RAMAN spectroscopy analysis of primary cultured neurons from epileptic rat brain

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Our understanding of epilepsy pathogenesis has been challenged by a need to separate 'lesions' causing epilepsy from 'lesions' produced by epilepsy. Several experimental evidences demonstrated that seizures might cause alterations in cell number, cell shape and organization of neuronal circuitry, thus setting up a likely identifiable seizure-genic focus. In this study, we have focalized our attention on molecular and biochemical changes occurring within single cortical adult neuron during chemically-induced epileptogenesis. To address this aim, we have isolated adult neurons from Wistar Rats following Brewer Protocols, (Nature Protocols 2007).

This protocol has produced yields of thousands of adult neurons collected from rat cortices of 1-month-old Wistar rats. Before sacrifice and brain sampling, animals were treated according to the pilocarpine-status epilepticus (SE) model; control rats received the same doses of all chemicals with the exception of pilocarpine (Russo et al.,2012 Neuroscience).

Brain samples were collected 24h after SE. Adult neurons isolated and maintained in short-term cell culture (48-96 hr) were submitted to RAMAN spectroscopy analysis.

During the past decade, progress in Raman spectroscopic instrumentation has increased the sensitivity of the measurement to such a degree that the acquisition of high-quality spectral data from biological tissue and cells is possible. The greatest benefit of this technique lies in its high sensitivity to subtle molecular and biochemical changes, as well as its capability for non-invasive sensing and free-labelling studies. Moreover, Raman spectroscopy provides details of the chemical composition of tissue. It is a non-destructive technique requiring no sample preparation, making it an attractive method for in vivo and in vitro characterization of biological tissues. Raman spectrum provides a molecular fingerprint, and the intensity of the Raman peaks is directly proportional to the concentration of the molecules.

We analysed the Raman spectra of Adult Cortical Neuron (Anti-tubulin III positive) isolated from short-term primary cell culture obtained by processing cortical brain tissue of epileptic and control rats.

The Raman spectroscopy is able to highlight interesting differences in terms of qualitative protein profile within the cytoplasm of Single Cortical Neuron of treated rats. The proteins have showed a preferential a-helix conformation respect to the control (Peak range 1650-1680 cm^{-1}). Moreover the treated cortical neurons are characterized by an increased amount of glutamate (Peak 854-942 cm^{-1}) respect to the neuron untreated characterized by the presence of tryptophan (Peak 1655 cm^{-1}). The Nucleic acid content (DNA/RNA) was overlapped between treated and untreated cortical neuron

Our data is in agreement with the expected increase in glutamate concentration after seizures; furthermore, it surprisingly highlights a high content in tryptophan contents which might have a role in seizure generation and maintenance (Russo et al., 2012 Neuroscience). Finally, changes in protein conformation need further studies to understand whether it is a modification of neurotransmitter receptors or voltage gated ion channels and therefore it might contribute to SE secondary damage, however, it could well be a defensive mechanism. In conclusion, Nucleic acid content is not an early consequence of seizures while protein conformation and neurotransmitters alterations are the major responsible of initial epileptogenic process underlying the development of spontaneous seizures in this animal model of temporal lobe epilepsy (Russo et al.,2012 Neuroscience)