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Magnetic Resonance Imaging of the Rat Brain after Epileptic Seizures – Preliminary Results

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Abstract: The magnetic resonance imaging (MRI) of the rat brain after the epilepsy seizures has been performed. As a first step, the model of the kainic acid (KA) induced seizures has been conducted to examine the possibilities of magnetic resonance imaging system kept in disposition. Seven Wistar albino rats, weighing about 300 g, were used in this study. We administered six of them with intraperitoneal injection of 10 mg/kg of KA. The control animal received corresponding volume of the saline. Every animal was examined under systemic anaesthesia induced by an intraperitoneal injection of thiopental sodium approximately 15 minutes before scanning. Diffusion-weighted imaging (DWI) has been used to acquire the coronary scans of the rat brain. The progress of hyper intense signal at the cerebral cortex and amygdale has been observed. Marked asymmetry of the signal intensity between hemispheres has been discovered. Subsequently the experimental model of audiogenic epilepsy will be conducted.

Introduction

Many animal models have been established to study the mechanism of epilepsy. The kainic acid (KA) induced seizures are one of the common models. KA is a cyclic analogue of glutamate that depolarises both pre- and postsynaptic cells by interaction with the non – NMDA type of glutamate receptor [1]. The kainic acid model reflects neuropathological changes seen in human temporal lobe epilepsy and allows investigating neuropathological changes induced by a status epilepticus and taking place during development of epilepsy [2].

The magnetic resonance imaging (MRI) is a powerful technique that enables sequential visualization of structural and functional changes occurring in living animals. This makes it possible to uncover the contribution of such changes to the development of epilepsy [3]. MRI has proven to be an effective non-invasive technique for identifying lesions in patients with temporal lobe epilepsy. It also has been suggested to be sensitive to transient functional or metabolic changes in brain tissue [4]. The imaging diagnosis in epilepsy is a non-invasive method for locating the epileptic focus and visualizing the potential epileptic brain damage.

It has been shown, that the diffusion-weighted images (DWI) showed improved contrast for oedematous tissue [5]. The diffusion of water molecules in vivo is limited by various tissue structures. DWI has been shown as a useful method to reflect conditions of brain oedema. The pathophysiological mechanism of the epileptic focus has been strongly believed to be an excitotoxicity theory. The glutamate and its analogues, especially kainic acid, induce excessive excitation of neurons that leads to cell death. The acute cytopathology resembles the excitotoxic type of damage induced by glutamate, which is acute swelling of dendrites and vacuolar degeneration of neuronal soma. Two types of glutamate neurotoxicity were recognized. The first type occurs quickly after glutamate stimulation and probably is the result of ionic fluxes that disrupt cell membranes. The second type occurs more slowly and may be related to lethal Ca^{2+} influx.

Prolonged glutamate application could permit an abnormally large Ca^{2+} influx into the neuron. Sufficient Ca^{2+} accumulation within the cytoplasm is known to be toxic [6]. This theory is thought to be a common mechanism for ischemia, anoxia and hypoglycaemia. Therefore, DWI, which is able to capture the changes in water diffusion, is expected to locate the epileptic focus [7].

The noise belongs to the important etiological agents that may cause different pathological changes. Broad issue of such influence is well known since 1960 [8]. This topic becomes more important in the time of noisy cities and loud music productions. Long-lasting influence of noise has been demonstrated as a causative agent in disorders in the nervous system and psyche. Sound intensity over 60–65 dB Sound Pressure Level (SPL) intrudes vegetative system, over 90 dB SPL hearing function, and sound over 120 dB SPL may cause destruction of the cells and tissues.

Human ear is mostly sensitive at the frequencies between 1000–4000 Hz, out of this range its sensitivity gradually decreases and the threshold of audibility is from 16 Hz to 20 kHz. The effects of the noise to the auditory system occur mainly after long time exposition, even after several years, when the alteration of tissues is irreversible. According to systemic impacts, the noise causes alterations in central nervous system and system circulation [9, 10]. The most influent are the sudden and unexpected sounds, when the typical stress reaction has been found.

Epilepsy is a phenomenon causing severe and continuous seizure activity such as that present in status epilepticus, or chronic spontaneous recurrent seizures. Clinically, the epilepsies are characterised by spontaneous recurrent epileptic seizures, which are caused by generalised paroxysmal or partial (focal) discharges in the brain. Epileptic seizures may be evoked by many different stimuli, for example light or sound. Audiogenic epilepsy in rats is one of common experimental model of convulsive epileptic seizures prepared with animals [11]. Genetically selected strains of rats susceptible to audiogenic seizures can be used for the experimental model of audiogenic seizures, the other way is to apply epileptic drug, such as kainic acid, that induces generalized seizures in rats, aggravated by sound stimulation [12]. Another possibility is to use animals after acoustic kindling. Primary sound – insensitive animals may be transformed by daily repeated stimulation. This repeated stimulation provokes modification in seizure phenotype.

