Synthes Research Award 2006

Neuroregenerative Strategies in Intracerebral Hemorrhage

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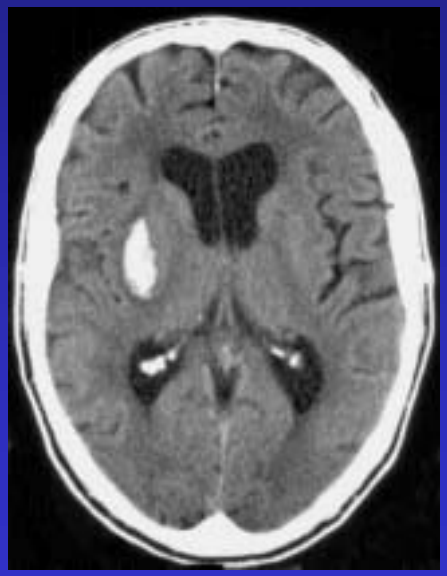
Non-traumatic intracerebral hemorrhage

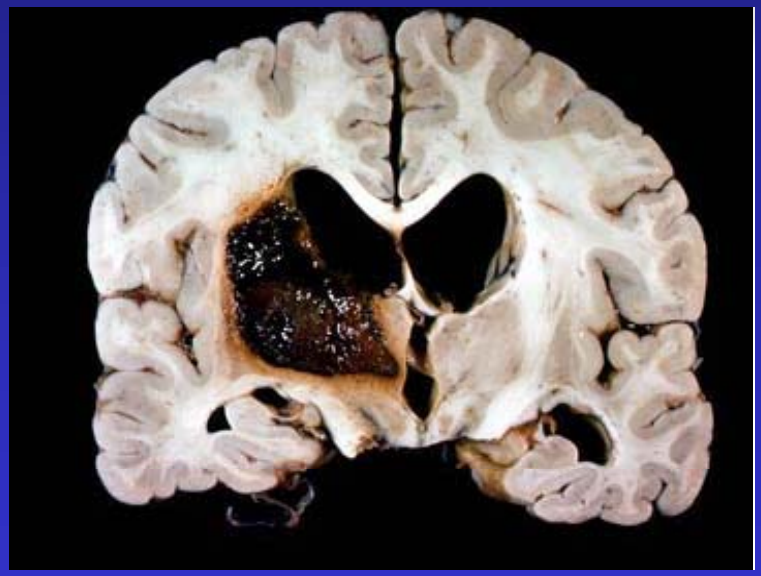
Incidence

12-15 / 100‘000 / year

< 45 years: < 2 / 100‘000 / year

> 80 years: 350 / 100‘000 / year





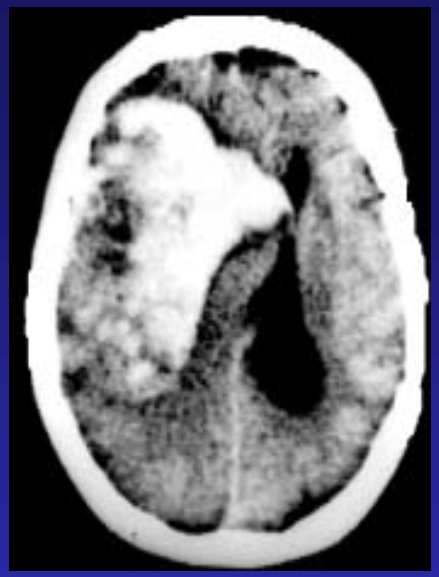
Main risk factor

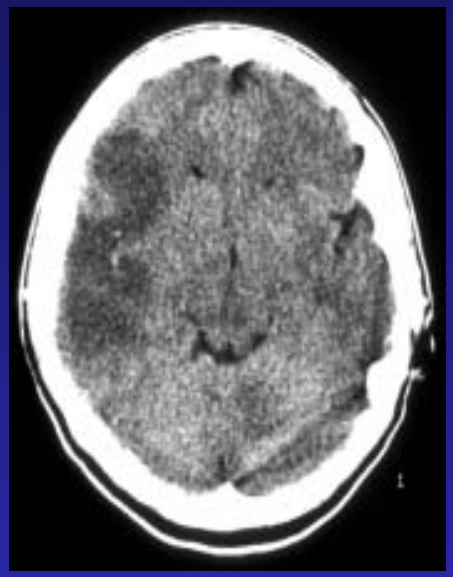
Chronic arterial hypertension

Non-traumatic intracerebral hemorrhage

Conservative treatment

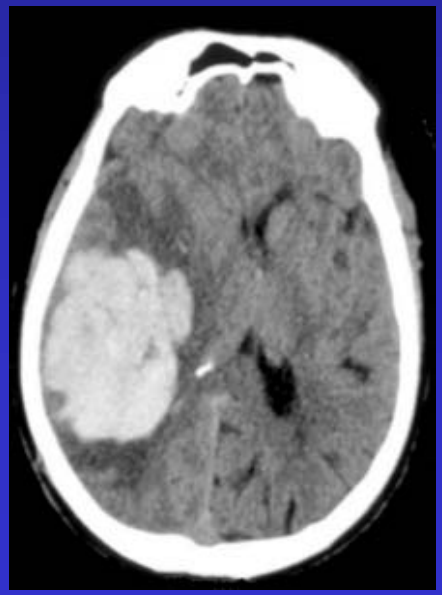
Persisting brain parenchyma defects

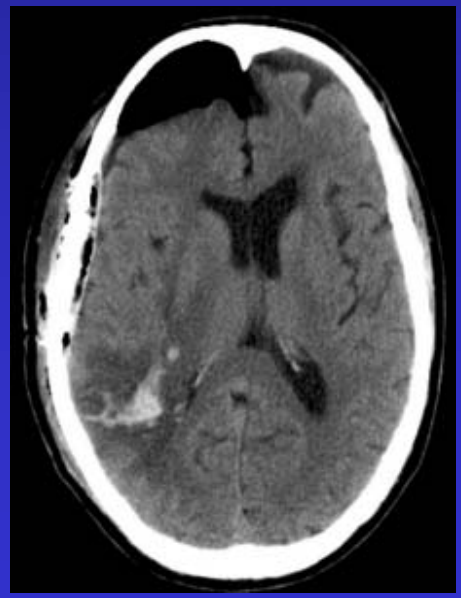




Surgical treatment

Persisting brain parenchyma defects





Early Surgical Treatment for Supratentorial Intracerebral Hemorrhage: A Randomized Feasibility Study

Zuccarello et al., Stroke 1999

Bad Outcome (GOS 1-3)	Surgical	44%
	Conservative	64%
Mortality	Surgical	22%
	Conservative	27%

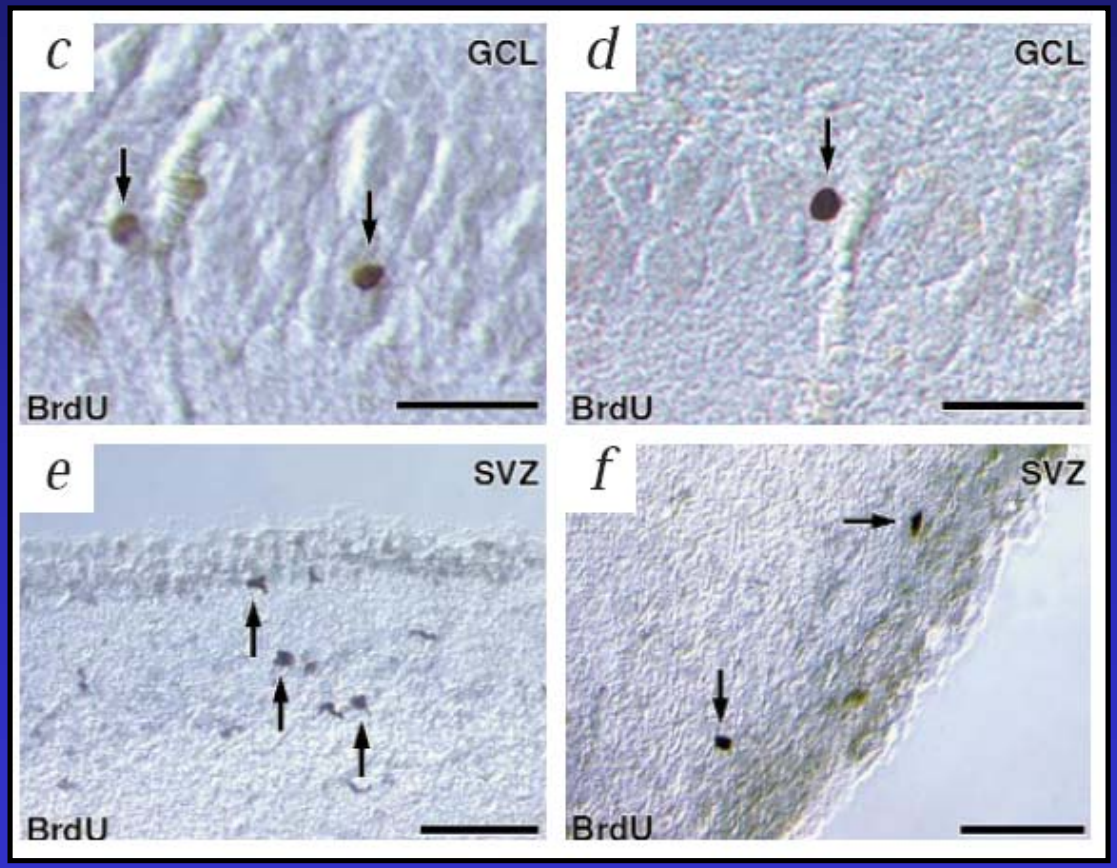
Surgical Treatment for Intracerebral Hemorrhage (STICH): A Single-Center, Randomized Clinical Trial

Morgenstern et al., Neurology 1998

Bad Outcome (GOS 1-3)	Surgical	50%
	Conservative	69%
Mortality	Surgical	24%
	Conservative	18%

