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Epileptic rat brain tissue analyzed by 2D correlation Raman spectroscopy

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ABSTRACT

Absence epilepsy is the neurological disorder characterized by the pathological spike-and wave discharges present in the electroencephalogram, accompanying a sudden loss of consciousness. Experiments were performed on brain slices obtained from young male WAG/Rij rats (2–3 weeks old), so that they were sampled before the appearance of brain-damaging seizures symptoms. Two differing brain areas of the rats' brain tissue were studied: the somatosensory cortex (Sc) and the dorsal lateral geniculate nucleus of the thalamus (DLG). The Raman spectra of the fresh brain scraps, kept during measurements in artificial cerebrospinal fluid, were collected using as an excitation source 442 nm, 514.5 nm, 785 nm and 1064 nm laser line. The average spectra were analyzed by 2D correlation method regarding laser line as an external perturbation. In 2D synchronous spectra positive auto-peaks corresponding to the C═C stretching and amide I band vibrations show maxima at 1660 cm^{-1} and 1662 cm^{-1} for Sc and DLG, respectively. The prominent auto-peak at 2937 cm^{-1}, originated from the CH_3 mode in DLG brain area, seems to indicate the importance of methylation, considered to be significant in epileptogenesis. Synchronous and asynchronous correlations peaks, glutamic acid and gamma-aminobutyric acid (GABA), appear in Sc and DLG, respectively. In the 1730–1600 cm^{-1} range occur cross-peaks which appearance might be triggered by glial fibrillary acidic protein (GFAP) activation.

1. Introduction

Animal modeling has directed to the understanding of pathogenesis and therapy of various central nervous system disorders [1]. Actually this seems to be most effective method of research in this field. The rat brain is composed of over a thousand functionally and anatomically independent structures and everyone is highly specialized [2–3]. Examination of the brain is a difficult task as all of the brain subdivisions only slightly differ from each other in biochemical composition [3–7]. Epilepsy is a common neurological disorder characterized by recurrent und unpredictable seizures that manifest as a general convulsion or as a short loss of consciousness. This behaviour is caused by hypersynchronous or abnormally excessive activity of neuronal networks in the brain [8]. Local imbalance between excitation and inhibition in the neuronal network within the central nervous system may be caused by mutations at the level of single molecule. WAG/Rij rats are well-validated genetic animal model of absence epilepsy, which main characteristic are a sudden loss of consciousness accompanied by generalized, synchronous changes in electroencephalogram (EEG) [8–10]. The pathomechanism of absence epilepsy it still not fully understood. Previous studies indicate the differences in electrophysiological functioning of lateral geniculate complex as well as the somatosensory cortex – areas in the brain where changes are the presumable cause of absence seizures in WAG/Rij rats [11–15].

The glial fibrillary acidic protein (GFAP) is the principal 8–9 nm intermediate filament in mature astrocytes of the central nervous system and plays an important role in modulating astrocyte motility and shape by providing structural stability to astrocytic processes [16]. The GFAP is expressed in a variety of pathologies associated with epilepsy in some parts of the brain, which may be relevant to many of the cytoarchitectural and developmental aberrations observed in patient with focal epilepsy [17]. Dutuit et al. have studied the genetic absence epilepsy rats from Strasbourg (GAERS) in which spontaneous absence seizures appear in the cortex and thalamus after one month of life. The

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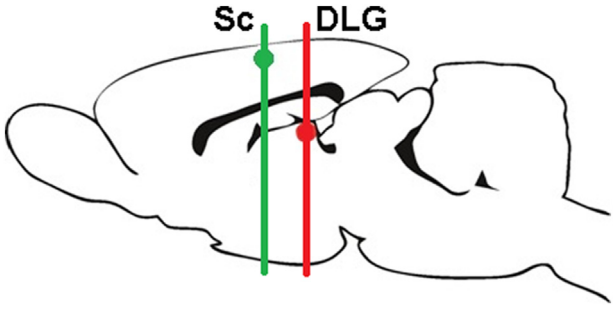

Fig. 1. Scheme of the rat's brain with marked areas of investigated brain structures: (A) the somatosensory cortex (Sc), (B) the dorsal lateral geniculate nucleus of the thalamus (DLG); marked areas which submitted the appropriate slices.

increase of GFAP expression in those brain structures appears in both adult and young GAERS, what indicate that reactive astrocytes are already present before the development of epileptic symptoms [18].

