Co-cultures of cerebellar slices from mice with different reelin genetic backgrounds as a model to study cortical lamination - Supplementary Material 2

Effect of section thickness and Purkinje neurons stacking on the definition of the centers of mass of cell nuclei in Voronoi analysis (sites) and GIS-based procedures

Voronoi’s analysis and GIS-based procedures rely on the possibility to calculate the X-Y coordinates of the centers of mass of cell nuclei, i.e. the nuclei of the GFP-tagged Purkinje neurons (PNs). It is possible to do so by manually clicking with a mouse on the centers of the PN nuclei using software such as the Voronoi Generator described in the main paper or using an automated (or semi-automated) image segmentation procedure. Both procedures have inherent advantages and disadvantages. Manual preprocessing of images is prone to operator’s errors, but, at the same time, permits a direct visual check that limits the errors deriving from the segmentation algorithms, the latter being ultimately validated by comparison with the visual procedure carried on the same material, as skillful human observers are the only existing gold standard for validating the automated analysis.

Automatic or semi-automatic image segmentation procedures are particularly useful and effective in the study of cell culture monolayers, e.g. 2, and/or tissues thin sections, e.g. 3 where the dispersion of cell nuclei along the thickness (Z axis) of the preparation is negligible and thus does not represent an issue for the identification of the individual nuclei.

Figure S2 shows the clustering of PNs in the translobular (parasagittal) and paralobular plane of the section. Plane orientation is shown in panel A. Sections are cut in the translobular plane (XY). Their nominal thickness was 400 μm at the cut, but with time in vitro, they tend to become thinner reaching about 60 μm after 10 days in vitro (B-C). In the confocal microscope reconstructions of Z-stacks (B-C), several cells may be piled one above the other in cultures from L7-GFP\textit{reln}^{+/+}/F1/ mice and most of the amassed cells are almost indistinguishable from each other in cultures from L7-GFP\textit{reln}^{−/−}/F1/ animals.

In translobular sections that were used for Voronoi and GIS-based analysis (panels D-E), cells below the plane of focus are somewhat blurred, even in confocal images. This occurs particularly inside the central mass of PNs that is typical of homozygous \textit{reln}^{+/+} mice. In these instances, it is very difficult to properly apply image segmentation to automatically/semi-automatically isolate the nuclei and calculate their centers of mass. For this purpose, some procedures have been developed e.g. that described by Bjornsson et al. 1. These authors have developed a method for automated quantification of 3D multi-parameter images of 100 μm thick vibratome sections of the adult rat hippocampus and cingulate cortex. The procedure, however, requires a spectrally resolved confocal microscope for image acquisition.

To overcome the problem of the scattering of PN nuclei along the Z-axis of the organotypic cultures we decided to use a manual procedure not considering the nuclei that were not distinguishable individually in subsequent analyses. This introduces a certain error that is random across all sections and thus has no significant effects on the outcome of the results of quantitative investigations.
Fig. S2: 3D organization of GFP-tagged PNs in culture slices. A: Simplified 3D representation of the cerebellar cortex showing the position of the PNs in the translobular and paralobular planes. The dendritic arbor of these neurons is mainly spread along the translobular plane, whereas is much compressed along the Z-axis in the paralobular plane. B-C: Computerized reconstruction of Z-stacks of confocal images showing the spread of GFP-tagged PNs along the Z-axis of the slice (slice thickness). Note that in the slice from an L7-GFPreln$^{+/+}$F1/ mouse, the PNs align towards the upper surface of the culture and are stacked to a pile of 3-4 cells (B and insert 1). On the other hand, in the slice from an L7-GFPreln$^{+/+}$F1/ mouse, the PNs form a highly compact mass of cells throughout the slice thickness (C and insert 2). The red arrowheads indicate the direction of the laser beam. D-E: Appearance of the PNs in translobular confocal sections of cultures from an L7-GFPreln$^{+/+}$F1/ mouse (D) or an L7-GFPreln$^{+/+}$F1/ mouse (E). Abbreviations: CM = central mass of clustered PNs in the depth of the medullary body; GCL = granule cell layer; ML = molecular layer; PCL = Purkinje cell layer; WM = white matter.
