Vibrational Spectroscopy 85 (2016) 48–54

Contents lists available at ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

The application of Raman microspectroscopy for the study of healthy rat brain tissue☆

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ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form 22 February 2016
Accepted 22 March 2016
Available online 23 March 2016

Keywords:
Rat brain tissue
Raman microspectroscopy
Somatosensory cortex (Sc)
Dorsal lateral geniculate nucleus of the thalamus (DLG)
Cerebellar cortex (Cc)
Principal component analysis (PCA)

ABSTRACT

The Raman spectra of three areas of Wistar rat brain tissue: the somatosensory cortex (Sc), the dorsal lateral geniculate nucleus of the thalamus (DLG) and the cerebellar cortex (Cc), excited with 785 nm and 442 nm laser line, were obtained. The no fixation method to arrange the samples was adopted, and fresh brain scraps were kept in artificial cerebrospinal fluid during the measurement. The CH stretching vibrations region was used to probe the state of the lipids and their arrangement in the studied brain areas. The obtained spectra were discussed by analyzing differences in particular spectral regions by evaluating changes in band intensity ratios as well as using principal component analysis (PCA). The I_{2880}/I_{2850} intensity ratio of the model unsaturated fatty acids, linoleic (LA) and the docosahexaenoic acid (DHA) were treated as a model of lipid composition and used for a comparison because of their similarity. The I_{2880}/I_{2850} intensity ratio allowed us to investigate the differences in the microenvironment of the studied areas of the brain. The I_{2930}/I_{2880} intensity ratio showed the specificity of the lipids disorder because they are a mixture of several hydrocarbon chains of different acyl region compositions. These differences were consistent with the lipid molecular architecture in the studied regions as shown in the PCA loadings plot. Some protein also seemed to differentiate the explored areas of the brain. The obtained results demonstrate that Raman spectroscopy can provide insight into healthy brain tissue composition and structure.

1. Introduction

The nervous system enables quick and accurate communication between distant areas of the body thanks to specialized cell functions. Neurons receive and process information and the response is to send the right signals. However, functional characteristics result more from the existence of the network of connections between neurons than the specificity of the construction of the individual cells, making it difficult to observe tissue differentiation [1].

Although available animal brain models do not exactly represent the biology of the human brain, they can offer significant insight into the understanding of the cellular and molecular mechanisms involved in the functioning of a healthy (human) brain. Research on animal brains may provide insight into many features of human brain disease and is employed to test new theories and therapies [2–5].

Vibrational spectroscopy techniques were made use of for the spectral analysis of the samples of the brain tissue [5–10]. The greatest amount of research seems to be dedicated to cancerous disease cases and to the determination of fine differences between normal and cancerous samples [5,7,11–13]. For this purpose spectroscopy and chemometric based analysis of tumour detection and classification of tissue samples were used [13,14].

One of the advantages of the Raman spectroscopic method is its non-destructive nature, combined with its capability to directly probe biochemical differences without the addition of stains or contrast agents, allowing for the acquisition of biochemical and structural data composed of complex materials [7,15].

☆ Selected paper from 8th International Conference on Advanced Vibrational Spectroscopy, 12-17 July 2015, Vienna, Austria.

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http://dx.doi.org/10.1016/j.vibspec.2016.03.017
0924-2031/

The aim of this study is to determine what changes in the individual parts of rat brain tissue, detained in artificial cerebrospinal fluid and with label-free identification, can be attained. The three areas of the brain: the somatosensory cortex (Sc), the dorsal lateral geniculate nucleus of the thalamus (DLG) and the cerebellar cortex (Cc) were specified. DLG is known to be a thalamic relay station of the visual pathway, conveying photic cues from the retina to the visual cortex. Sc is a complex cortical area processing touch information from the whole body, while Cc is involved in motor control.

2. Experimental

2.1. Samples

In order to obtain living brain tissue, acute brain slices were prepared according to the developed method [16,17]. Animals were held in a 12 h/12 h light/dark condition (light on 8.00 am, light off 8.00 pm) with water and food ad libitum in the Jagiellonian University Animal Facility. Male Wistar rats (14–18 days old) were decapitated between 1 and 2 h Zeitgaber Time (ZT, 9.00–10.00 am). The block of brain tissue was quickly removed from the skull and immersed in ice-cold artificial cerebrospinal fluid (ACSF). Then, the tissue was placed on a cold plate of a vibroslicer (Leica VT1000S, Heidelberg, Germany) and coronal slices of 300 μm in thickness were cut. Further procedures were performed on the slices containing three different neuronal structures: the dorsal lateral geniculate nucleus of the somatosensory cortex (Sc), the thalamus (DLG), and the cerebellar cortex (Cc), 3.48 mm, 4.08 mm and 13.76 mm from the bregma point on the rat skull, respectively [18] (Fig. 1).

