

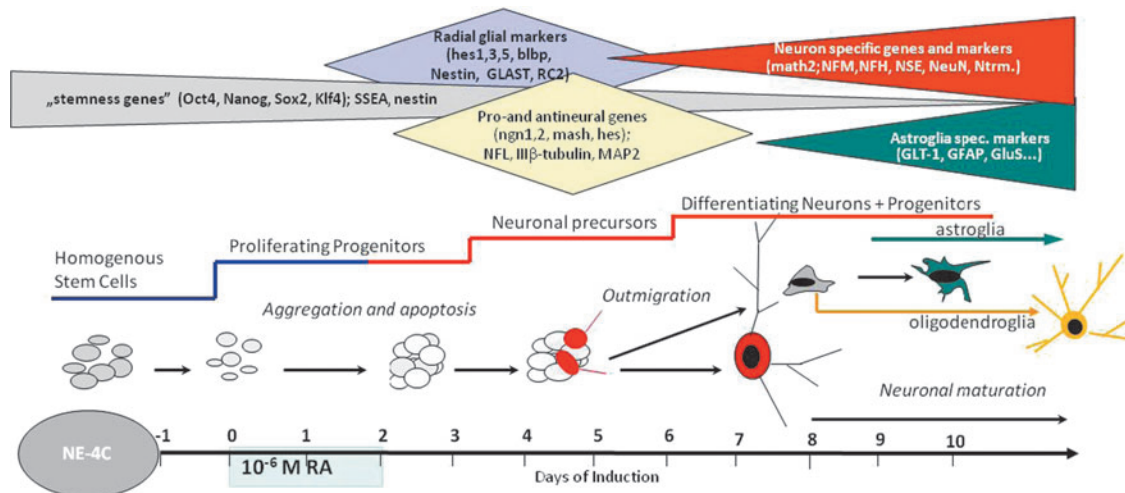
## Supplementary Data

### Supplementary Data 1

NE-4C embryonic neuroectodermal stem cells (ATTC-CRL-2595) [30], if induced by *all-trans* retinoic acid, generate neurons through highly reproducible morphological and biochemical stages.

By the 3rd day of induction, compact aggregates are formed, and the first cells with neuronal characteristics ( $\beta$ -III-tubulin, MAP2, process elongation) appear inside of the aggregates. By the 5th–6th days, several cells migrate out of the aggregates and provide a cellular basal monolayer for the further development of maturing neurons. By the 7th day, about 50% of the cells display neuronal characteristics, including synapsin and NeuN immunoreactivity and  $\text{Na}^+$ -based action potentials [27]. GFAP-immunoreactive astrocytes appear by the 9th day of induction and their number increases with time. Oligodendrocyte formation is even further delayed and needs ascorbic acid and thyroid hormone treatment.

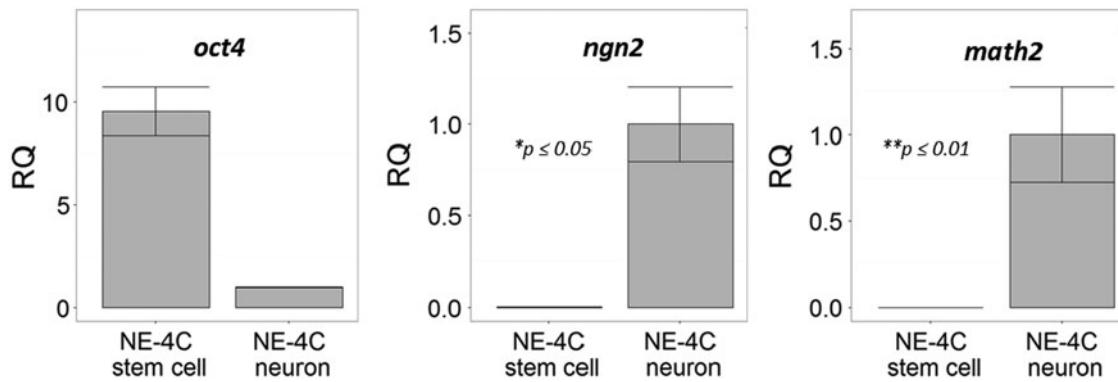
SSEA-1- and oct4-positive cells are present in all stages of in vitro differentiation indicating the persistence of some stem-like cells in the differentiated cultures.



