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Transcriptomics of Stroke in Aged Rodents and its Relevance for Neurorehabilitation Strategies

EPIDEMIOLOGY OF STROKE

Main Causes of Death

1. Cardiovascular Disease

2. Cancer

3. **Stroke** (main cause of disability)

- 80 % cerebral ischemia, 20% intracerebral hemorrage

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Epidemiology of Stroke

- In Germany stroke mortality rate:
  100/100000 persons per year
- In East Europe far higher
  Romania 210/100000 persons per year

- Whereas in Western Europe the numbers decline stepwise
- The incidence of stroke is still increasing in Eastern Europe

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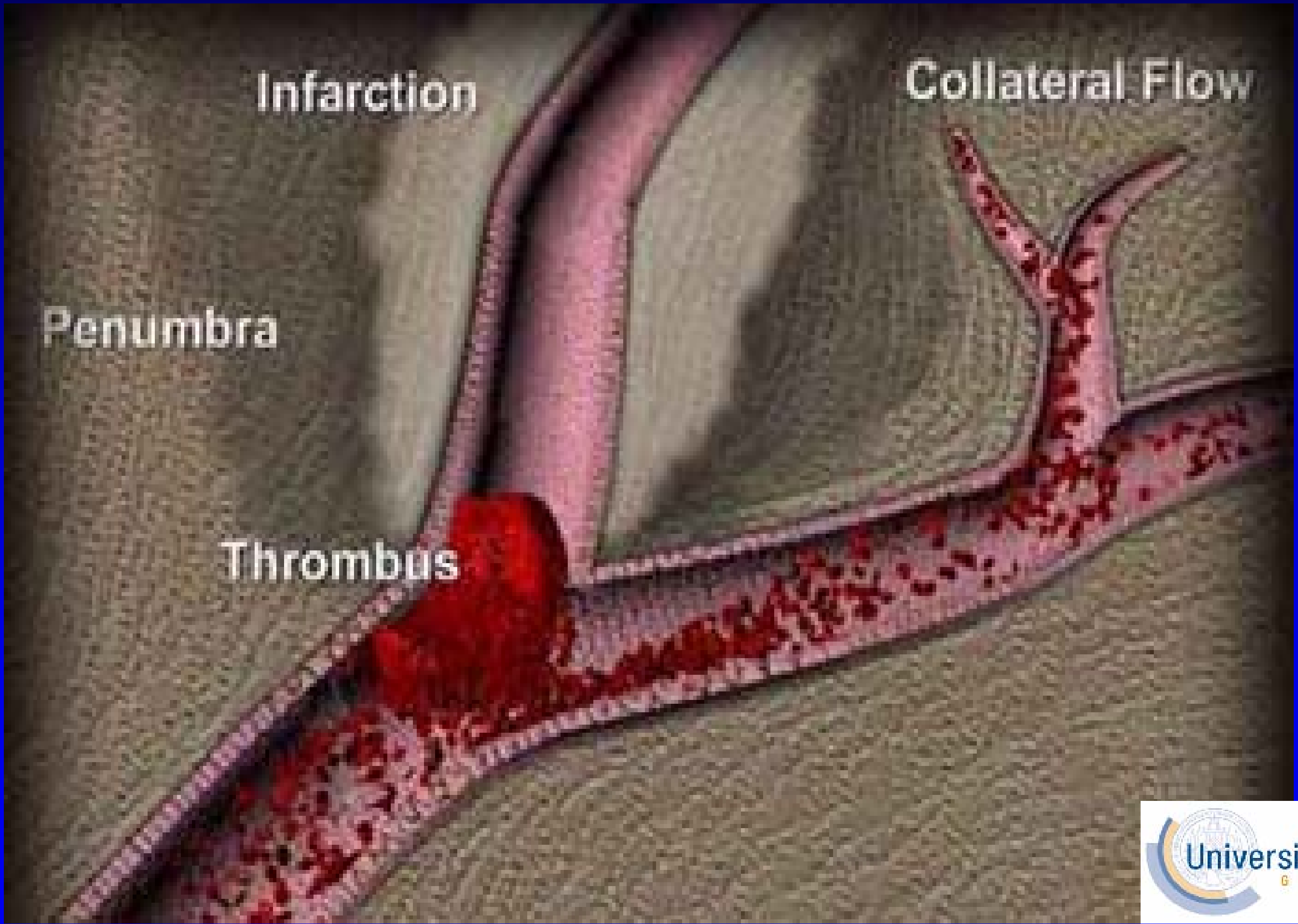
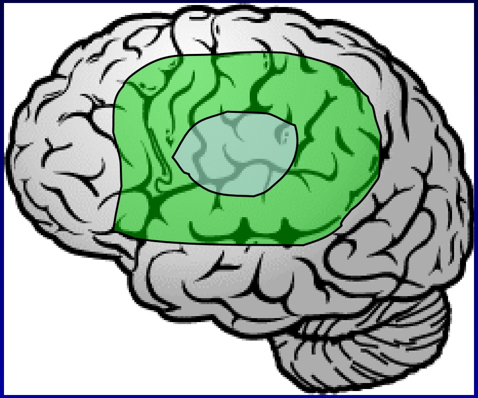
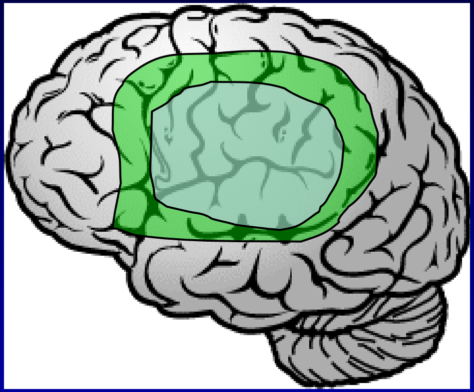
Intracerebral vessel occlusion has a strong *AGE* dependency

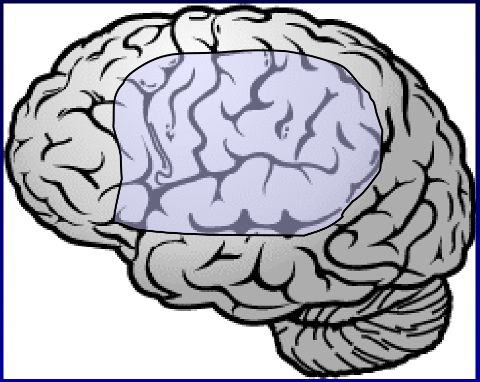


Ischemic Penumbra

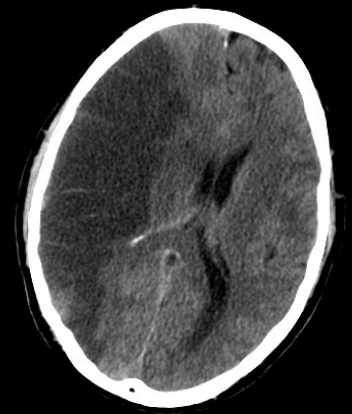


hours





MRI Image of Stroke



Ginsberg MD u. Pulsinelli WA: Ann Neurol. 1994;36:553-554

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Overwiew:

1. **Stroke Model in Aged Rodents**
2. **Genomics of Stroke in Aged Rodents**
3. **Strategies to improve neurorehabilitation in aged rodents**
4. **Conclusions**

Why use aged animals to study rehabilitation after stroke?