Regeneration: Neurogenesis in the adult human brain

BrdU-immunostaining of the subventricular zone in adult human brain tissue



Eriksson et al., Nat Med 1998

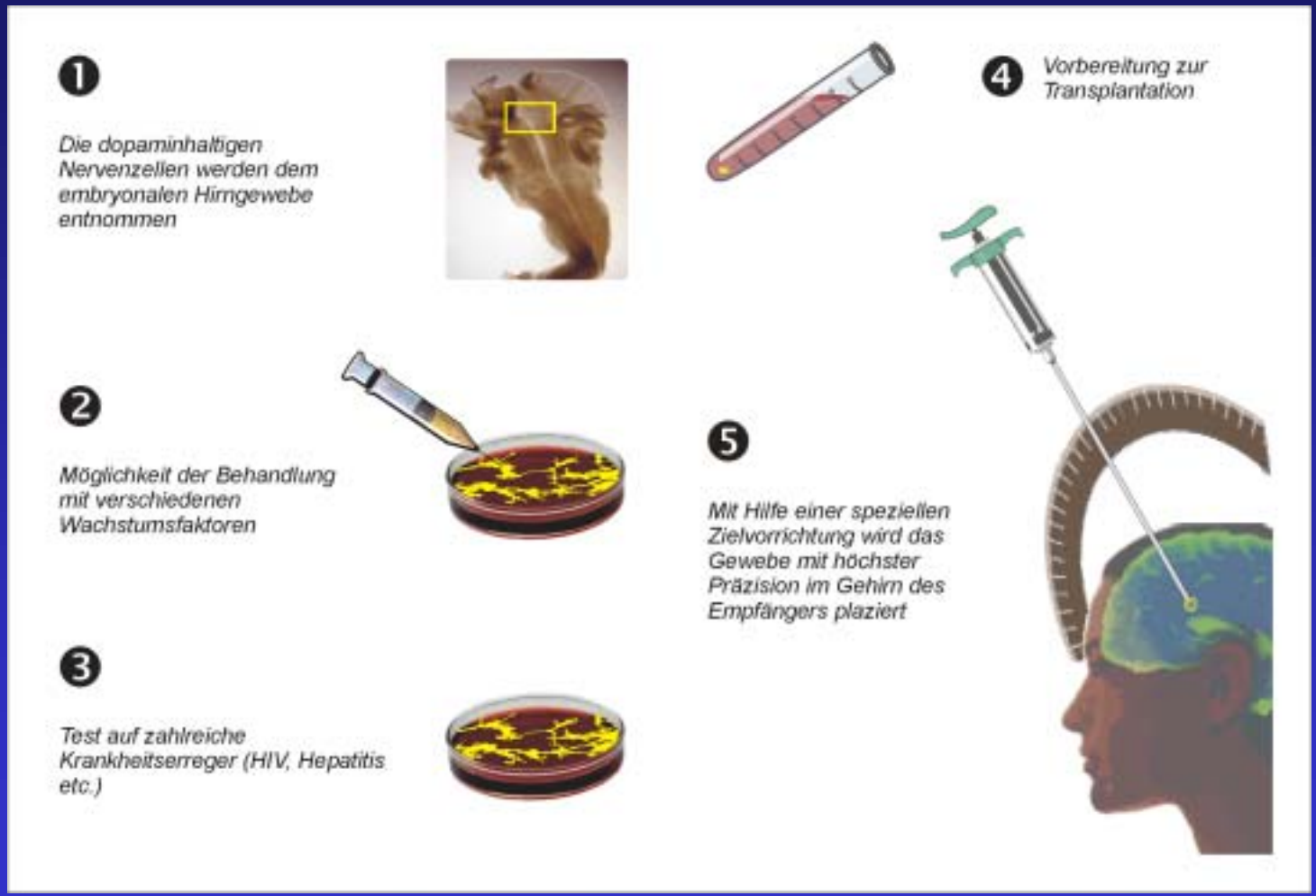
Experimental concepts for the treatment of ICH

Transplantation of stem cells or neuronal precursors

Treatment with neuroprotective factors

Activation of endogenous neurogenesis

Transplantation of embryonic precursor cells



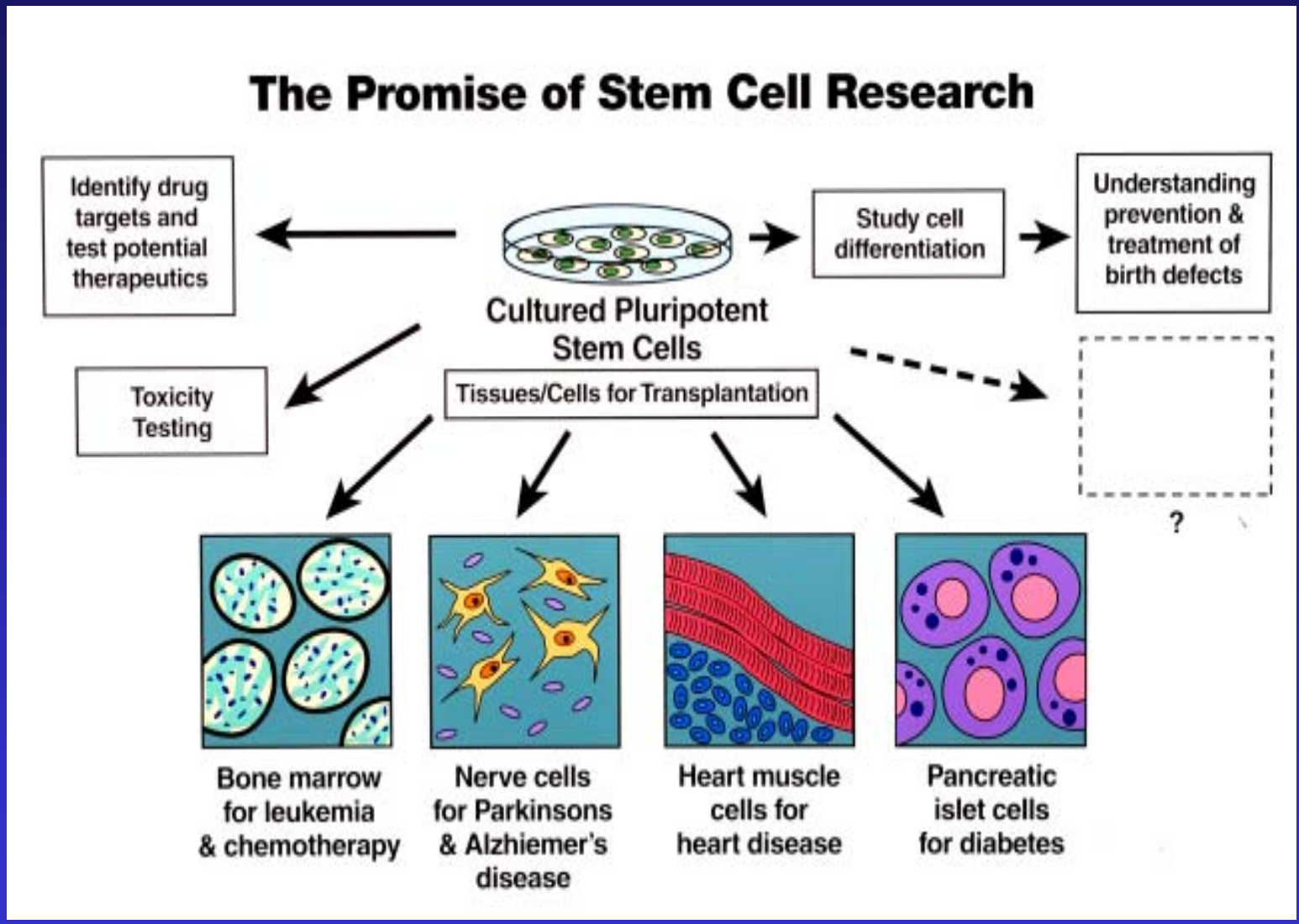
Andres et al., Ars Med 92:428-32 (2002)

Limitations of neural transplantation

- Limited availability of embryonic tissue due to ethical and logistical problems

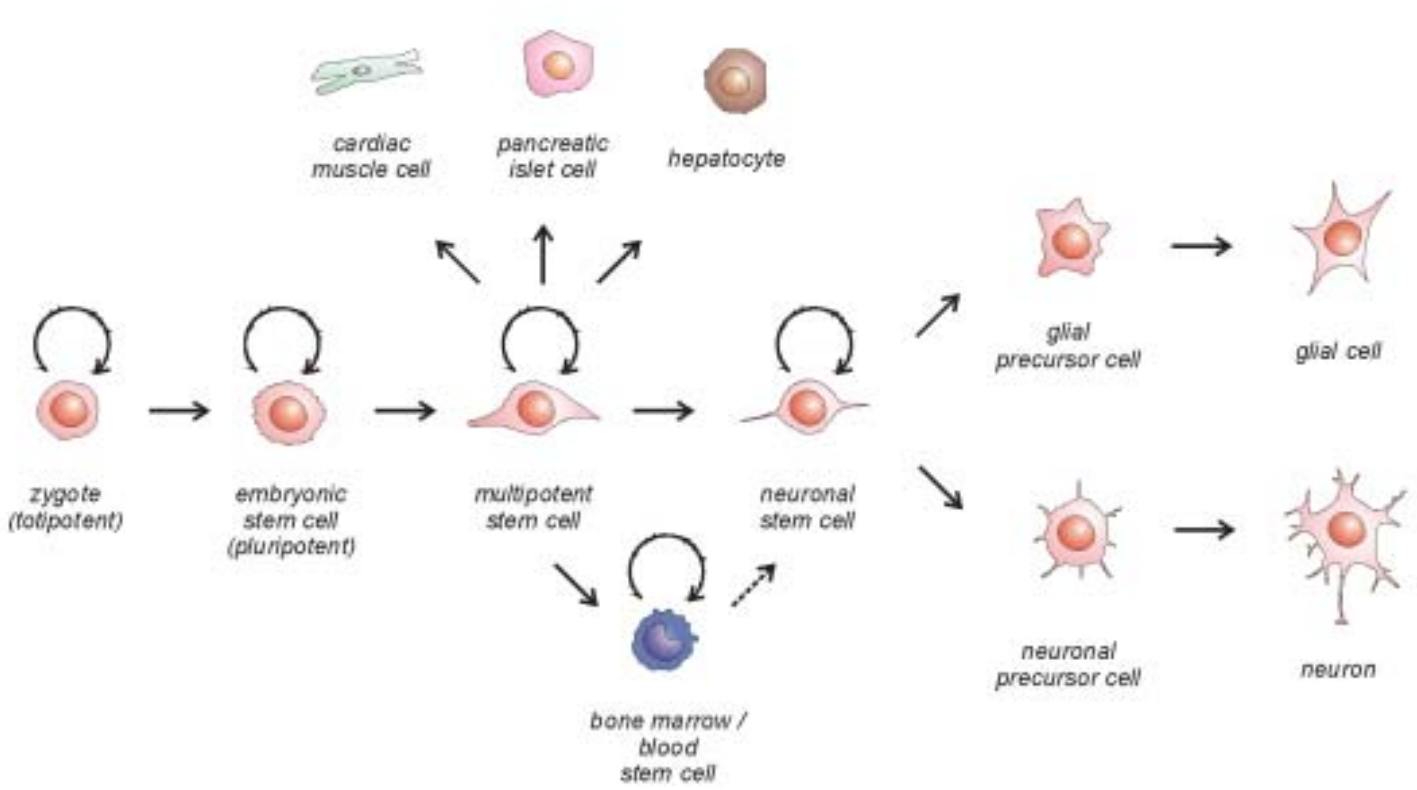
- Insufficient survival of the transplanted cells