The schedule of neuron formation by NE-4C

Modified forms of the scheme have been presented earlier in Varga et al. [22] and in Madarász [14].

The neuronal development of RA-induced cells was evidenced by qPCR assays on stemness (oct4), proneural (ngn2), and neuronal (math2) gene expression.



Total RNA samples were prepared from NE-4C stem cells and from NE-4C-derived neurons on the 10th day after the onset of induction. The individual gene amplification values ( $n=3$ ) were normalized to that of *hprt* in the same preparation, and then, the gene/*hprt* ratio of stem cells was related to the gene/*hprt* ratio in NE-4C neurons ( $=1$ ).

### Supplementary Data 2

The Cell Metabolism Analyzer XF 96 allows detecting the effects of various mitochondrial blocking agents during continuous measurement of  $O_2$  consumption and extracellular proton production of cells. In assay mode, the device reduces the sensing volume to 2.28  $\mu\text{L}$  fluid volume above the cells and produces a gas-tight measuring well. The oxygen content in the cell-covering fluid decreases with time in parallel with the  $O_2$  consumption of cells. The device records the oxygen content in every 15 s for 3 min and then introduces atmospheric  $O_2$  into the cell-covering media by opening up and mixing the wells for 3 min. The oxygenation and assay steps alternate. Through ports in the assay plate, solutions can be introduced to the assay space with a 3-min mixing period. Acidification (pH changes) of the extracellular medium was measured in parallel with oxygen content in each well. The data were plotted as OCR (oxygen consumption rate: pmole  $O_2$  consumption/min) and ECAR (extracellular acidification rate mpH/min) as a function of time.

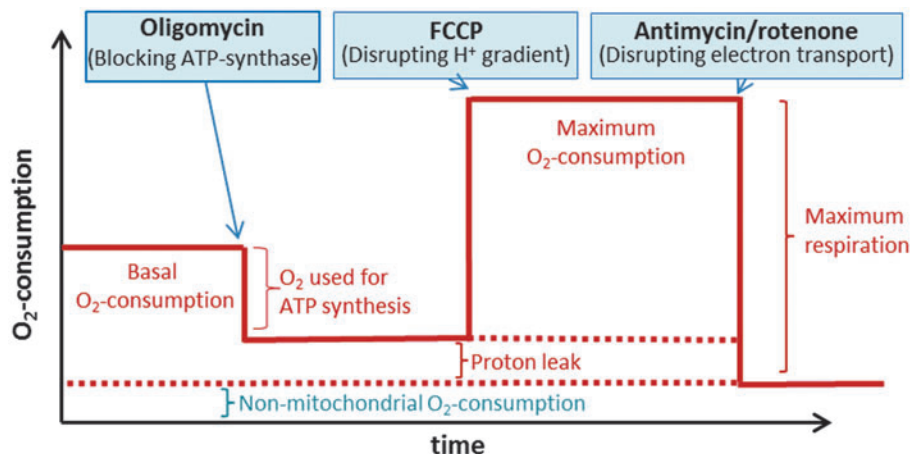
For comparing the reactions of cells in different wells (do not always contain the same amount of cells), OCR and ECAR values measured after metabolite or inhibitor addition were related to the OCR and ECAR values recorded in the nontreated state of the same well (100%) and were plotted as relative OCR% and ECAR% values.

The mitochondrial blockers used in the experiments included oligomycin, FCCP or DNP and antimycin (in case of previous DNP testing, antimycin was added with rotenone).

*Oligomycin* blocks ATP synthase resulting in reduced hydrogen ion transport and accumulation of hydrogen ions in the intermembrane space of mitochondria. As a consequence, the electron transport chain will be blocked and the oxygen consumption decreased.

*FCCP* (fluoro 3-carbonyl cyanide-methoxyphenyl hydrazone) is a mobile ion carrier that transports the hydrogen ions through the inner mitochondrial membrane, resulting in heavy increase in the oxygen consumption.

Antimycin blocks the mitochondrial complex III (cytochrome c reductase). By blocking the electron transport chain, it abolishes the mitochondrial oxygen consumption.



The scheme of effects of mitochondrial blocking agents.