Although it is well known that aging is a risk factor for stroke, the majority of experimental studies of stroke have been performed on young animals, and therefore may not fully replicate the effects of ischemia on neural tissue in aged subjects.

In this light, the aged post-acute animal model is clinically most relevant to stroke rehabilitation and cellular studies, a recommendation done by the STAIR committee and more recently by the Stroke Progress Review Group.

STAIR Criteria
(Stroke Academic and Industry Round Table)

- **Reproducibility of results proven in many different laboratories wordlwide**
- **Efficiency in many different species**
- **Testing on aged animals**
- **Efficiency in both transient and permanent ischemia**
- **Establish the therapeutic time-window**
- **Establish the dose-efficiency relationship**
- **Monitoring of physiologic parameters during the experiments**
- **Does the infarct volume correlate with functional recovery ?**
- **Longterm studies of the above parameters (min. 4 Weeks)**

STAIR 1999

Beam- Young Control

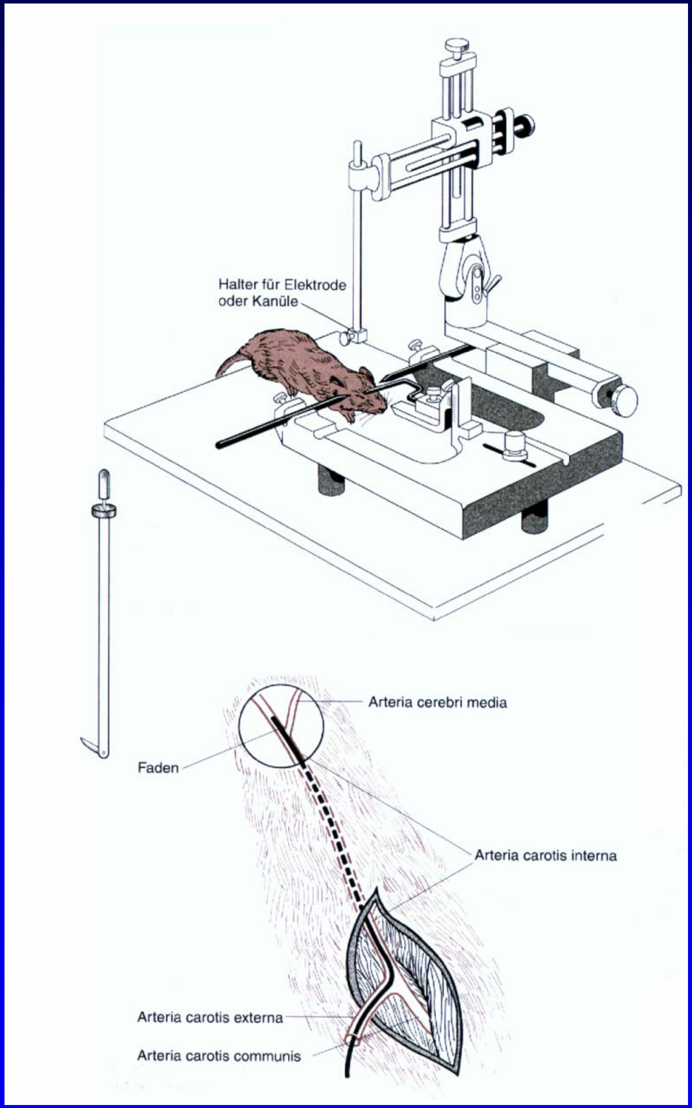
Beam- Young Stroke

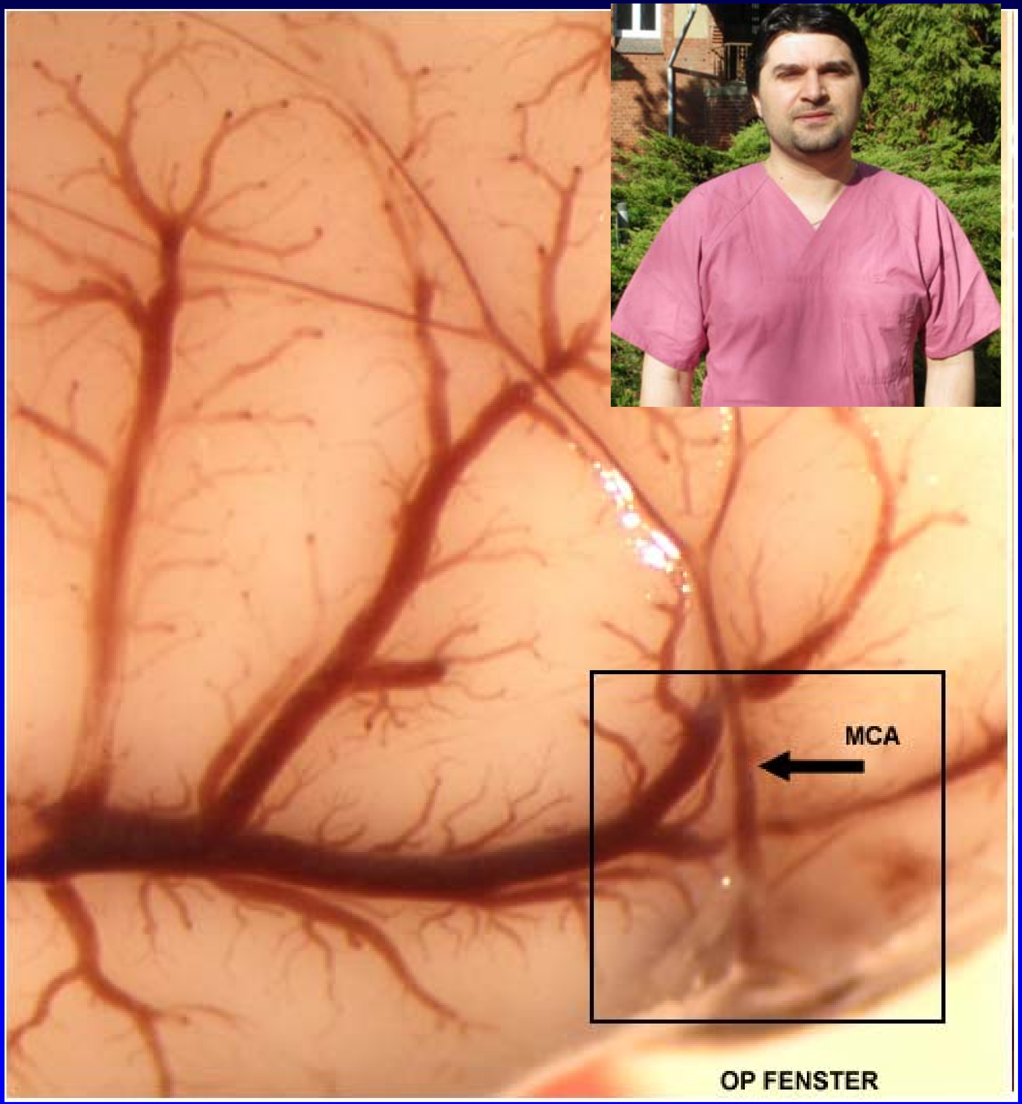
Beam- Old Control

Beam- Old Stroke

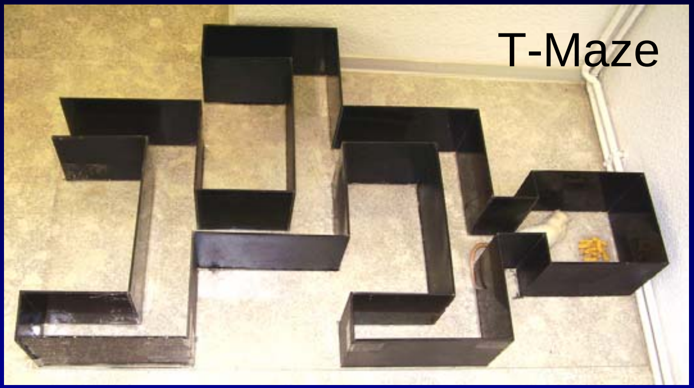
Brain of a Patient after MCA Occlusion

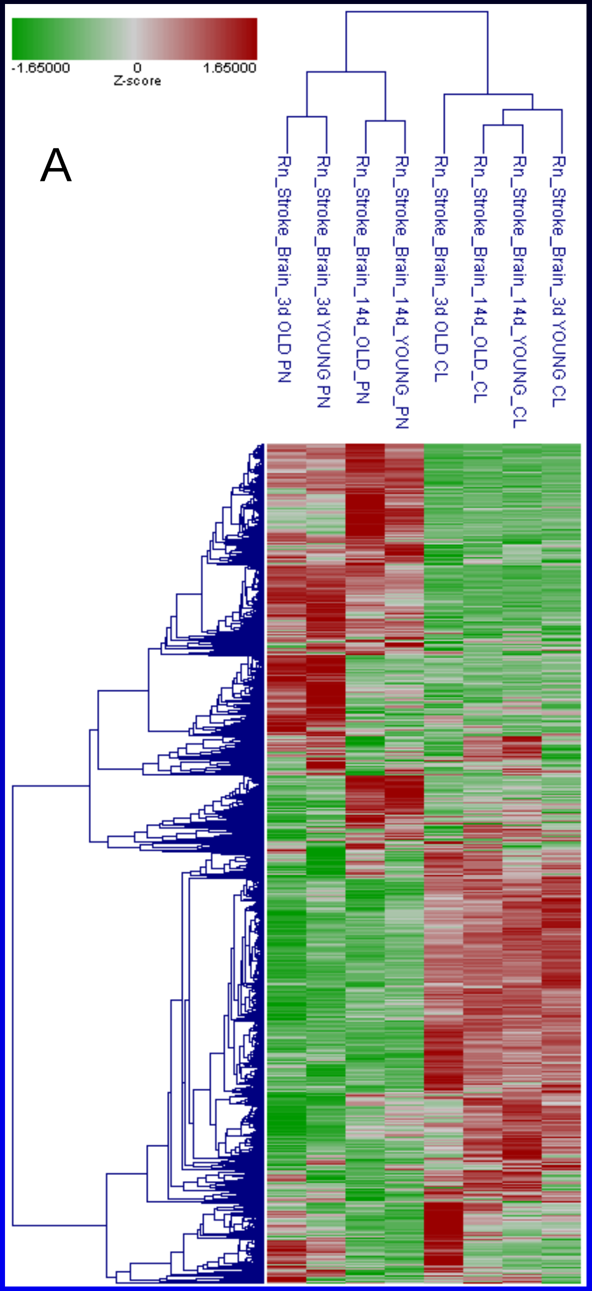
Rat Modell for Cerebral Ischemia





Summary of functional tests after stroke

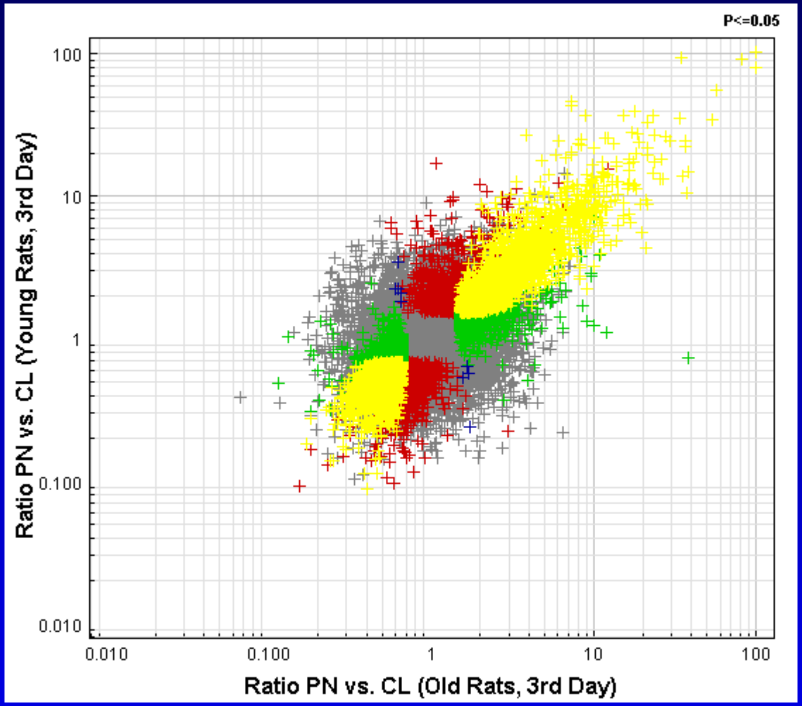




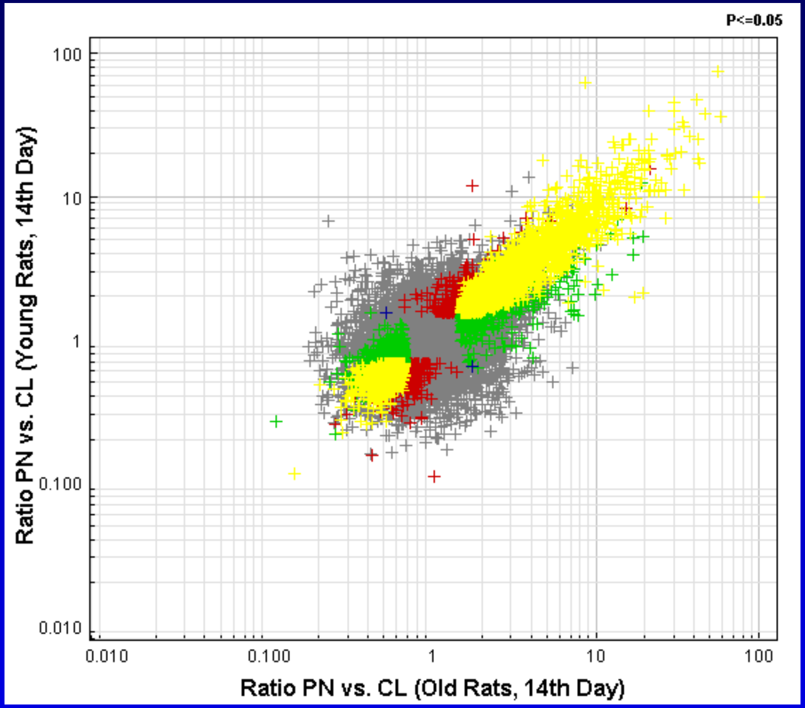
Changes of gene expression in response to stroke.
(A) Dendrogram of two-dimensional hierarchical clustering analysis of all 9,494 transcript-specific probe sets which indicate differentially expressed genes

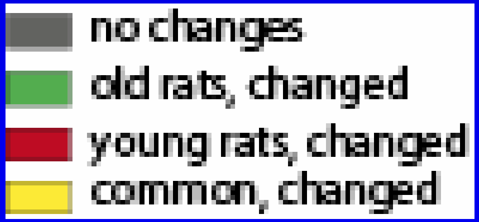
Transcriptomics of Stroke: Scatter Plots

