Supporting information

Cholesterol biosynthesis and uptake in developing neurons

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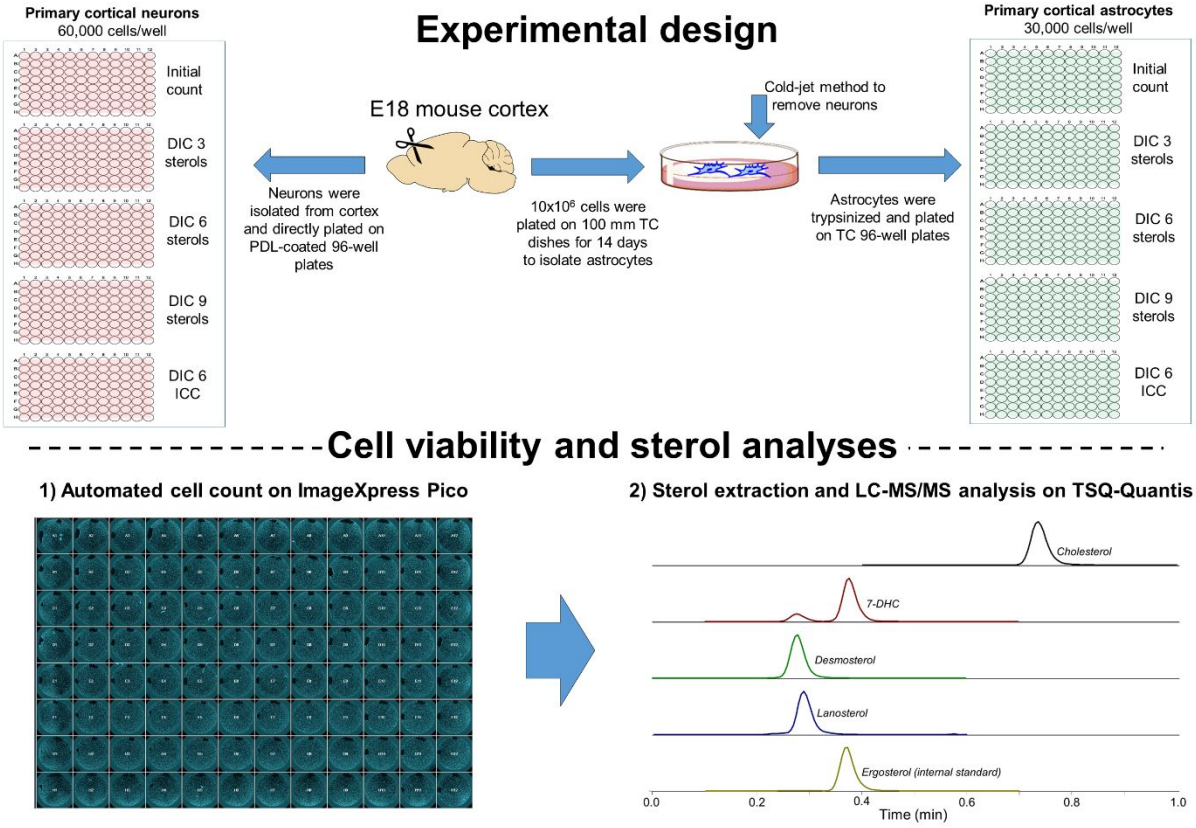
SUMMARY

Supplemental Figure 1



Supplemental Figure 1. Experimental design. Primary cortical neurons and astrocytes were isolated from E18 cortices. Neurons were immediately plated on 96-well plates, whereas astrocytes were cultured for 10-14 days to increase cell population. Astrocytes were then subjected to the cold-jet method to remove neurons, trypsinized, counted and plated on 96-well plates. Cells were cultured for 3, 6 or 9 days in defined cholesterol-free medium. At the endpoint, Hoechst dye was added to the plate and cells were imaged on an ImageXpress Pico where cells were counted. 200 µL of methanol containing the internal standards was added to each well and the samples were analyzed by LC-MS/MS for their sterol profile.

Supplemental Table 1. Neuronal and astrocytic cell count per well at DIC6.

	Neurons in neuronal cultures	Astrocytes in neuronal cultures	Astrocytes in astrocytic cultures	Neurons in astrocytic cultures
Cell count (mean ± SEM)	42,311 ± 795	1,062 ± 32	33,153 ± 3,834	4,123 ± 480
% of total cells per well	97.6%	2.4%	88.9%	11.1%

To determine the neuronal and astrocytic content in each well, 4 wells were imaged at 4x, taking 4 images per well to cover the whole well, for a total of 16 images. Cell count values were then averaged and used to determine the content of both cell types in the same well and assess the purity of our cell culture preparations. To determine the astrocytic content in neuronal cultures, astrocytes were counted using the cell counting algorithm in CellReporterXpress, counting only cells that were FITC positive (GFAP). Astrocytic and neuronal nuclei were counted using the same algorithm, but set to count DAPI positive nuclei from the Hoechst stain. Analogously, to determine the neuronal content in astrocytic cultures, neurons were counted using the cell counting algorithm in CellReporterXpress with the parameters set to count only Cy3 positive (MAP2) cells that would indicate neurons.