Dynamics of astroglia-induced neurogenesis

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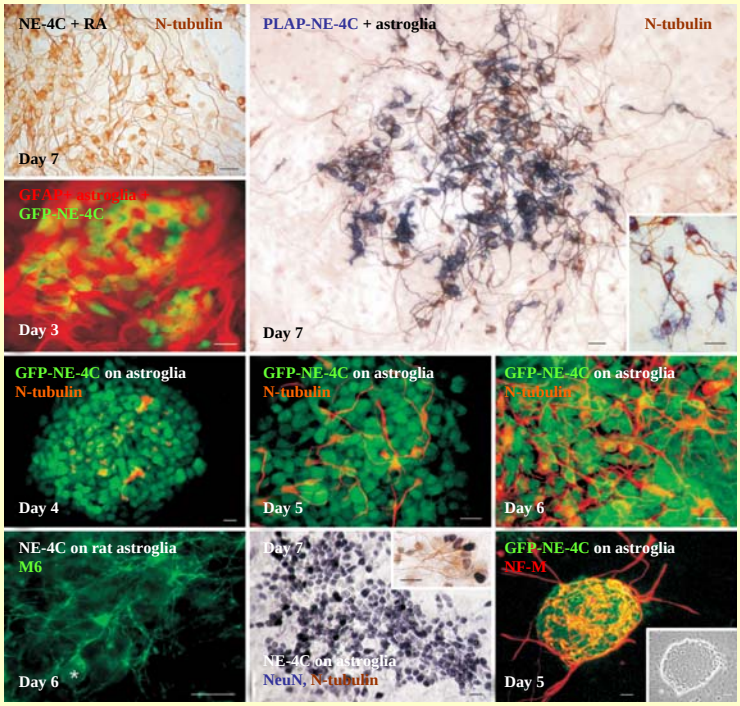
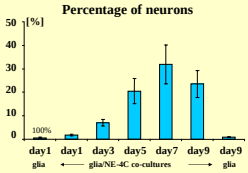
Introduction

As an alternative to their stem cell function, glial cells participate in the formation and maintenance of neurogenic niches both in the developing and in the adult brain. Astroglial cells support or restrict the migration and differentiation of neural stem cells depending on the developmental stage of the progenitors and the physiological state of the astrocytes. In the present study we show that **astroglial cells instruct non-committed, immortalized neuroectodermal stem cells [NE-4C cells; 1,2,3,4] to adopt a neuronal fate,** while they fail to induce neuronal differentiation of embryonic stem (ES) cells under similar culture conditions. Astrocytes induce neuron formation by neuroectodermal progenitors both through direct cell-to-cell contacts and via short-range acting humoral factors. Statistical analyses of time-lapse microscopic recordings show that direct contacts with astrocytes hinder the migration of neuroectodermal progenitors, while astroglia-derived humoral factors increase their motility. In *non-contact co-cultures* with astrocytes, altered adhesiveness prevents the separation of frequently colliding neural stem cells. In *contact co-cultures* with astrocytes, on the other hand, the restricted migration on glial surfaces keeps the cell-progenies together resulting in the formation of clonally proliferating stem cell aggregates. Neuron formation takes place inside compact stem cell assemblies formed 30-60 hours after the onset of glial induction. Astrocytes selectively support the migration of maturing neurons as well as the elongation of neurites.

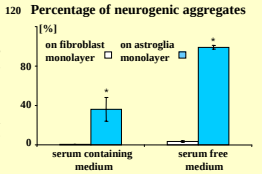
Refs.: 1) Schlett K. and Madarász E. 1997. J Neurosci Res. 47: 405-415; 2) Czirók A. et al. 1998. Phys Rev Lett 81: 3038-3041; 3) Schlett K. et al. 2000. J Neurosci Res 60: 184-194; 4) Demeter K. et al. 2004. Neurology, in press

1. Astrocytes induce neuron formation by NE-4C immortalized neuroectodermal stem cells in *contact co-cultures*

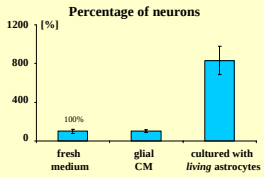




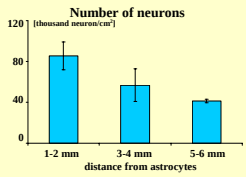
Astrocytes induced neuronal differentiation of NE-4C cells in *contact co-cultures*. The total number of NE-4C neurons increased rapidly during the first week of co-culturing. Fibroblasts did not induce neuron formation. The rate of neuron formation was dependent on the presence of serum factors.



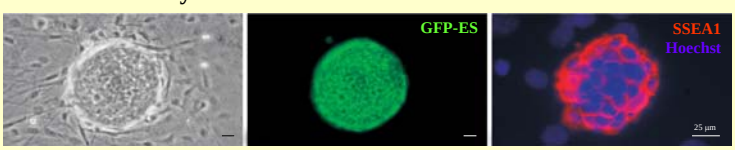
2. Astrocytes induce neuron formation by NE-4C cells in *non-contact co-cultures*



Addition of glial conditioned medium (GCM) did not induce neuronal differentiation of NE-4C cells. A significant increase was observed, however, if NE-4C cells were grown in common fluid environment with *living* astrocytes. The density of NE-4C neurons decreased sharply with the distance from living astroglia.