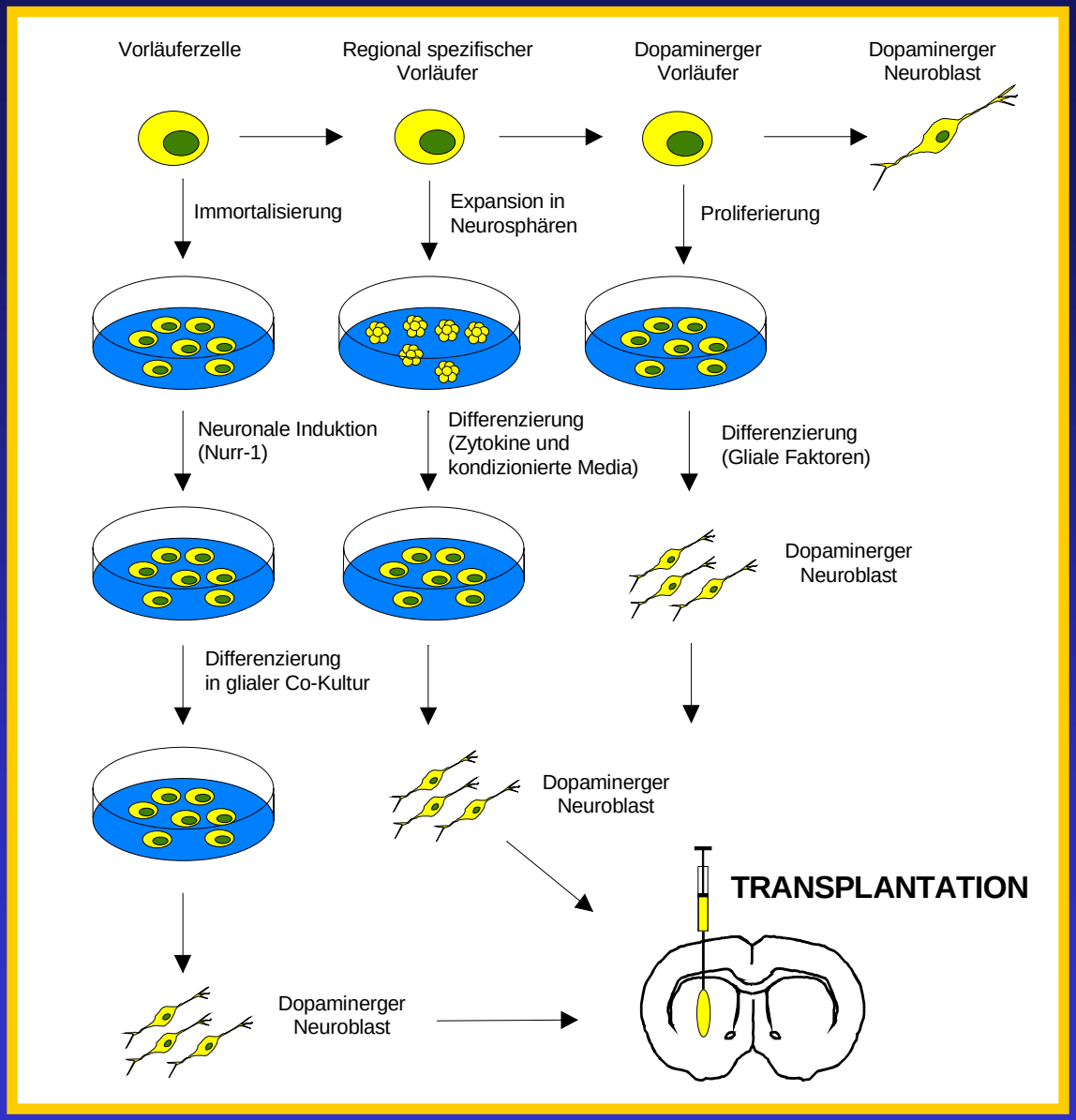
- Suboptimal graft integration into the host brain



Adapted from *Cell*

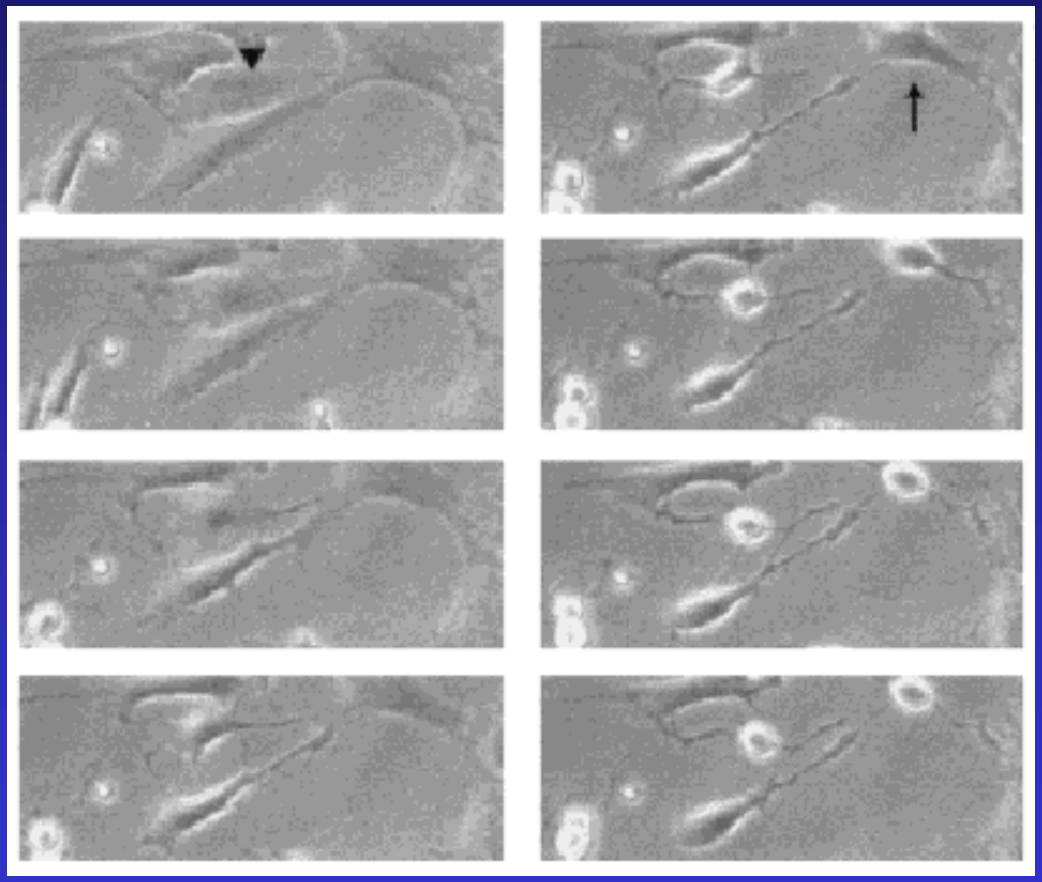
Stem cell differentiation towards neuronal lineage





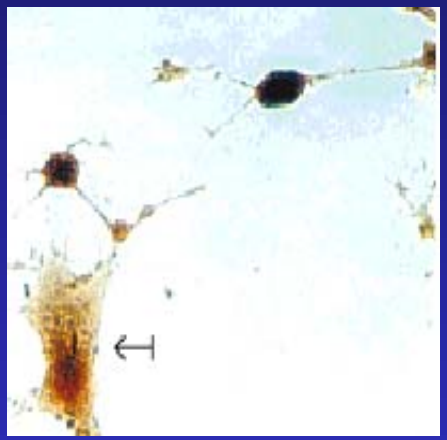
After Björklund and Lindvall, Nature Neurosci, 3(6): 537 (2000)

Neuronal differentiation of mesenchymal bone marrow stem cells

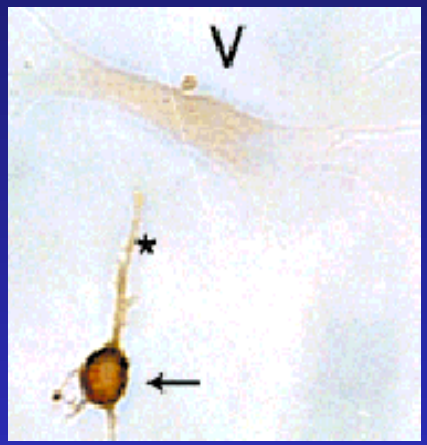


Woodbury et al., J. Neurosci. Res. 61:364-370 (2000)

