**Role of Cell to Cell Connections in the course of in vitro Neuron Formation:**

**Gap Junction Communication by NE-4C neuroectodermal stem cells**

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### Introduction

Fate determination of neural stem cells is governed by signals derived from their microenvironment via multiple forms of contact and humoral cell-to-cell interactions. Gap junctions are involved in the control of both, cell proliferation and differentiation. In the primary germinative layer of the neural tube, clusters of neuroectodermal cells are interconnected by gap junctions (to Turco and Kriegstein, 1991).

Nowadays, it is thought that communications through gap junctions may contribute to the maintenance of the proliferative state of stem cells (Cheng et al., 2004; Deisseroth et al., 2004; Devel et al., 2002). Gap junction coupling is a common feature of progenitor cells throughout the whole period of neural tissue development.

NE-4C – a p53-deficient, immortalized neuroectodermal stem cell line (Schlett K., Madarasz E.: Retinoic acid induced neural differentiation in a neuroectodermal cell line immortalised by p53 deficiency. J.Neurosci.Res.47 (1997)) – was successfully used to study the effect of gap junctions on neuronal cell fate determination by NE-4C model cells.

In the recent studies, gap junction coupling and the importance of gap junction communication were investigated in the course of in vitro induced neuronal development.

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### Retinoic Acid (RA) induced neuronal differentiation

**Non-induced NE-4C progenitor cells**

- Alexa Fluor Hydrazid 594
- SSEA-1
- Nestin
- Hiß Tubulin

**Maturating NE-4C neurons**

- Connected cells
- RA-Day 4
- RA-Day 8
- RA-Day 12

**Astroglia induced neuronal differentiation**

NE-4C progenitors in contact with astrocyte monolayer

- Phase contrast & DiI

**Gap junction communication by NE-4C neuroectodermal stem cells**

- 30 µM CRX blocks the dye spreading between a single astrocyte (loaded with Dil and calcine) and non-induced NE-4C cells.

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### Conclusion

1. NE-4C neural stem cells communicate with each other via gap junctions.
2. With the advancement of neuronal development, differentiating cells cease gap junction communication with neighboring cells.
3. Gap junction coupling persists until substrate-attached, non-differentiated cells.

### Retinoic Acid induced neuronal differentiation

1. Retinoic acid (RA) or by the presence of primary astrocytes. In the course of in vitro neuron formation induced either by all-trans retinoic acid (RA) or by the presence of primary astrocytes. The cells are gap junction coupled as it can be detected by dye spreading. The cells were loaded with dyes either electrophysiologically (Alexa Fluor Hydrazid 594) or by previous dye uptake (DiI & calcine) in a separate culture.

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### Summary

At the beginning of induction, cells arrange themselves into compact aggregates. The majority of cells are still proliferating. The first Hiß tubulin positive cells appear within the aggregates. The cells within the clusters, remained coupled and form gap junctions.

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### Astroglia induced neuronal differentiation

#### Gap junction communication

- CRX induced gap junction communication interferes with the neuron formation, depending on the developmental stage of NE-4C cells.
- Blockage of gap junction communication during the initial period (6-3 days) of astrocyte induced neuron formation, when the majority of NE-4C cells are still proliferating, reduces both the number and size of neuronal aggregates.
- Blockage of gap junction communication in the later phase (3-6 days) of astrocyte induced differentiation, when neuronal maturation of committed NE-4C precursors takes place, results in an increase in the number of neurons.

The observations indicate that communication through gap junctions supports the maintenance of the proliferative, progenitor state of NE-4C neuroectodermal cells.