In current study two different brain structures from epileptic rats brain were investigated and compared with our previous studies, of healthy Wistar rats' brain tissue [19]. The first structure, the dorsal lateral geniculate nucleus (DLG), is located in the thalamus and is a crucial neuronal structure of the visual pathway, involved in conveying photic cues from the retina to the visual cortex [2–3,20–21]. The DLG is a collection of nuclei in the diencephalon with neurons that project to neocortex [20–21]. The cytoarchitecture of DLG in rats is rather homogeneous, but comparing to other mammalian species it is not clearly laminated [21]. The second structure of interest is the somatosensory cortex (Sc), which is a complex cortical area. Sc consists of a topographic map of the body and is responsible for the sense of touch, as here the sensory information from the entire body is processed [2,22–25]. Somatosensory cortex consists of three components: granular, septal and dysgranular, which are functionally distinct and they have different connections with different response properties of neurons [25]. The DLG and Sc structures are widely investigated as a model of absence epilepsy [26–32].

Raman spectroscopy is widely used as noninvasive diagnostic method which can detect small changes at the molecular level at early stages of disease, e.g. in the diagnosis of cancer, as well as in the monitoring of therapy [33–43]. This analysis can be performed directly on a tissue, without the additional sample preparation, what allow to apply this technique for the examination of biological materials. Raman spectra almost as fingerprint provide information about the molecular composition, molecular structures and molecular interactions in tissues [33–43].

The aim of this work was to verify if Raman spectroscopy can be used to detect brain abnormalities responsible for epilepsy at the molecular level. Predisposition for seizures resulted from genetic disturbances, that can be derived from animal model, is analyzed. This research is aimed at finding changes in the individual parts of epileptic rat's brain tissue that can be resolved by 2D correlation Raman spectroscopy.

2. Experimental

2.1. Samples

To eliminate the impact of nonspecific tissue changes due to the electrical discharges during the epileptic activity, all of the measurements were performed on the young brain tissue, before the appearance of

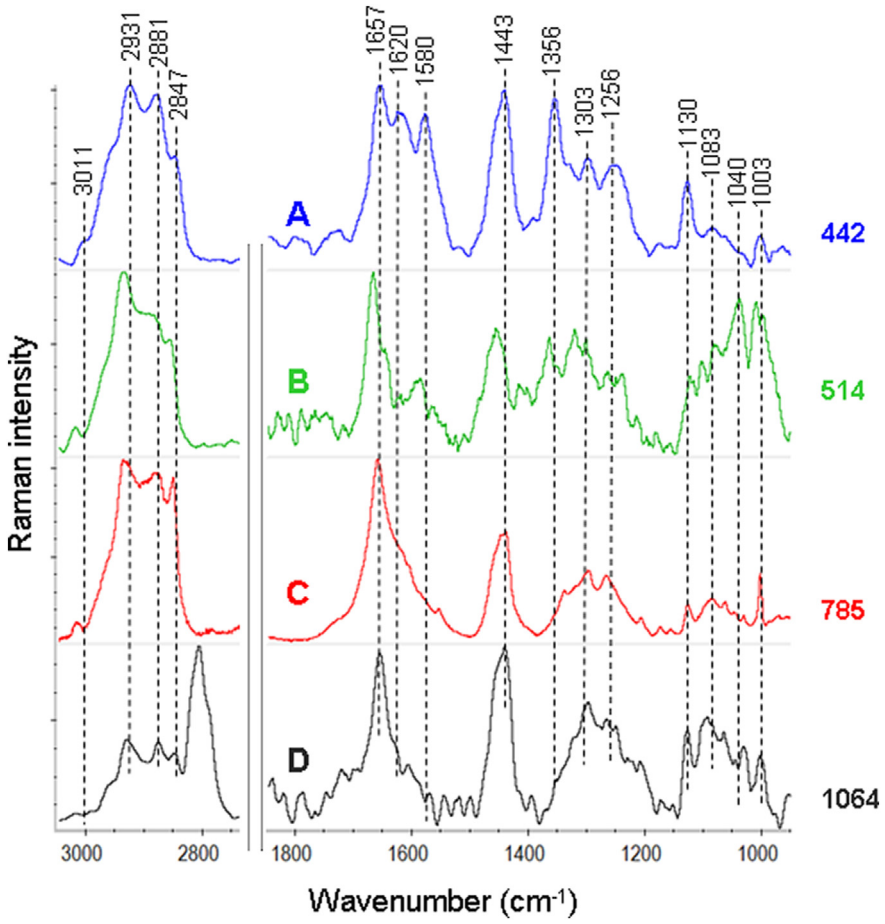

Fig. 2. The averaged Raman spectra for the somatosensory cortex (Sc) excited with: (A) 442 nm; (B) 514 nm; (C) 785 nm and (D) 1064 nm laser line in the ranges of 3050–2750 cm^{-1} and 1850–950 cm^{-1} (intensity (A) ×5,0; (B) ×5,2; (C) ×0.4; (D) ×3,4, in comparison to the appropriate intensities in 3050–2750 cm^{-1} range).