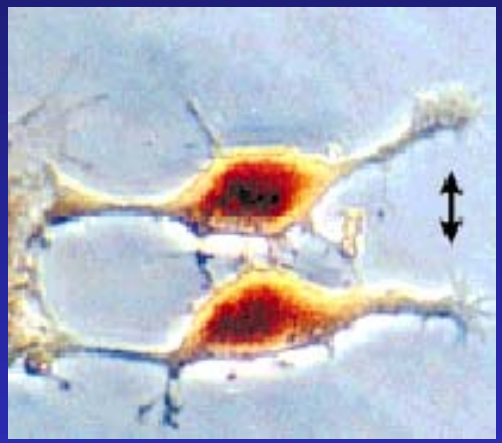
Expression of neuronal marker proteins



NSE



tau



growth cone

Woodbury et al., J. Neurosci. Res. 61:364-370 (2000)

Factors that influence the success of neuronal grafting

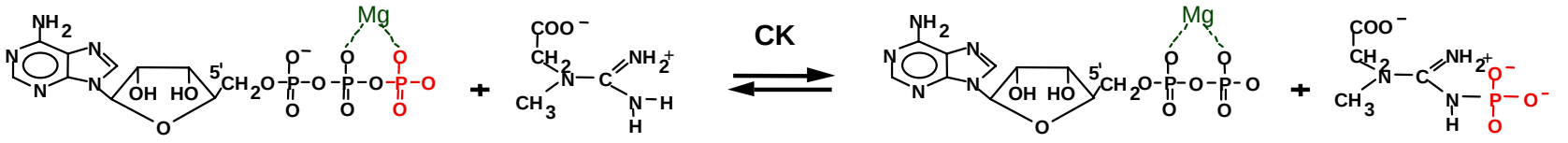
Predetermined factors
- Age and immunological state of the host

Technical factors
- Type of the transplanted cells
- Number of transplanted cells
- Storage and conditioning of the cells
- Location of the implantation site

Pretreatment
- Neural growth factors
- Nutritional factors
- Antioxidants
- Antiapoptotic substances

Creatine treatment

The creatine / phosphocreatine circuit and the phosphocreatine pool



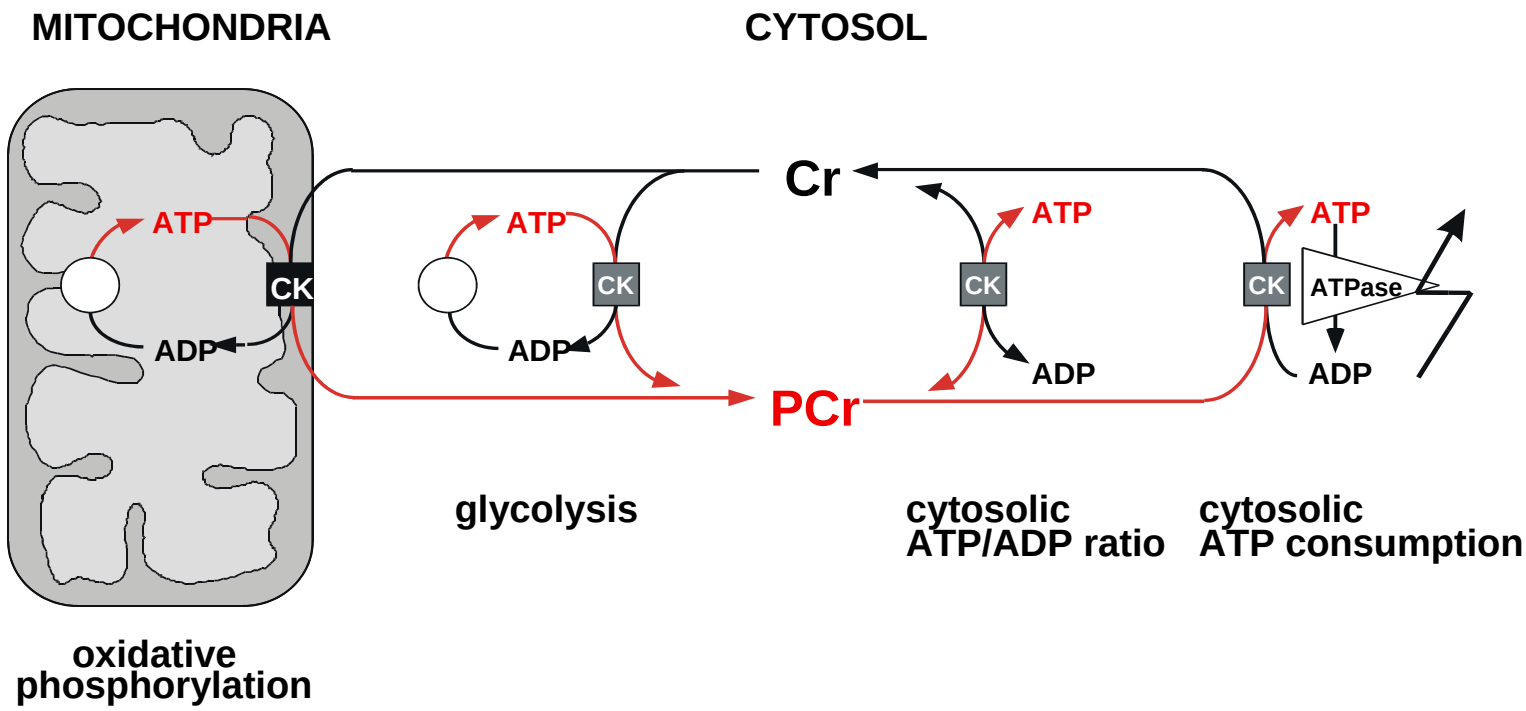
MgATP + Creatine ⇌ MgADP + Phosphocreatine

$\Delta G°_{ATP}$ = -7.3 kcal/mol

$\Delta G°_{PCr}$ = -10.3 kcal/mol

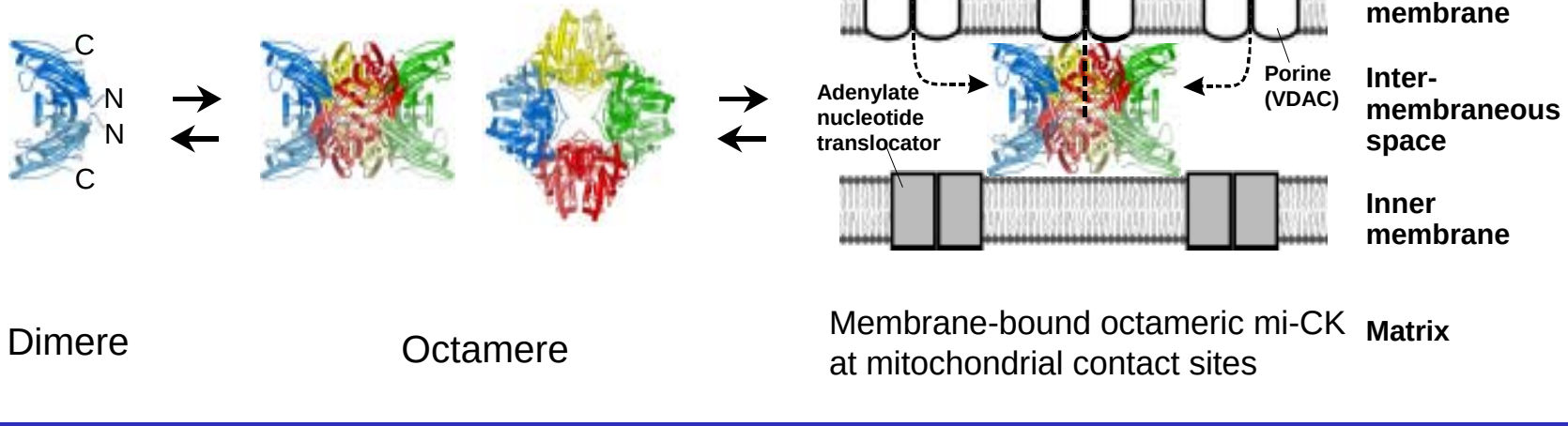
Creatine supplementation leads to an increase in cellular ATP reserves

The creatine-/phosphocreatine shuttle



Enhanced energy transfer at higher substrate concentrations

Higher cytoplasmic creatine levels inhibit the mitochondrial permeability transition