Audiogenic seizure is the generalized tonic-clonic seizure evoked by sound stimulation. Repetitive high intensity (110 dB SPL) sound stimulation induces a forebrain-kindling phenomenon in animals predisposed to sound induced seizures. Wistar audiogenic rats (WARs) have been reported to develop a mixed brainstem-limbic seizure pattern, after more than five to ten stimuli. Besides the original brainstem wild running and tonic-clonic seizures, new behavioural patterns appear resembling those of electrical amygdala kindling [13].

Important fact is that rat's hearing is immature before 3rd week of life. Acoustic kindling has to begin in this critical period, when the external auditory meatus is opened and the ear is irritable to excessive sound stimulation. Wistar rats are the

most susceptible to acoustic kindling 14th and 15th postnatal day [14]. The behaviour during seizures can be scaled according to Racine [15] scale (classes 0 to 5) of limbic seizures as follows:

0 = immobility, 1 = facial automatisms, 2 = head nodding, 3 = unilateral forelimb clonus/bilateral forelimb clonus, 4 = bilateral forelimb clonus and rearing, and 5 = rearing, falling and generalized convulsions.

The MRI allows us to investigate the localization and the development of the pathological changes in the rat brain after epileptic seizures. Our aim is to characterise both the metabolic derangement and brain injury associated with seizures, using functional (diffusion weighted) and structural (T_1, T_2) neuroimaging.

Materials and Methods

We perform the MR imaging by the nuclear magnetic resonance SISCO 85/310 system (Figure 1) provided by the Joint laboratories of biomedical engineering Czech Technical University and Charles University in Prague. The MRI system is dedicated for experimental imaging on small mammals. The system specification: superconducting coil 2 Tesla (85 MHz for 1 H), gentry diameter 20 cm, gradient strength 70 mT/m.

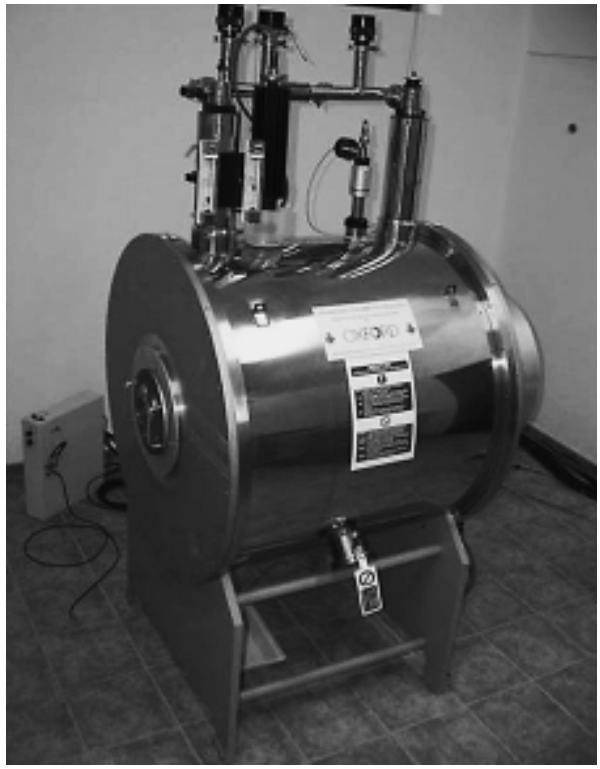

Figure 1 – The nuclear magnetic resonance SISCO 85/310 system.

Seven male Wistar albino rats, weighing about 300 g, were used in this study. Six of them were treated with intraperitoneal injection of 10 mg/kg of the kainic acid. The seventh one, the control animal, acquired intraperitoneal injection of the corresponding volume of the saline. Within 30 minutes after the injection appeared in the first group seizure symptoms like facial twitching, head nodding, wet-dog shakes, instability.

We examined every animal under systemic anaesthesia induced by an intraperitoneal injection of thiopental sodium approximately 15 minutes before scanning. We scanned the control animal one hour after saline injection. Subsequently we acquired the first pictures 60 minutes after intraperitoneal injection of KA on animal one, then we scanned the second animal 4 hours after injection, the images of third animal were carried out 7 hours after KA injection, the other images were acquired after 25 hours, 31 hours, and 48 hours. We evaluated the coronary slices at the –1 mm to –1.5 mm from zero coordinate, the bregma point. The signal intensity as a mark for the affected oedematous tissue, the symmetry between hemispheres and the time progression of the changes had been assessed.

