -4.0mm from sagittal suture and -5.3mm from brain surface. MRI data were acquired 3, 5, 7 and 10 days after surgery, at 4.7 T using a Varian ^{UNITY}INOVA console. Animals were anesthetized with 1.0% halothane in N_2O/O_2 during all the experiments. Standard T_1-weighted (TE=2.7 ms, TR=120 ms) 3D gradient echo -imaging was performed using an adiabatic 70° BIR-4 excitation pulse to minimise flip angle -dependent contrast variation due to inhomogeneity of the transmitter/receiver quadrature surface-coil used. Volume of 25x 25*35 mm was covered with 192*64*256 points, with 2 averages leading to total acquisition time of 49 minutes/animal. Signal intensities of the regions of the interests were measured and normalised with adjacent muscle tissue.

Results

All animals that experienced STE developed spontaneous seizures 2-3 weeks later. Significant decrease in MRI slice-volumes of amygdala and enlargement of ventricles was detected bilaterally in epileptic animals ($p \leq 0.01$). Enhanced T_1 signal in the hippocampus and thalamus ipsilateral to injection site were found. Control animals showed increased MEMRI signal in all subfields of hippocampus, including granular cell layer ($p \leq 0.05$). Epileptic rats had always stronger signal in hilus, CA3 and CA1 (but not CA2) regions of hippocampus, probably due to mossy fiber sprouting [4]. Epileptic animals had also stronger MEMRI signal than controls in a dorsal thalamus. Additionally, in ipsilateral thalamus the signal enhancement was more pronounced in both animal groups ($p \leq 0.05$).

Conclusions

Trans-neuronal tracers may be used to detect activity-induced changes of connectional networks in epilepsy like mossy fiber pathways reorganization in hippocampus which is characteristic feature of epilepsy.

References

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Fig.1: Epileptic rat (#1) shows severe atrophy in temporal lobe structures and enlargement of ventricles. Hippocampal regions CA3 and CA1 are strongly enhanced, but CA2 shows only modest T_1-signal enhancement in the ipsilateral side than in contralateral side. MRI of control rat (#10) shows no signs of brain damage.