The reference samples of the fatty acids, linoleic acid (LA) and *Cis*-4,7,10,13,16,19-Docosahexaenoic acid (DHA) and lipids, 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol were purchased from Sigma-Aldrich (Poland).

2.2. Raman microspectroscopy

The Raman spectra were obtained using a Renishaw inVia spectrometer equipped with a Leica microscope. The StreamLine fast Raman imaging technique with a water immersive objective (×60, NA = 0.75) was used to characterize the brain tissue samples while a 785 nm excitation laser source was used. StreamLine uses a laser line instead of a laser spot to collect imaging data. Spreading the laser power over a line reduces the laser power density on the sample, thus preventing laser induced modification, reducing fluorescence and allowing for higher total laser powers to be used. This capability enabled the imaging of large brain tissue areas with high speed and resolution. The scattered light was dispersed by a diffraction grating with 1200 grooves/mm and 2400 grooves/mm for the 785 nm and 442 nm laser, respectively. The signal was recorded using a Peltier-cooled charged coupled device (CCD). Spectral data were processed with Renishaw Wire 3.2 software and spectra were recorded in the reflection mode from the surface in the 3300–200 cm^{-1} region with an acquisition time of 10 s and a collection of six accumulations for the 785 nm and four for the 442 nm laser, respectively. Each sample was measured three times in different spots to check its homogeneity. The laser power applied while recording the Raman spectra was 10% of its maximum power (3–5 mW).

Ten animals were tested, the current paper presents the results of three recently studied Wistar rat brain tissue samples. No fixation method to prepare the samples was used and fresh brain scraps were kept in artificial cerebrospinal fluid while measurements took place.

2.3. Principal component analysis

Principal component analysis (PCA) allows for the determination of the variance between the Raman spectra recorded from each of the brain slices with a 785 nm laser line using Unscrambler X software packages (v. 10.3, CAMO Software, Oslo, Norway). The Raman spectra were smoothed using a Savitzky–Golay smoothing algorithm (thirteen smoothing points), baseline corrected and unit vector normalized.

The first principal component contains the highest percentage of variation, which indicates that PC-1 is associated with the direction of the maximum variation in the dataset.

3. Results and discussion

3.1. Some aspects of cellular building blocks of the studied brain regions

The mammalian brain is known to be one of the most complicated biological structures ever known. Over a thousand brain structures have been identified in the rat brain [19], which comprise an excellent model for neuroscience research. The functional and structural distinctness of various brain parts stems from both phylogeny (the process of the nervous system evolution) and ontogeny. That is why, in our study we aimed to choose three different brain regions as a small sample from the overwhelming

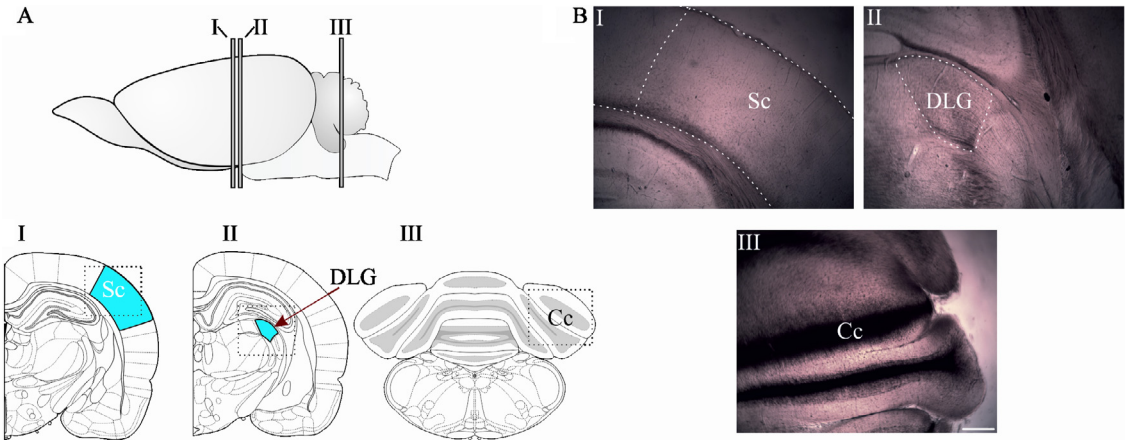

Fig. 1. Coronal slices from three different brain structures: (I) the somatosensory cortex (Sc), (II) the dorsal lateral geniculate nucleus of the thalamus (DLG), and the (III) cerebellar cortex (Cc). Schemes adapted from the Paxinos and Watson atlas of the rat brain [17]. Photomicrographs of corresponding tissue slices (white bar: 100 μm).