

Immunohistochemical characterization of axon terminals of the adult rat cerebellar cortex

Anna Rizzi, Loredana Lorusso, Giovanni Ciccimarra, Vincenzo Benagiano

Dipartimento di Scienze Mediche di Base, Neuroscienze e Organi di Senso – Università di Bari

Aim of the present study was to immunohistochemically characterize the glutamatergic terminals in the cerebellar cortex. The study was carried out on adult rat, using immunohistochemistry for the vesicular transporters of glutamate, VGLUT1 e VGLUT2, and synaptophysin.

Results. Terminals positive for VGLUT1 e VGLUT2 were observed in the molecular layer (ML) and granular layer (GL). ML. VGLUT1-positive terminals appeared very numerous, homogeneously distributed throughout the layer with a distribution pattern resembling that of the parallel fiber terminals. VGLUT2-positive terminals appeared displaced along distinct spiral lines extending from the deeper part of the layer up to the its superficial part, likely corresponding to synapses between climbing fibers and Purkinje neuron dendrites. No colocalization of VGLUT1 and VGLUT2 was observed in ML. Double labelling for VGLUT1 and synaptophysin and, respectively, VGLUT2 and synaptophysin revealed colocalization, suggesting the axon terminal nature of the immunolabelled elements. Interestingly, some elements appeared synaptophysin positive, but negative for VGLUT1 and VGLUT2.

In GL, VGLUT1 and VGLUT2 positive terminals displayed similar distribution patterns, They appeared clustered in restricted regions of the layer, scattered within granule neurons, at the level of synaptic glomeruli. Most of these terminals showed a colocalization of VGLUT1 and VGLUT2. However a part of them shows positivity for VGLUT2 and negativity for VGLUT1. All the VGLUT1 and VGLUT2 positive elements also displayed positivity for synaptophysin. Finally, like in ML, some elements appeared synaptophysin positive, but negative for VGLUT1 and VGLUT2.

Conclusion. The results indicate that the glutamatergic terminals in the cerebellar cortex may be differentiated combining Immunohistochemistry for VGLUT1 and VGLUT2. Moreover, the results identify subpopulations within terminals of the parallel, climbing and mossy fibers. In particular, a subpopulation of mossy fiber terminals, displaying positivity for VGLUT2 and negativity for VGLUT1, are different from the vast majority of mossy fiber terminals, which display for both VGLUT1 and VGLUT2, but similar to climbing fiber terminals. It is intriguing to hypothesize that these mossy fibers may constitute a contingent of mossy fiber originated in the inferior olivary nuclear complex.

Keywords

Rat cerebellar cortex, climbing fibers, mossy fibers, vesicular glutamate transporters