Dimere

Octamere

Membrane-bound octameric mi-CK at mitochondrial contact sites

MPT inhibition by stabilization of mi-CK in the octameric state

Advantages of creatine for the treatment of CNS disorders

Endogenous substance

Specific transport across the blood-brain-barrier

High bioavailability

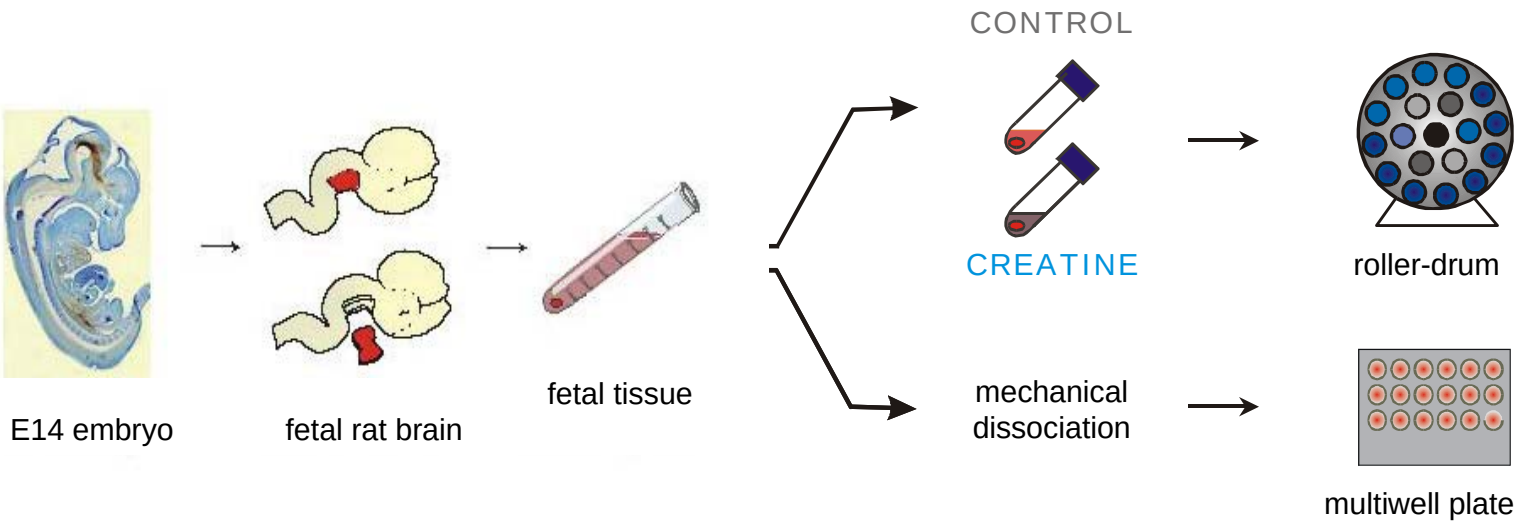
Low toxicity

No relevant side effects

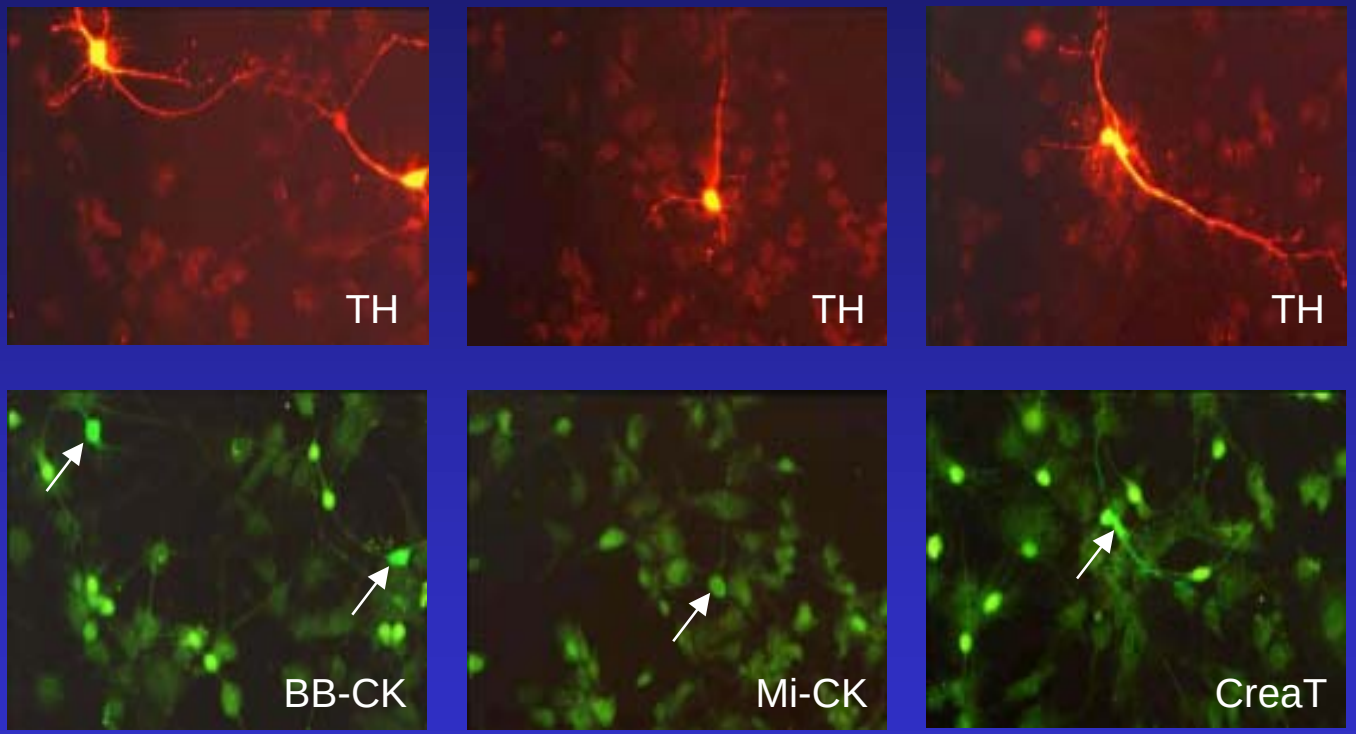
Long term therapy is safe

Inexpensive drug

Effects of creatine on cultured neuronal precursor cells