3 days post-stroke

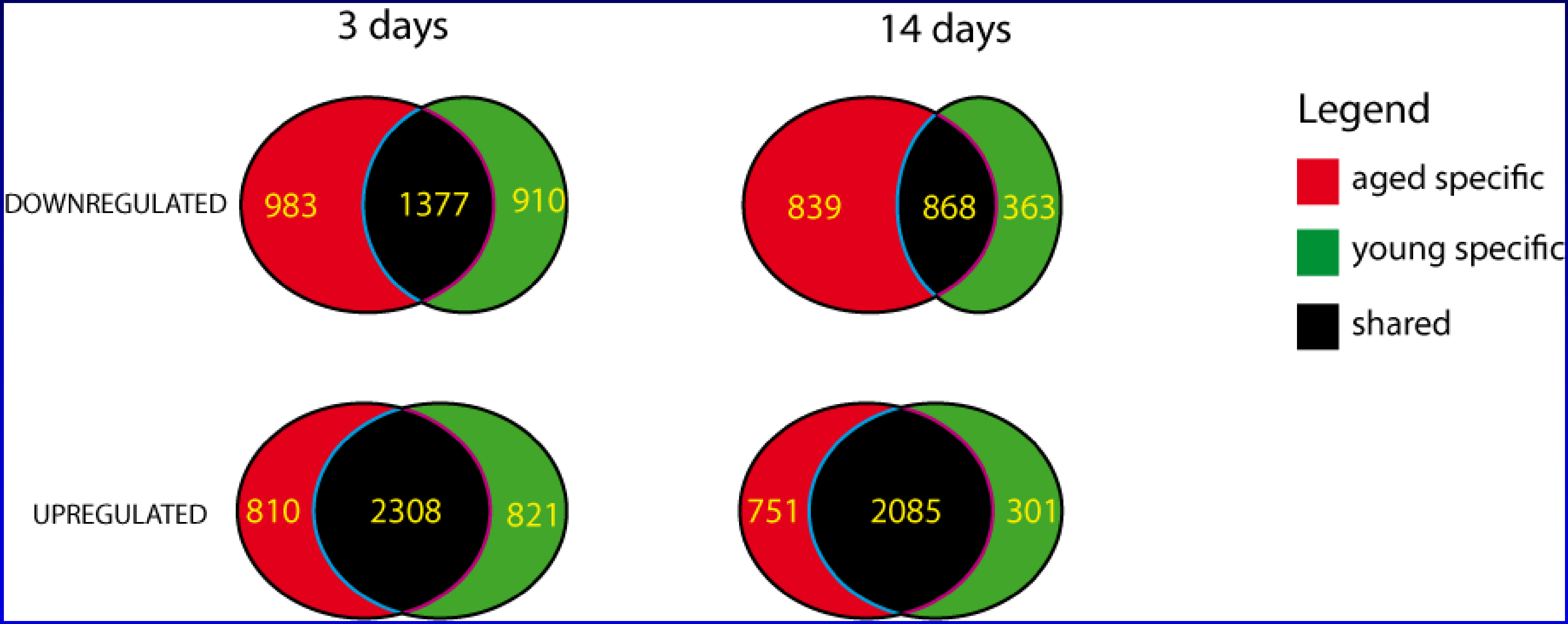


14 days post-stroke

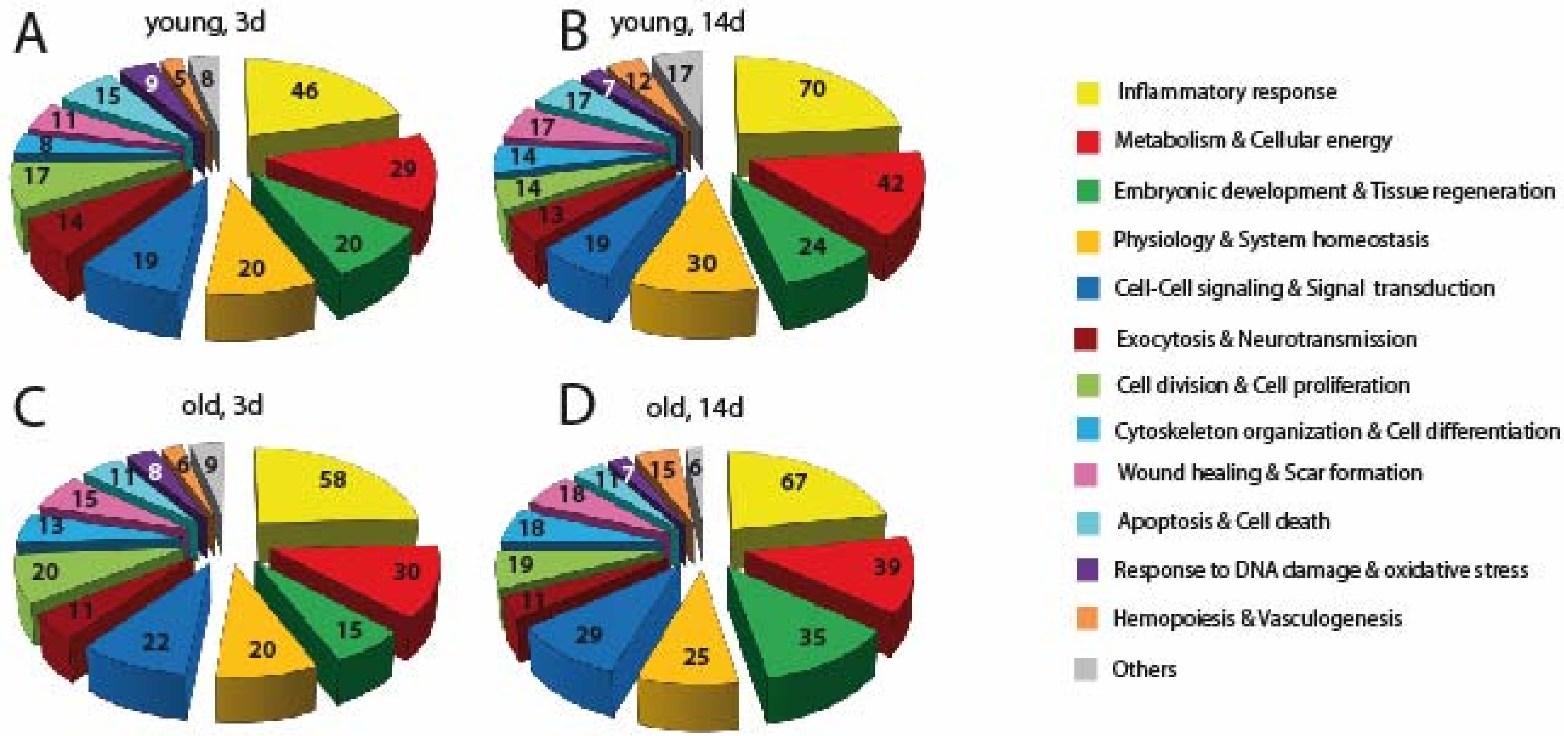




Transcriptomics of Stroke: Venn Diagramm

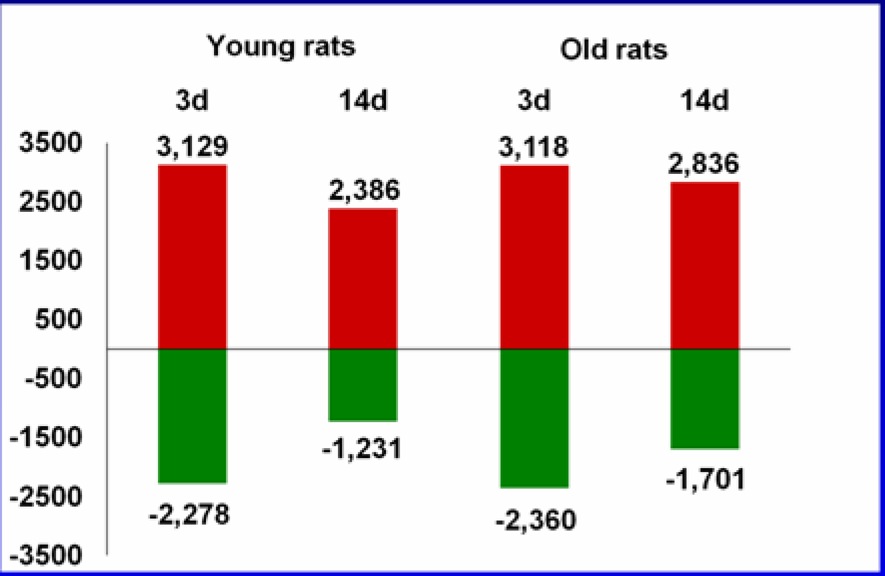


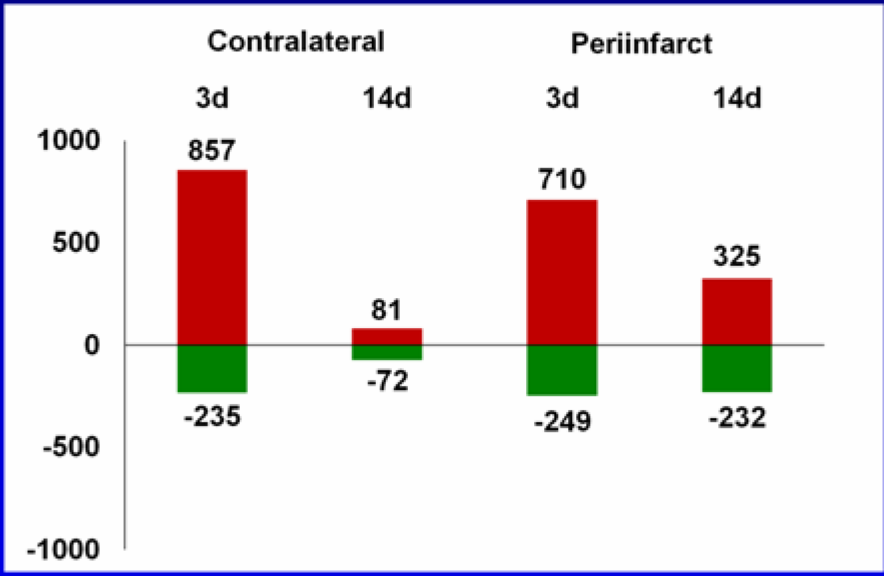
