

LOCALIZATION OF SYNAPSIN AND PSD-95 IN DEVELOPING POSTNATAL RAT CEREBELLAR CORTEX

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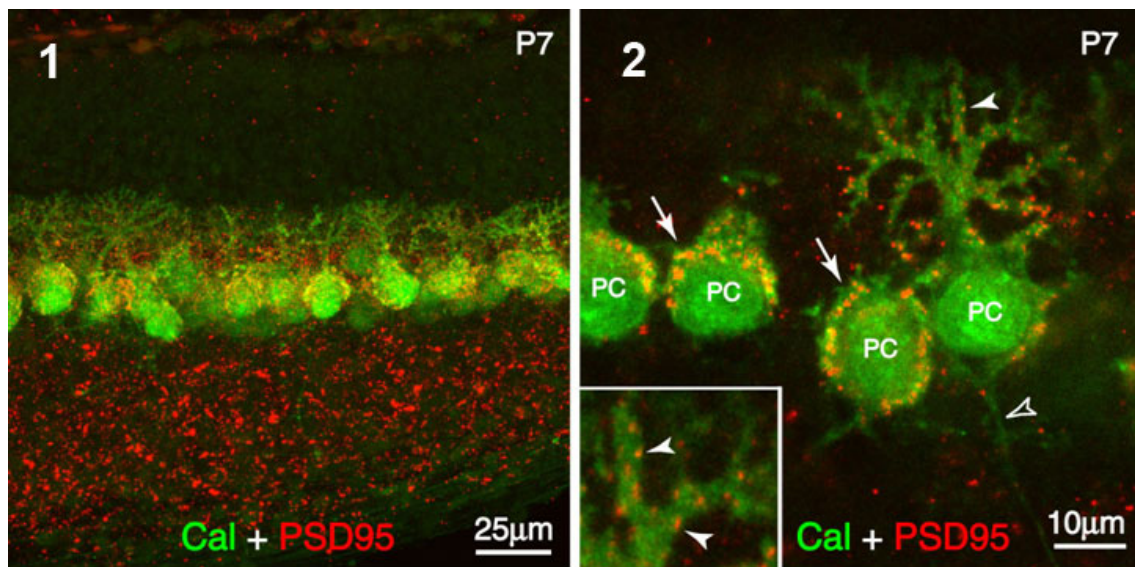
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Synapses in the CNS are highly specialized sites of rapid signaling between neurons. Although the protein components of pre- and post-synaptic structures are now being elucidated, the distributions and roles of specific synaptic proteins in different neural systems during development remain to be determined. Here we examined the distributions of two prominent synaptic proteins, synapsin-I (Syn-I) and PSD-95, in developing rat cerebellum during peak periods of synaptogenesis.

The comparative localization of two prominent synaptic proteins, Synapsin-I (Syn-I) and PSD-95, was investigated in slices of developing [postnatal day (P)3-P21] rat cerebellar cortex using double or triple-label fluorescence immunohistochemistry and multi-channel laser scanning confocal microscopy. During the first postnatal week, Syn-I and PSD-95 immunoreactive (IR) puncta were strongly concentrated in the Purkinje Cell (PC) layer where they circumscribed irregularly shaped PC somata, forming pericellular nests that likely correspond to early climbing fiber synapses. PSD-95 and Syn-I puncta also were found along the shafts and at the tips of growing PC dendrite branches labeled with calbindin. During the second postnatal week, synaptic puncta were lost from the PC layer while many new puncta were added to the molecular layer (ML) and glomerular layer (GL). At P10, about half of the PCs were circumscribed by PSD-95 or Syn-I puncta, whereas at P14 none of the PCs were circumscribed. During the third postnatal week, PSD-95 and Syn-I were most strongly localized to many small puncta in the ML and to large clusters at mossy fiber rosettes in the GL where PSD-95 often encircled Syn-I clusters. At P14, some large clusters in the GL contained only PSD-95 or Syn-I, but not both. No PSD-95 staining of presynaptic terminal *pinceaux* was observed during the first 3 weeks of postnatal development. Thus, in relation to the somatodendritic compartment of PCs, there is a developmental shift in PSD-95 localization whereby, first, it is concentrated on PC cell bodies and short dendrites in the PC layer (P3-P7), then it is lost on a subset of PC cell bodies (P7-14), and finally it becomes localized exclusively on PC dendrites (>P14). Syn-I IR hotspot clusters are correlated topographically with developing mossy fiber presynaptic endings or mossy rosettes. Small Syn-I labeled puncta surrounding the glomerular regions may be Golgi cell presynaptic endings, and some Syn-I IR puncta may correspond to climbing fiber presynaptic endings surrounding Golgi cell somata. The numerous PSD-95 puncta in the ML were correlated with the PC dendritic PSDs making synaptic junctions with parallel and climbing fibers. These findings are consistent with previous studies of mature cerebellar synaptic architecture using transmission and scanning electron microscopy (1), as well as confocal laser microscopy (2). PSD-95 IR puncta in the glomerular region were interpreted as localized at mossy fiber granule cell synapses, typical excitatory synapses.

References

- 1) Castejón, O.J. Scanning Electron Microscopy of Cerebellar Cortex. Kluwer Academic Pub. New York. (2003).
- 2) Castejón et al. J. Submicrosc. Cytol. Pathol., 33,289-300 (2001)



Figs 1. Calbinding staining of Purkinje cells.

Fig. 2. PSD95 positive immunostaining circumscribing Purkinje cell bodies (arrows). The arrowheads show the PSD 95 localization at the level of Purkinje dendrites.

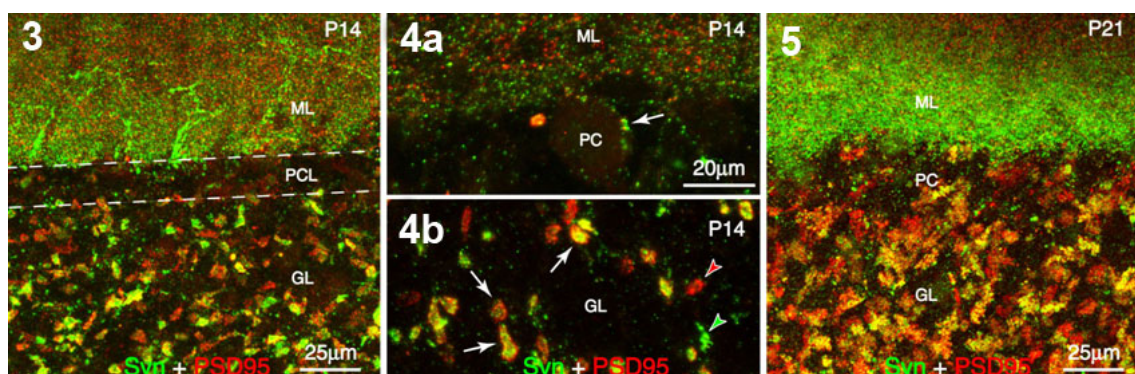


Fig.3. Syn I and PSD immunostaining at P14 in the molecular and granular layer.

Fig. 4a. Strong Syn I and PSD immunostaining at P14 of molecular layer, and weak at the Purkinje cell layer (arrow). Fig. 4b. Corresponding Syn I and PSD label at the glomerular layer.

Fig. 5. Strong Syn I labeling of molecular layer, and of PSD95 in the granular layer.