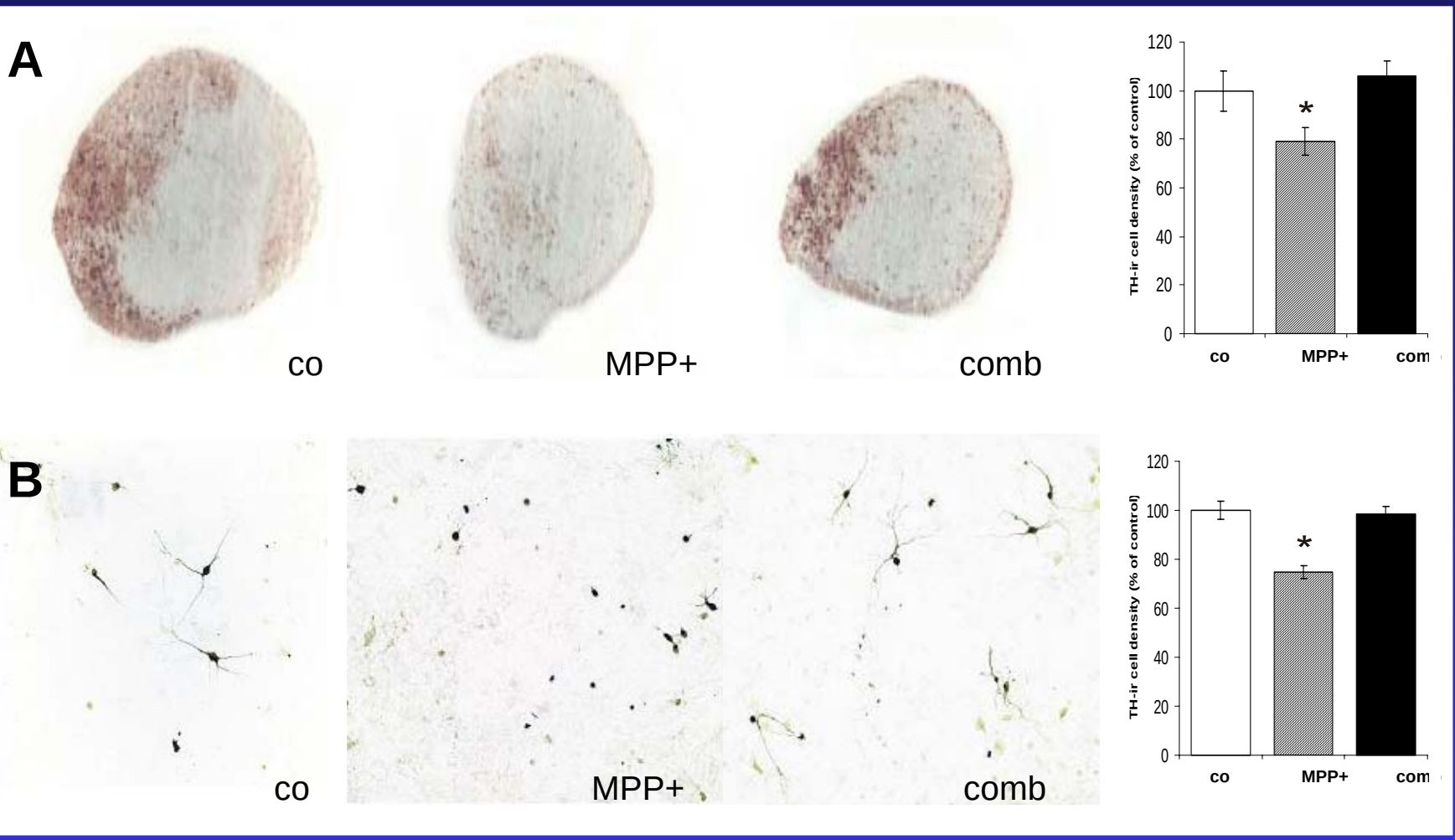


CK expression in embryonic mesencephalic cell cultures



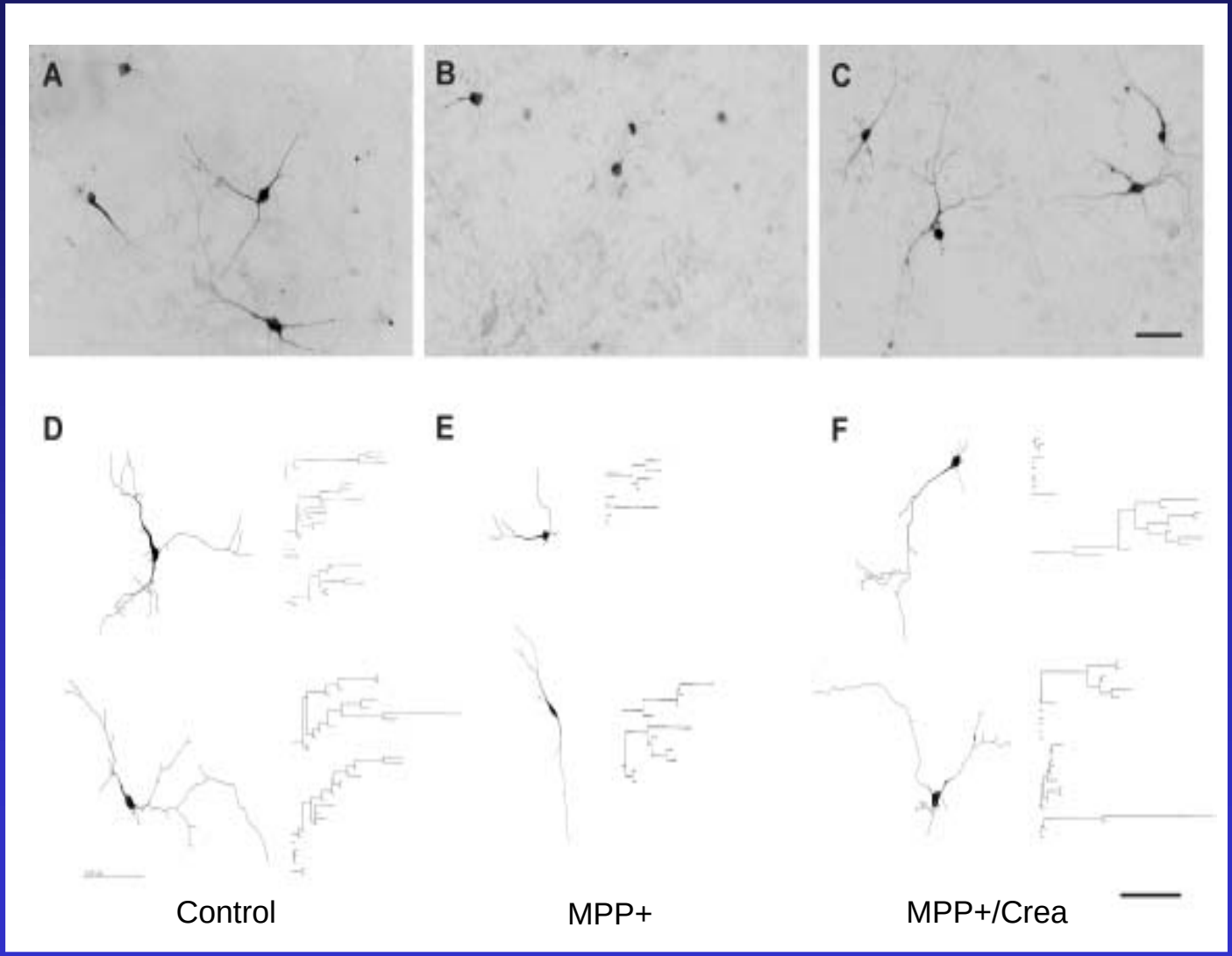
Andres et al., Neurosci 133:701-731 (2005)

Creatine protects dopaminergic neurons against MPP+ toxicity



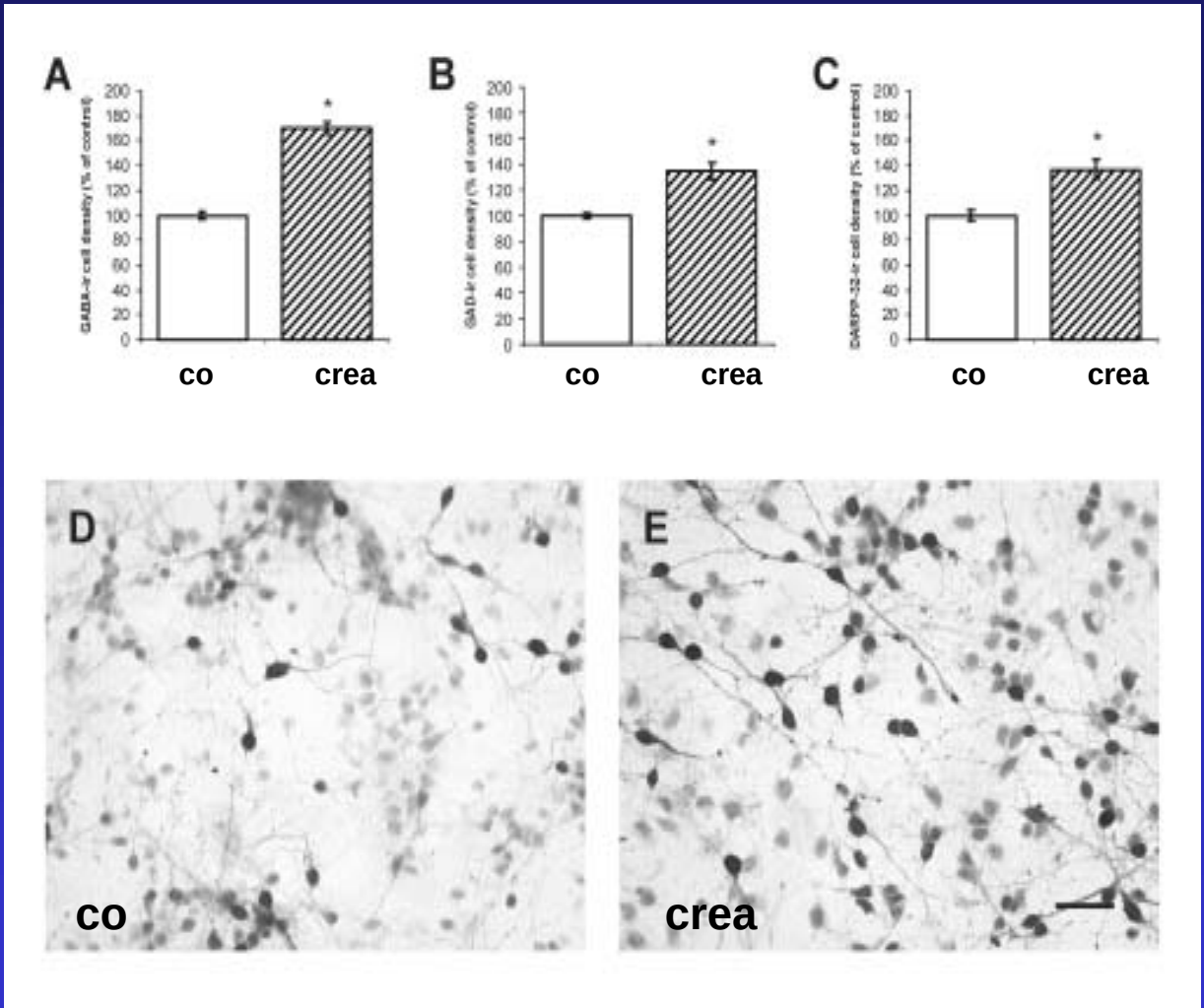
Andres et al., Cell Transplant (2005)

Creatine protects neuronal morphology against MPP^+ toxicity



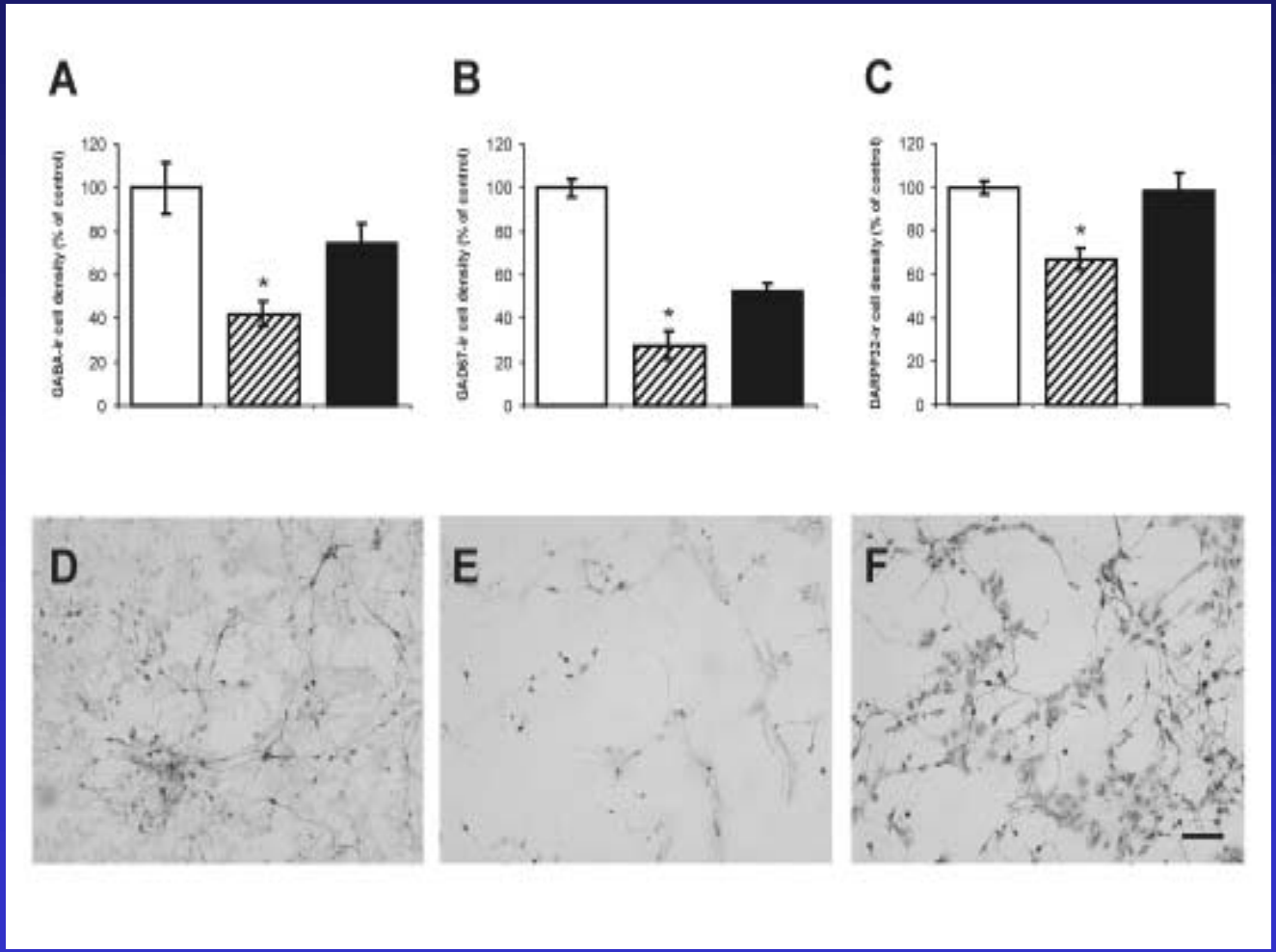
Andres et al., Neurosci 133:701-731 (2005)