Transcriptomics of Stroke: Pie Charts



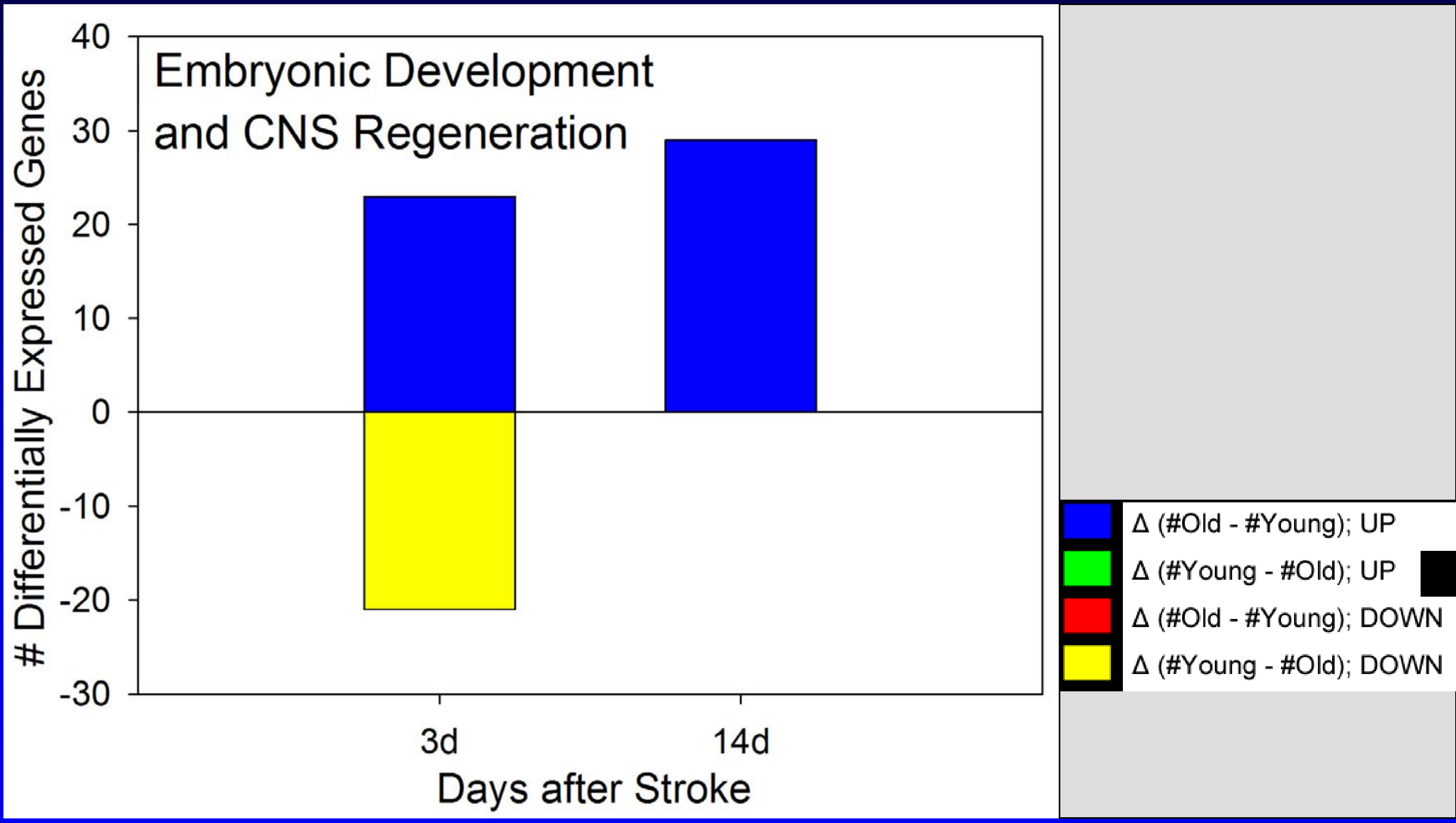
Graphical representation of significantly affected/changed biological process GO-terms per fundamental process

Transcriptomics of Stroke. Powerful effect of age





