Astroglia - neural stem cell interaction

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Introduction

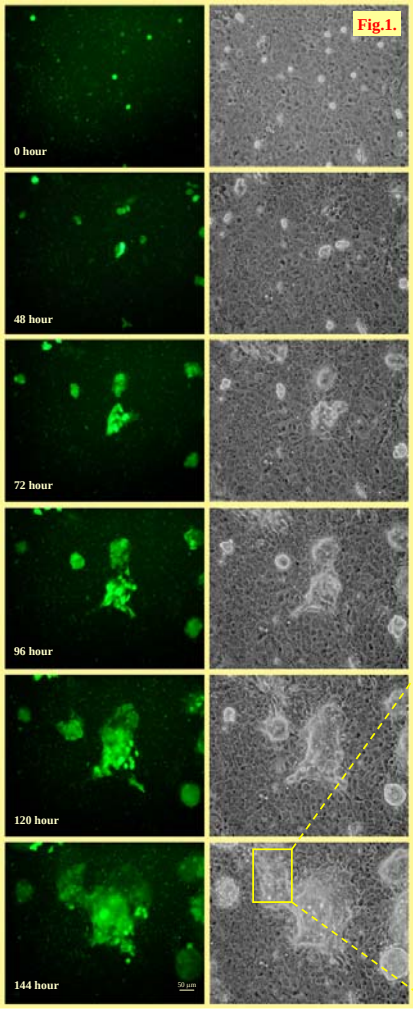
Fate determination of multipotential stem cells is governed by signals derived from their microenvironment. One of the major components of the cellular milieu surrounding *neural* stem cells are cells of the *astroglial lineage*.

Astrocytes have been shown to promote the survival and maturation of young neurons and neuronal precursors by many authors (Banker 1980; Temple and Davis 1994; Környei et al. 1999). More recent reports have demonstrated that astrocytes induce neurogenesis by adult neural stem cells (Lim and Alvarez-Buylla 1999; Song et al. 2002) and embryonic stem cells, *in vitro* (Nakayama et al. 2003). Though these observations confirm, that astroglial cells has the potential to instruct non-comitted stem cells to adopt a neuronal fate, little is known about the factors responsible for the instructive effect.

To study astroglia induced neurogenesis we used primary or secondary mouse and rat astroglial cells in combination with an immortalized neuroectodermal stem cell line, NE-4C (Schlett and Madarász 1997). Here we present data on the development of neurogenetic foci in GFP-NE4C/astroglia co-cultures based on long term time-lapse video-recordings. We show, that NE-4C neural stem cells communicate with astrocytes through gap junctions and inhibition of gap junction function results in reduced neuron formation.

I. Astrocytes induce neuronal differentiation of immortalized neuroectoderm-derived stem cells

We have recently shown, that cultured astroglial cells instruct neural stem cells of the immortalized neuroectodermal stem cell line, NE-4C, to adopt a neuronal fate (Környei et al., 2003). To follow the fate of individual neural stem cells on the surface of astroglial monolayers we made long term time-lapse video recordings (**Fig.1.**).



The current of events from the initial stem cell attachement on astrocytes to neuron formation follows a well determinable spatial and time schedule. In brief:

i, Initially, cluster formation occurs, as a result of clonal proliferation of the individual neural stem cells (**Fig.1.**).

ii, Neurons are formed within the stem cell colonies. A significant amount of non-differentiated cells remain within the clusters (**Fig.2.**).

iii, Later on, neurons outmigrate from the colonies and form networks on the surface of astroglia. The density of neurons reaches a maximum at around DIV5 (**Fig.3.**).

These observations strongly support the view, that homotypic stem cell to stem cell contact interactions are essential in astroglia-induced neurogenesis.