Creatine promotes survival of GABA-ergic striatal cells



Andres et al., J Neurochem 95:33-45 (2005)

Creatine protects GABA-ergic neurons against 3NP-toxicity



Andres et al., J Neurochem 95:33-45 (2005)

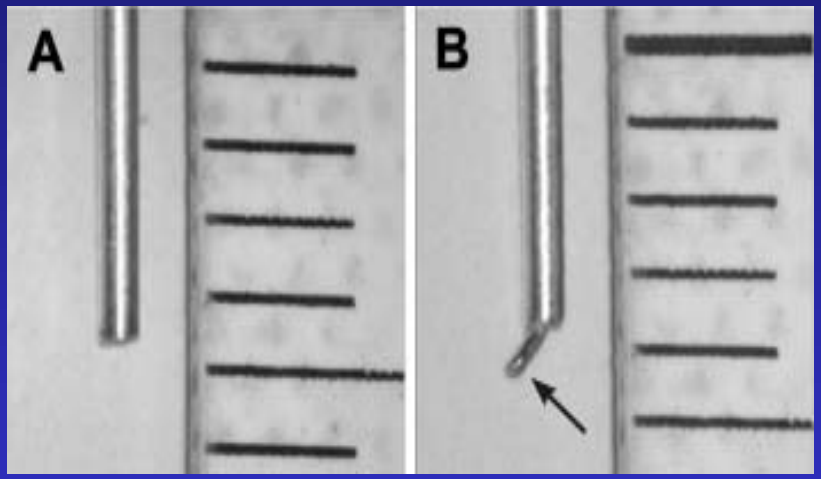
Effects of creatine: Summary

Creatine kinase isoenzymes are expressed in mesencephalic and striatal precursor cells and in the human brain.

Creatine is a potent endogenous neuroprotective factor for dopaminergic mesencephalic and GABA-ergic striatal neurons.

Elevated intracellular creatine levels promote the differentiation of neuronal precursors towards specific phenotypes.

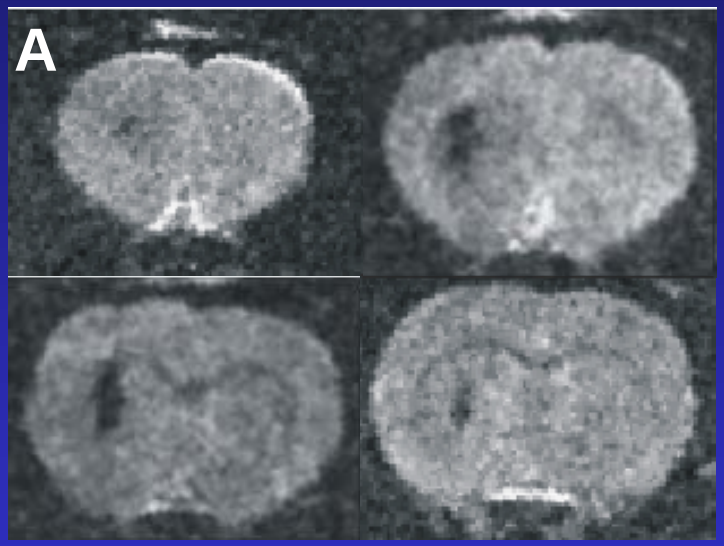
Experimental model of deep striatal ICH

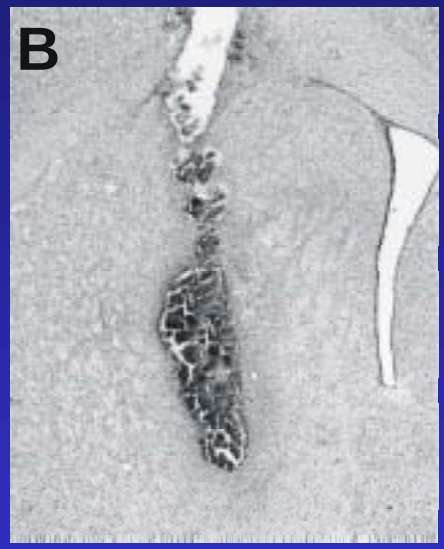


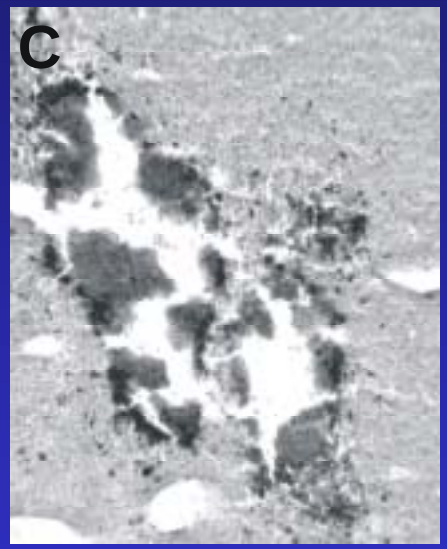
Mikroknife cather

Barth et al., Restor Neurol Neurosci, in press (2006)