Transcriptomics of Stroke. Disregulation of gene expression required for Embryonic development & CNS regeneration

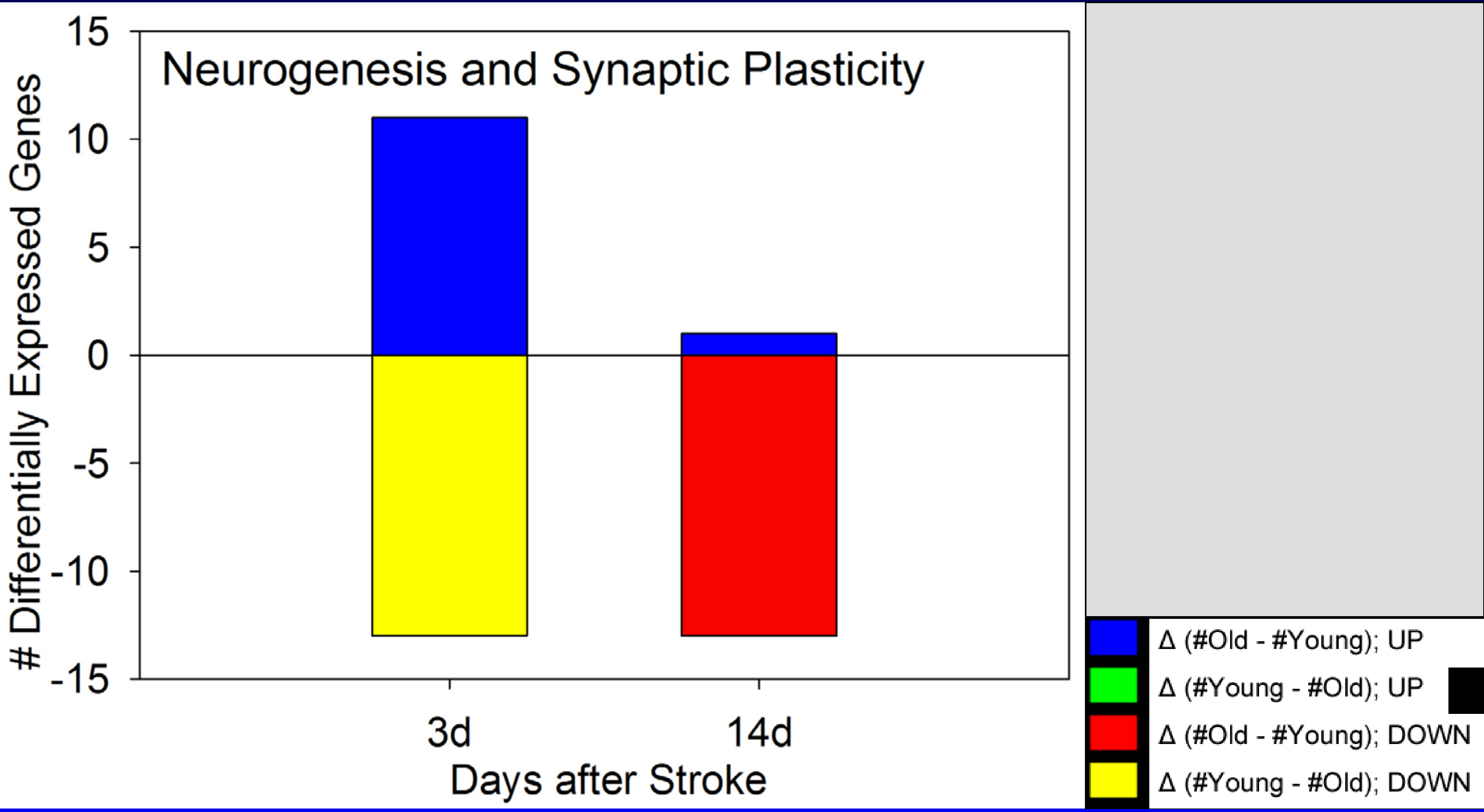


Transcriptomics of Stroke. Disregulation of gene expression required for Embryonic development & CNS regeneration. Examples of regulated genes