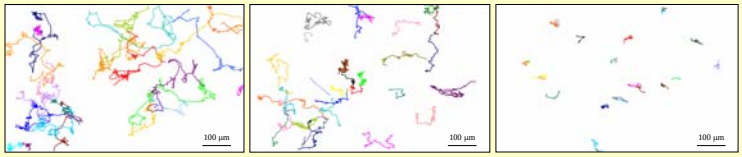


3. Astrocytes do not initiate differentiation of ES cells



The initially single ES cells seeded onto glial monolayers proliferated and formed dense aggregates. During the two week period of co-culturing neuronal differentiation was not observed.

4. Sectereted glial compounds enhance, while contacts with astrocytes hinder the motility of non-comitted NE-4C cells



average speed	average speed	average speed
20,2+-2,8 µm/h	12,2+-2,8 µm/h	4,7+-1 µm/h

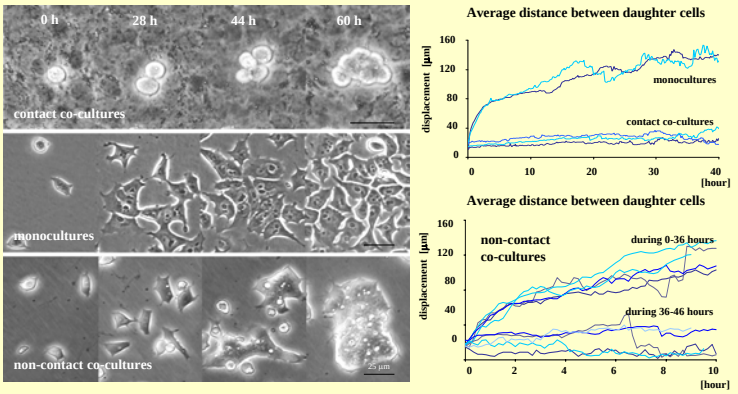
Analyses of time-lapse microscopic recordings show that astroglia-derived humoral factors increase the motility of neuroectodermal progenitors, while direct contacts with astrocytes hinder their migration.

5. Astrocytes alter the rate of proliferation of NE-4C cells

astroglia/NE/4C non-contact co-cultures	time elapsed between cell divisions *NE-4C monocultures*	*astroglia/NE-4C contact co-cultures*
cell cycle length 10,5+-2 hour	cell cycle length 15,2+-3,2 hour	cell cycle length 20,8+-5,2 hour

In *non-contact co-cultures*, the duplication time decreased significantly in comparison to NE-4C monocultures, indicating the immediate action of some soluble, mitogenic factors. Attachment onto astrocytes seemed to delay the moment of the first cell division and to extend the cell cycle, at least at the beginning of co-culturing.

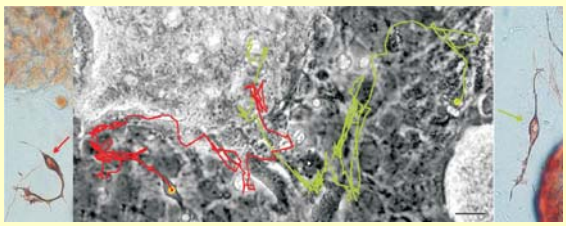
6. NE-4C cells form aggregates in the presence of astrocytes



In *monocultures*, non-induced NE-4C cells migrate randomly and display random collisions and separations. Also, the daughter cells depart from each other after each division. This leads to the formation of a confluent NE-4C monolayer. On the surface of astrocytes, however, the progenies of individual NE-4C neural stem cells can not separate from each other, resulting in the formation of compact cell assemblies. In *non-contact co-cultures* with astrocytes, altered adhesiveness prevents the separation of frequently colliding neural stem cells, after ~36 hours from the onset of induction.

7. Outmigration of neuronal precursors from neurogenic aggregates is supported by astroglial cells

According to the time-lapse recordings, some cells displaying polarized shape and extending processes left the aggregates by rapid translocation of the cell body. These cells later elongated long,, neurite-like processes. The outmigrating cells did not divide and were later identified as neuronal precursors by staining for neuron-specific tubulin.



Conclusion

The data indicate that *in vitro* maintained parenchymal **astrocytes**

i. **secrete factors, which initiate aggregation of neural stem cells**. The initial aggregate formation by NE-4C cells may be a consequence of some rapid changes in their adhesive properties and indicate an early response to astroglia-derived inducing factors. **Aggregation is an early, inevitable step of neuronal cell fate commitment**.
ii. **secrete factors, which enhance the rate of cell proliferation**, leading to increased cell density and speeding up the formation of the aggregates.
iii. **restrict the migration of non-comitted neural stem cells**. The restricted migration indicates that the set of adhesion molecules on NE-4C cells is not compatible with the adhesive surfaces provided by the astroglial environment.
iv. **support the migration of postmitotic neuronal presursors** in contrast to non-comitted cells, indicating that astrocytes can selectively support or restrict the migration and differentiation of neural stem cells depending on the developmental stage of the progenitors.
v. **do not support the neuronal differentiation of individiually plated ES cells**. Stem cell populations should reach a - yet non-defined - stage of cell fate commitment in order to respond to tissue-specific differentiation signals.