Results

The evaluation of the coronary scans:

1. The control animal: there was no signal abnormalities in the image of the animal treated with saline (Figure 2a).
2. One hour after KA administration: enhancement of the signal in the area of piriform cortex and amygdale bilaterally (Figure 2b).
3. Four hours after KA administration: results very similar to the previous ones (Figure 2c).
4. Seven hours after KA administration: hyper intense signal in the area of amygdale, almost whole piriform cortex is affected; the results are similar from both hemispheres (Figure 2d).
5. Twenty-five hours after KA administration: after 18 hours from the last acquisition we can see reduction of signal intensity in the left hemisphere, on the other hand, the right amygdale and piriform cortex is still affected, the hyper intense signal occurs in the area of somatosensory cortex (Figure 2e).
6. Thirty-one hours after KA administration: similar results as on the image acquired after 25 hours, with reduction of signal intensity in the area of the amygdale (Figure 2f).
7. Forty-eight hours after KA administration: obvious asymmetry between hemispheres, infliction of the piriform cortex persist in the left hemisphere, almost without signal abnormalities in the area of amygdale, in the right hemisphere persist hyper intense signal in the area of amygdale, piriform cortex and high intense signal from the somatosensory cortex (Figure 2g).

The image of the control animal (Figure 2a) was matched to the images of the animals treated with KA. We recognized the progress of hyper intense signal at the cerebral cortex and amygdala. In addition, as can be seen on Figures 2b–2g, there were obvious asymmetry of the signal intensity between the hemispheres of the scanned animals. The hyper intense areas on our slices were the ectorhinal and temporal cortex. The asymmetry occurred mainly 7 hours after KA administration and later. The hemisphere with the hyper intense signal was repeatedly the right hemisphere.

Discussion

Our ambition was to acquire and analyse the magnetic resonance images (MRI) of the rat brain after the epilepsy seizures, as well as to investigate the possibilities of