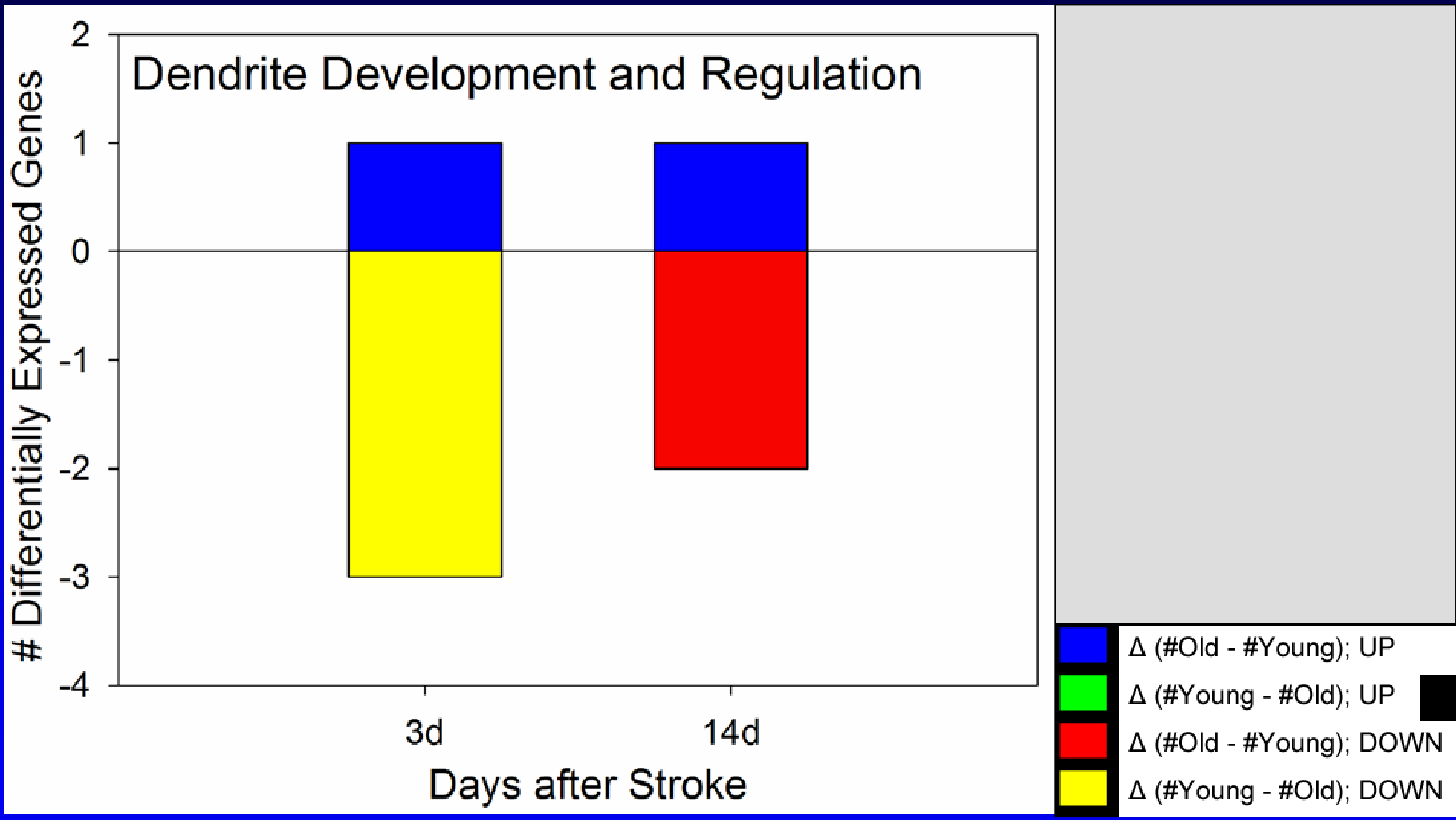
Embryonic Development and CNS Regeneration

PCR	Assession number	3 days				14day				Naive	
		Young PI vs CL		Old PI vs CL		Young PI Vs CL		Old PI Vs CL		Young vs Old	
		fold change	SD	fold change	SD	fold change	SD	fold change	SD	fold change	SD
Aldh1a2	NM_053896	7.62	1.01	9.62	0.05	10.67	0.05	12.54	0.23	1.03	0.09
Cdk5rap2	XM_575844	9.71	1.24	5.43	0.00	2.70	0.12	1.95	0.22	1.02	0.45
Crabp2	NM_017244	5.99	0.38	5.15	0.89	9.88	0.79	7.14	1.00	0.74	0.08
Cthrc1	NM_172333	12.60	1.62	14.38	0.28	4.59	0.02	11.00	1.51	1.70	0.53
Mafb	NM_019316	5.05	0.57	9.33	2.02	4.90	0.29	8.06	0.71	1.94	0.22
Nr2f2	NM_080778	1.93	0.38	2.17	0.34	3.37	0.40	5.03	0.23	5.47	0.72
Rbp1	NM_012733	11.43	1.52	11.52	1.47	5.85	0.03	8.87	0.13	0.72	0.11
Tpm3	NM_057208	1.13	0.14	1.69	0.13	0.48	0.13	1.12	0.15	1.19	0.07
Wnt4	NM_053402	0.77	0.05	1.23	0.02	2.77	0.17	4.11	0.14	3.26	1.18