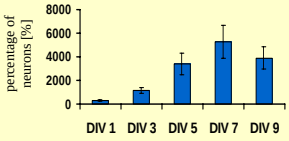
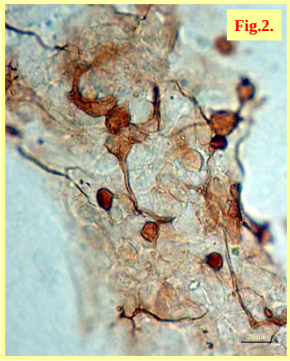


Fig.3. Percentage of Tuj1+ neurons developed in astroglia-NE-4C co-cultures. [glia DIV1=100%]



Conclusion

Our results show, that **i**, astrocytes support the attachement and proliferation of NE-4C neuroectodermal stem cells; **ii**, astroglia induced neuron formation occurs inside the expanded NE-4C cells clusters; **iii**, the neural stem cells exhibit reduced migratory activity on the surface of astroglial cells; **iv**, the neural stem cells can communicate with astrocytes via gap junctions; **v**, blockage of gap junctional communication results in reduced neuron formation.

The data indicate, that the microenvironment presented by astroglial cells supports the survival and proliferation of the neuroectoderm-derived multipotential NE-4C cells, however, restricts migration of these cells. As a result, neuroectodermal progenitors form clusters, which serve as sites of neuron formation. Within the clusters, NE-4C neural stem cells communicate with each other via gap junctions (Tárnok et al. 2002). Besides, they can form gap junctions with the underlaying astroglial cells, as well. Though the blockage of gap junctional communication of the NE-4C cells did not infuence the rate of retinoic acid induced neurogenesis (Tárnok et al. 2002), it reduced the rate of astroglia-induced neuron formation. These data indicate, that astrocytes can take an active part in the regulation of neurogenesis and gap junctional communication plays and important role in this process.

II. Influence of astroglia on the migratory activity of imortalized neuroectodermal stem cells

Immortalized neuroectodermal stem cells (NE-4C cells) exhibit a random migratory activity if cultured on poly-L-lysine-coated surfaces, as it was shown previously (Czirók et al. 1998). During their walk the individual cells randomly collide and separate. Upon induction with all-trans retinoic acid (RA), the cells slow down, as a result of "sticking" together due to alterations in their cell surface properties (Schlett et al., 2000).

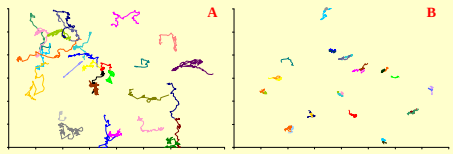


Fig.4. Migratory routes of NE-4C cells on pLL-coated plastic surfaces (30 cells) (**A**) and on astroglial monolayer (30 cells) (**B**). The trajectories represent 24 hour long routes. The fields of view cover 600 x 800 µm.

In order to study the influence of astroglial cells on the motility of NE-4C neuroectodermal stem cells, we analyzed cell displacement in both astroglia/NE-4C co-cultures and monocultures of NE-4C cells (plated onto pLL coated dishes). We determined the positions of individual GFP-NE4C cells on every second image of time-lapse recordings (in every 10 minutes in real time).

Cellular trajectories (**Fig4.**) and time/displacement functions (**Fig.5.**) were calculated from these data. The results show, that neural stem cells exhibit reduced migratory activity on the surface of astroglial cells. The formation of stem cell clusters are rather due to the clonal proliferation of single neural stem cells than encountering, and subsequent adhesion of the migrating progenitors.

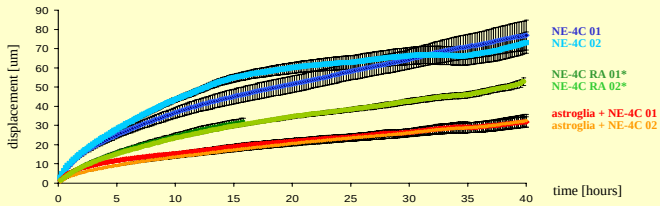


Fig. 5. Displacements of NE-4C cells under various culture conditions are plotted as a function of time. ** Data on all-trans retinoic acid (RA) treated cells were taken from the paper by Schlett et al. (2000).