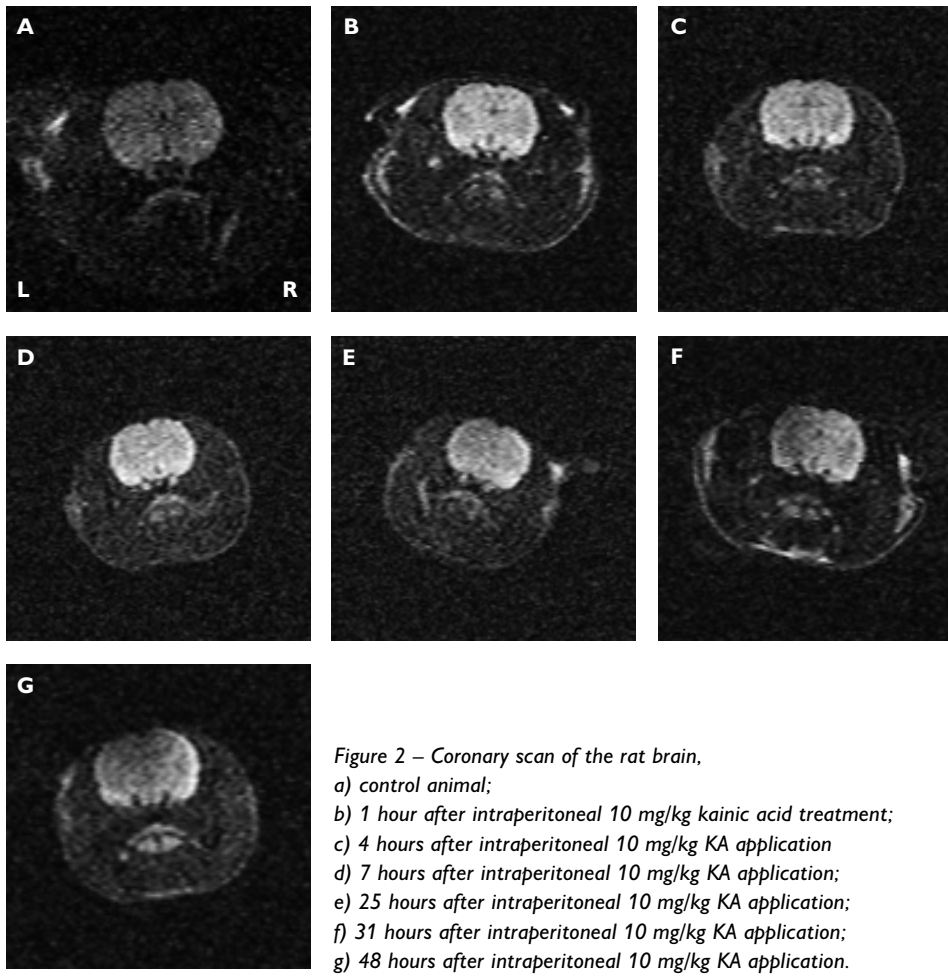

Figure 2 – Coronary scan of the rat brain,
a) control animal;
b) 1 hour after intraperitoneal 10 mg/kg kainic acid treatment;
c) 4 hours after intraperitoneal 10 mg/kg KA application
d) 7 hours after intraperitoneal 10 mg/kg KA application;
e) 25 hours after intraperitoneal 10 mg/kg KA application;
f) 31 hours after intraperitoneal 10 mg/kg KA application;
g) 48 hours after intraperitoneal 10 mg/kg KA application.

the MRI system we have in disposition. We decided to acquire coronary images of the central part of rat brain after intraperitoneal KA administration as an example of epilepsy seizures and status epilepticus in adult Wistar albino rats. We decided to use diffusion-weighted MRI as suitable method to investigate functional changes in the brain tissue during early period after KA treatment.

The interpretation of such findings is not clear. According to systemic application of the KA, there were no differences in the accessibility of the drug between the hemispheres. These findings reflect transient cytotoxic and vasogenic oedema induced by the seizures. The asymmetry in oedema may result from the different viability and responsibility of the affected cells to the excitotoxic acting of the KA. The asymmetrical behavioural response in the rat after kainic acid injection into to

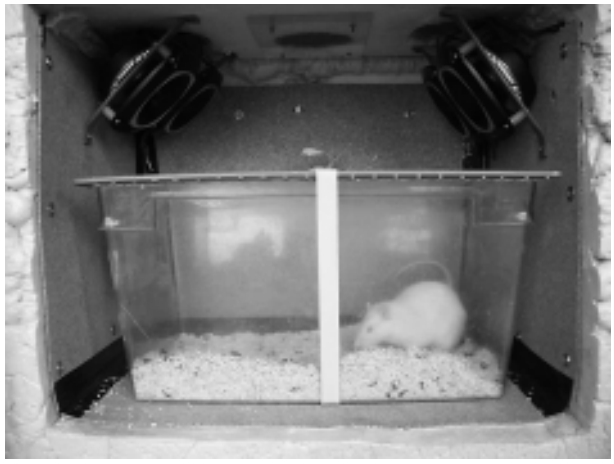

Figure 3 – The stimulation chamber.

Figure 4 – The Le Son STN-400 tweeter.

the frontal cortex was described [16]. The kainic acid injected into the right frontal cortex of rats produced a significantly greater spontaneous hyperactivity than identical injections into the left hemisphere.

We attested the possibilities of the MRI system. We would like to continue in the use of the MRI in the research of acoustic evoked seizures. In our onward research, we would like to conduct the model of audiogenic epilepsy. Audiogenic seizures are a model of generalized tonic–clonic seizures. We will induce the seizures susceptibility by the audiogenic kindling protocols. The Wistar rats will be used in the model of reflex epilepsy with seizures induced by daily high-intensity sound stimulation (110 dB SPL). The acoustic kindling will take place in stimulation chamber (Figure 3) with available video recording system and possibility to change the frequency and the intensity of the applying sound. The chamber is equipped with the Le Son STN-400 Tweeter (Figure 4). We will scale the behaviour of the animals during and after the seizures according to Racine scale. We will evaluate the pathological changes in the central nervous system; our region of interest includes hippocampus and auditory cortex, with magnetic resonance imaging and histochemical methods

Our plan is to conduct Wistar rats and their sensitivity to sound stimulation and compare the results with stimulation of the other accessible rat inbred strains.

Consequently, we will evaluate the pathological processes presented in limbic structures and auditory cortex. In the first instance, we will perform brain MR imaging on such treated animals. Simultaneously, we will evaluate the processes in the region of interest, hippocampus and auditory cortex, using Hoechst staining, Fluoro Jade-B staining, NADPH – diaphorase measurement.

Our aim is to analyze morphological changes of the nervous tissue after repeated sound stimulation conducting into epileptic seizures of the experimental animals, evaluate its behaviour and compare the results of the DWI with the results of histochemical analysis.

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