MR follow-up

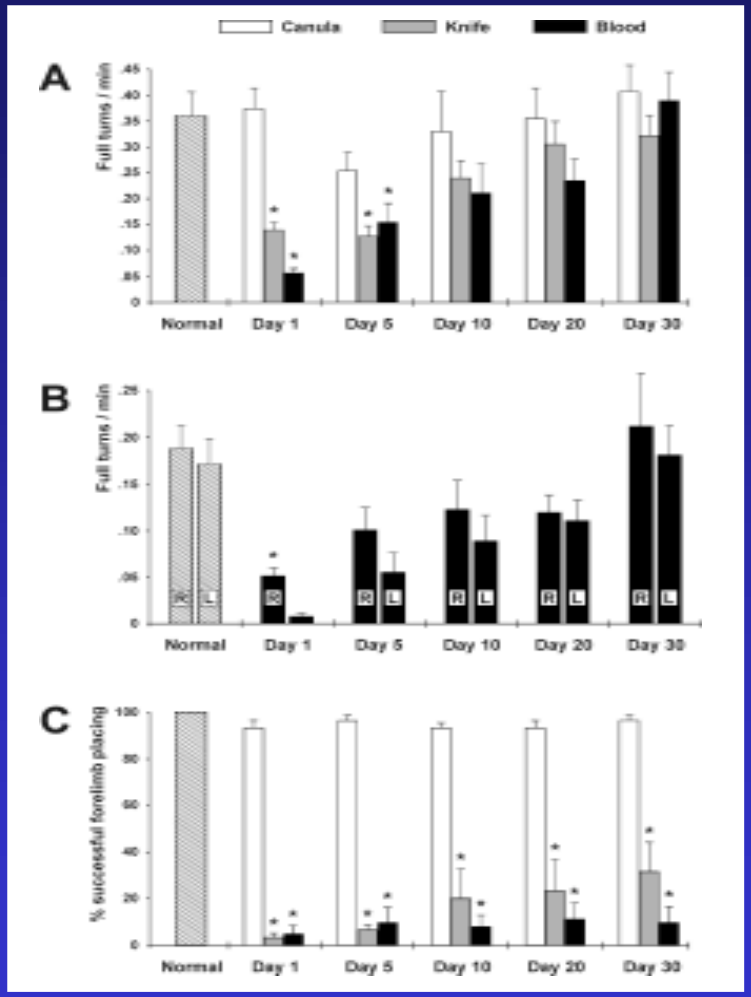




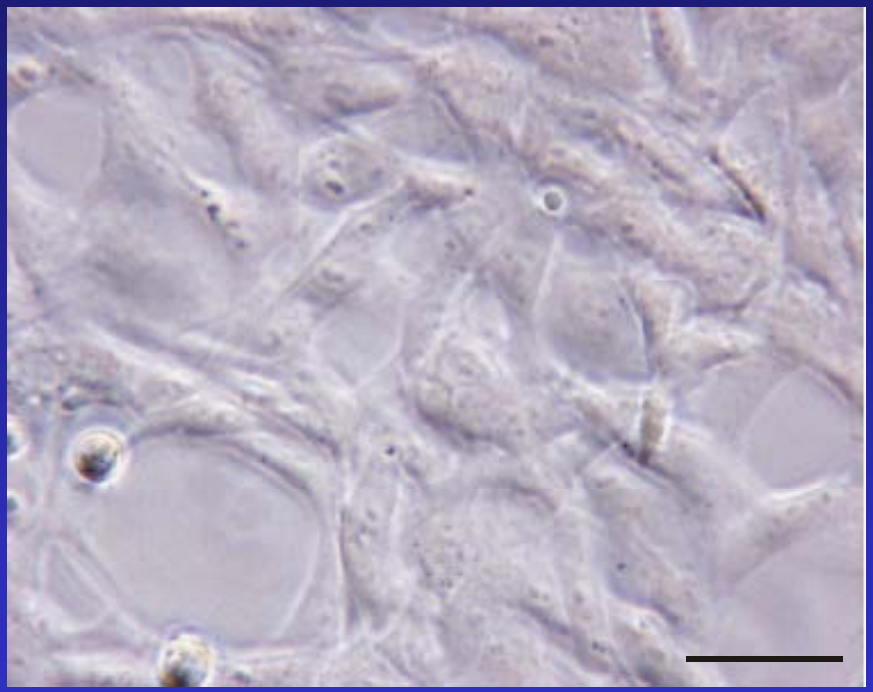


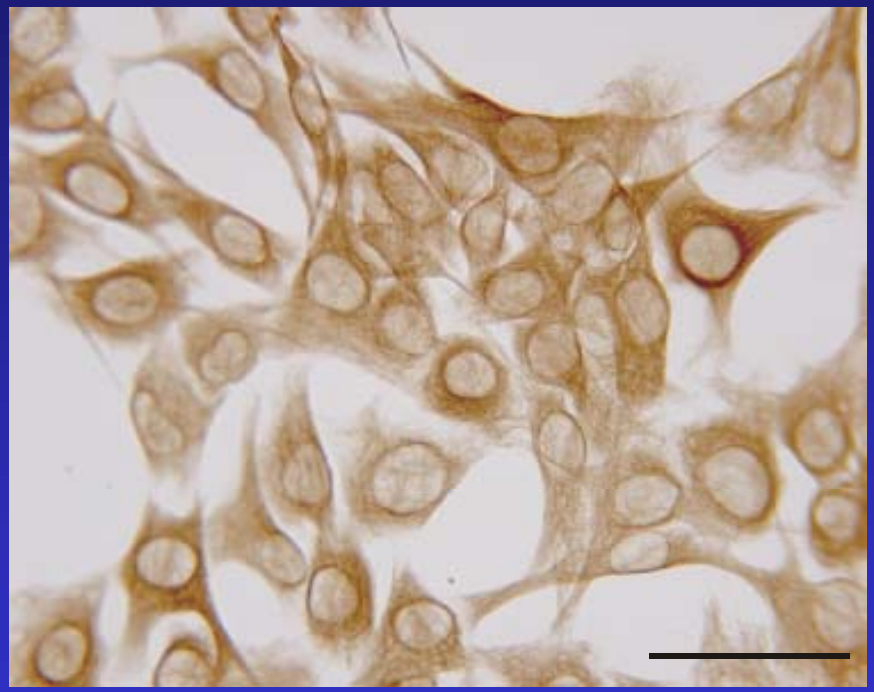
Barth et al., Restor Neurol Neurosci, in press (2006)

Behavioral analysis

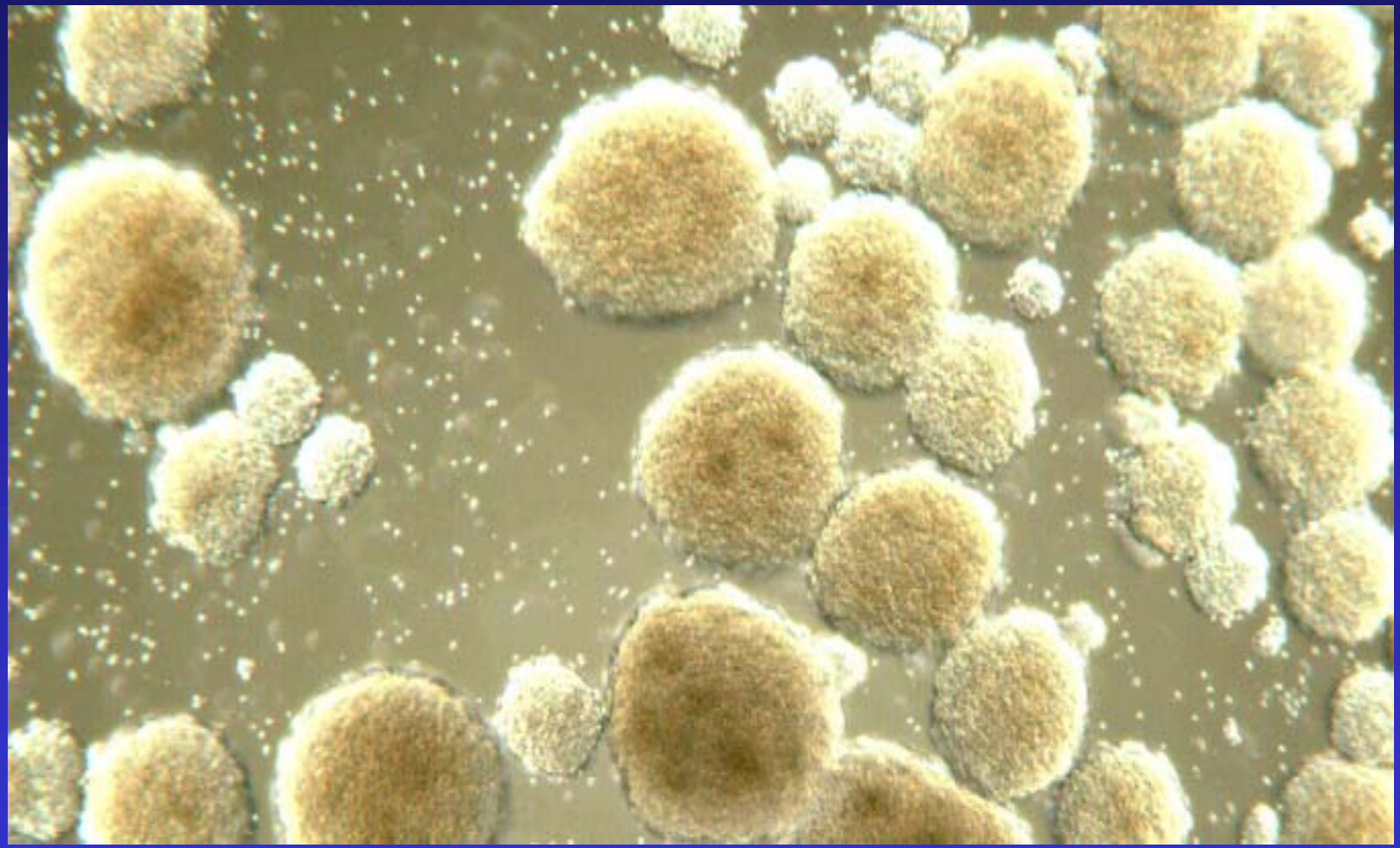


Transplantation of RN33B neuronal precursor cells

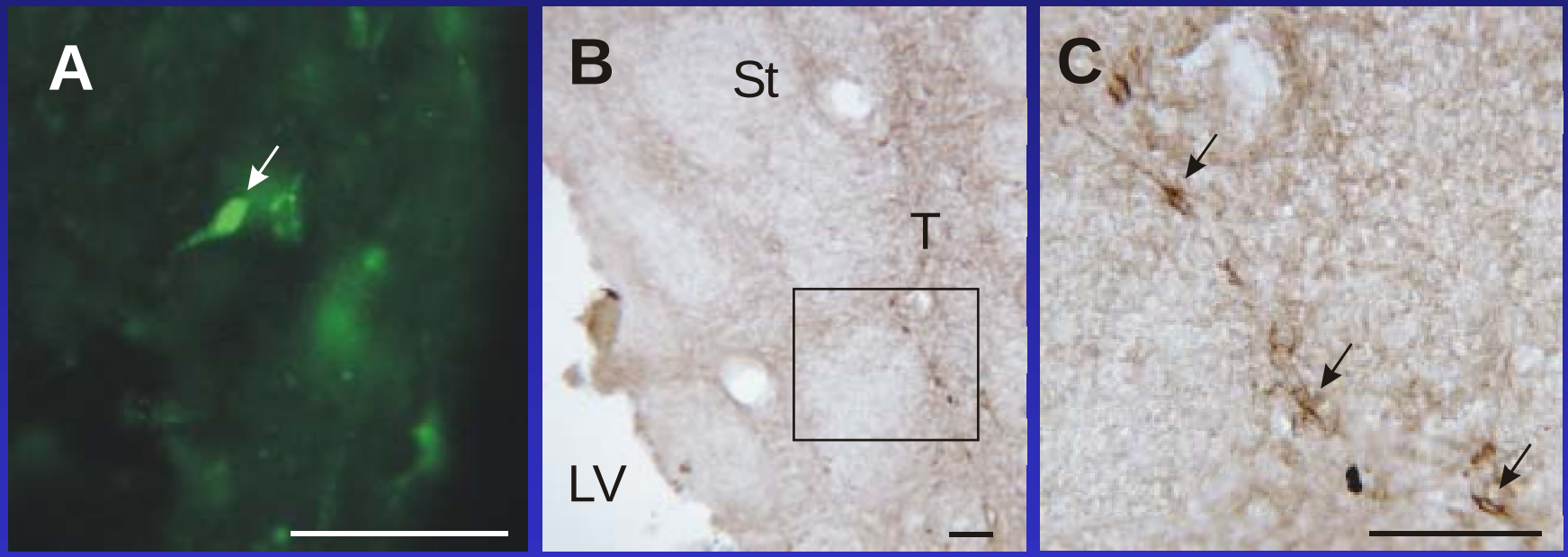




Cultured RN33B neuronal precursor cells



Intracerebral transplantation of RN33B precursor cells



Conclusions

Spontaneous ICH is a common clinical problem associated with severe residual neurological deficits.

Our experimental rat model of ICH allows the study of potential therapeutic concepts *in vivo*.

In a pilot study, stem cell transplantation in ICH was technically feasible. The cells survived in the brain and showed neuronal differentiation.

The transplanted cells have the potential to replace the function of damaged neurons.

Treatment with the neuroprotective drug creatine may improve the outcome after ICH and influence the survival of transplanted cells.

Aims of the research project

Aim I

To show that neural progenitor cells transplanted into the perihematomal region are able to survive, differentiate, establish host connections and improve the functional outcome after ICH.

Aim II

To investigate the effects of experimental ICH and stem cell transplantation on endogenous neurogenesis in the injured brain.

Aim III

To demonstrate that creatine administration provides neuroprotection against ICH and improves neuronal survival as well as functional outcome after transplantation of RN33B cells.

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