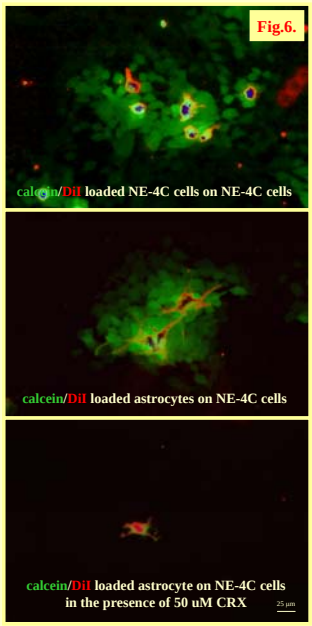
III. Gap junctional communication between astrocytes and neural stem cells: GJ blockage reduces astroglia-induced neurogenesis

We loaded separate cultures of NE-4C immortalized neural stem cells and primary astrocytes with **calcein-AM** and **DiI**. Inside the cells, **calcein AM** was hydrolyzed by endogenous esterases into the highly negatively charged green fluorescent **calcein**. After 20 min. loading and 30 min. washing we trypsinized the cells and plated them onto non-labeled NE-4C-, astroglia- or fibroblast monolayers (**Table I.**).

	loaded NE-4C cells	loaded astrocytes
plated onto NE-4C cells	+	+
plated onto astroglia	+	+
plated onto fibroblasts	-	nd

Table I. Calcein dye spreading through gap junctions.

Within 2 hours **calcein** readily spread through gap junctions from NE-4C cells to NE-4C cells or from astrocytes to NE-4C cells (**Fig.6.**) and vice versa. Dye spreading was blocked if the co-cultures were treated with the gap junction blocker carbenoxolone (CRX; 50 µM) (**Fig.6.**). No calcein spreading was observed between neural progenitors and primary mouse fibroblasts. The data demonstrated, that astroglial cells and non-induced NE-4C neuroectodermal progenitors can communicate through gap junctions.



The role of gap junction mediated cell-to-cell communication in glia-induced neurogenesis was investigated by blocking the gap junction channel function with carbenoxolone (CRX). In previous studies CRX was shown not to perturb cell survival and retinoic acid-induced neurogenesis if applied daily, in a concentration of 25 µM (Tárnok et al. 2002).

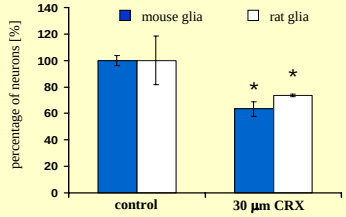


Fig.7. Percentage of Tuj1+ neurons in astroglia/NE-4C co-cultures at DIV7 [control=100%].

During a 7 day period of co-culturing we treated the cells with CRX in every second day. As a result of the repeated CRX administration a significant – approximately 30-35% - decrease in the total number of neurons was observed, both in mouse glia/mouse NE-4C and in rat glia/mouse NE-4C co-cultures (**Fig.7.**). The size of the NE-4C clusters was not reduced, indicating, that the reduction in neuron formation was due to the lack of neuronal differentiation and not to a decrease in the number of stem cells.

References.: Banker G.A. Science 1980, 209: 809-810; Czirók A. et al. Phys Rev Lett. 1998, 81: 3038-3041.; Környei Zs. et al. J. Neurosci Res. 2000, 61: 421-429; Környei Zs. et al. et VI. IBRO Congress of Neuroscience, Neural Stem Cells and Brain Repair Satellite Symposia; Lim D.A. and Alvarez-Buylla A. PNAS 1999; 96(13):7526-31.; Nakayama T. et al. Neurosci Res. 2003, 46(2):241-9.; Schlett K. et al. Int J Dev Neurosci. 1997, 15: 95-804; 11., Schlett K. and Madarász E. J Neurosci Res. 1997, 47: 405-415; Schlett K. et al. J Neurosci Res. 2000, 60: 184-194.; Song H. et al. Nature 2002, 417:39-44; Tárnok K. et al., Eur. J. Cell Biology 2002, 81:403-412; 16., Temple S. and Davis A.A. Development. 1994,120(4):999-1008.