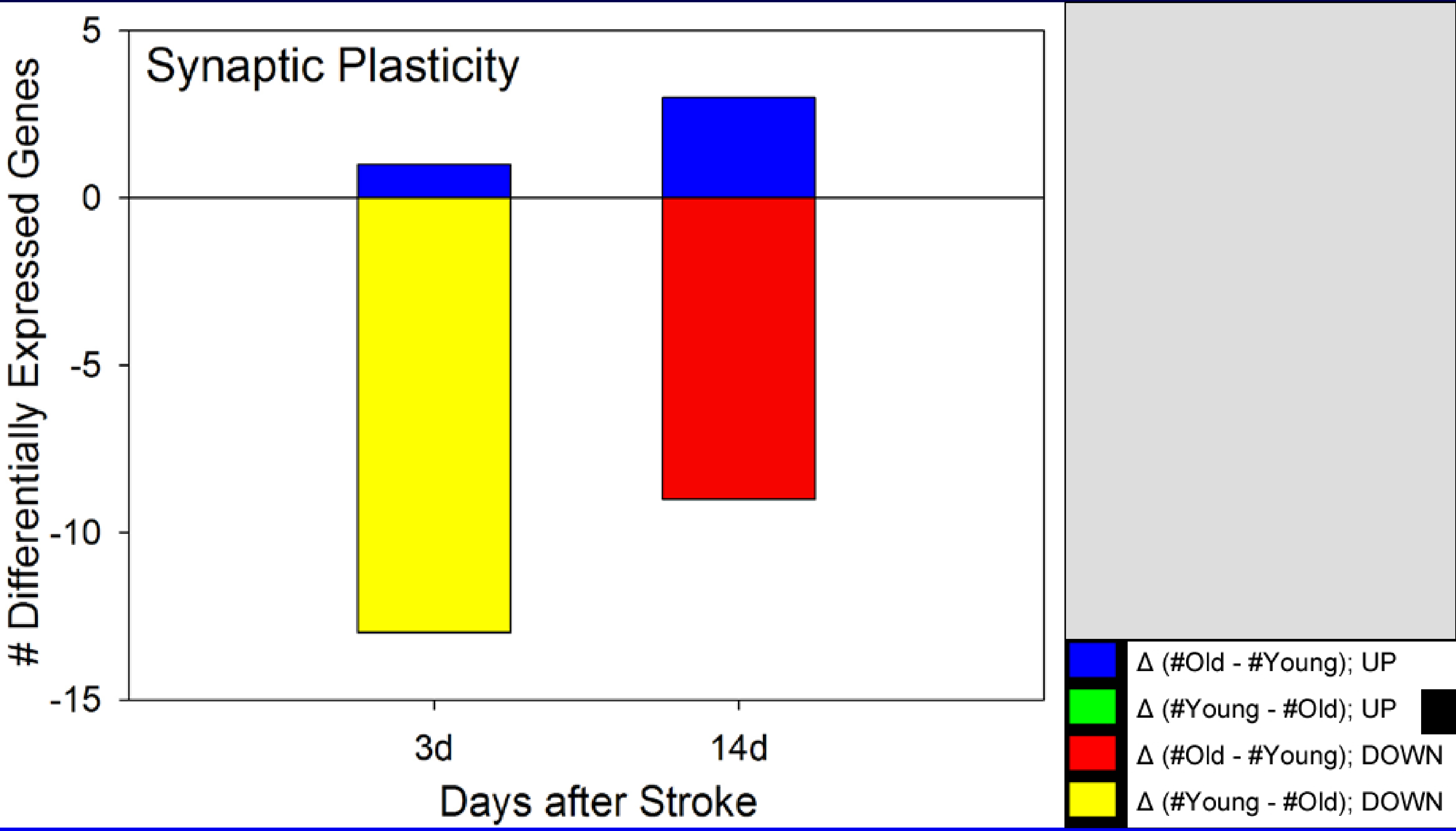
Transcriptomics of Stroke. Disregulation of gene expression required for Neurogenesis & Synaptic plasticity



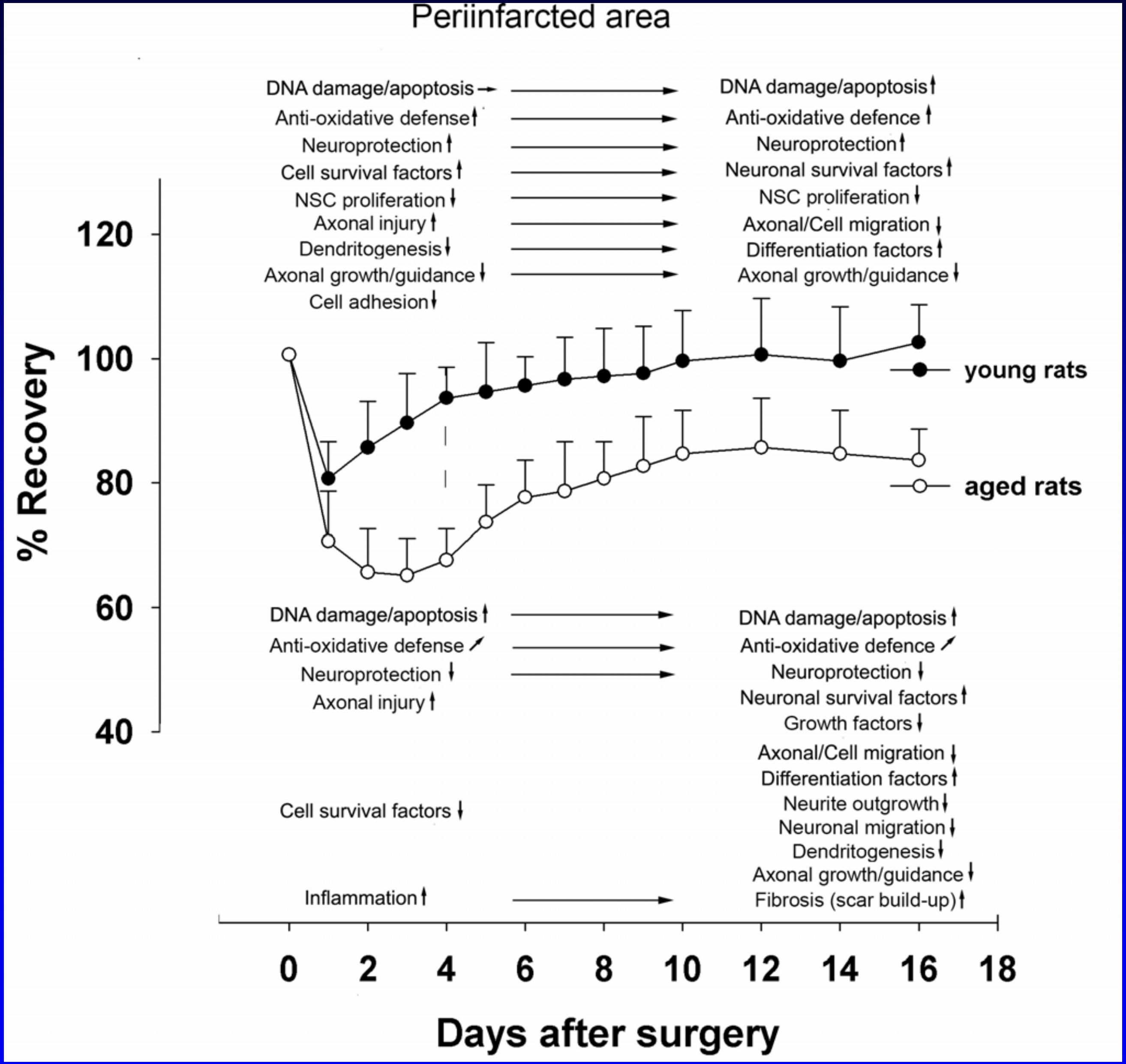
Transcriptomics of Stroke. Disregulation of gene expression required for Embryonic development & CNS regeneration



Transcriptomics of Stroke. Disregulation of gene expression required for Embryonic development & CNS regeneration



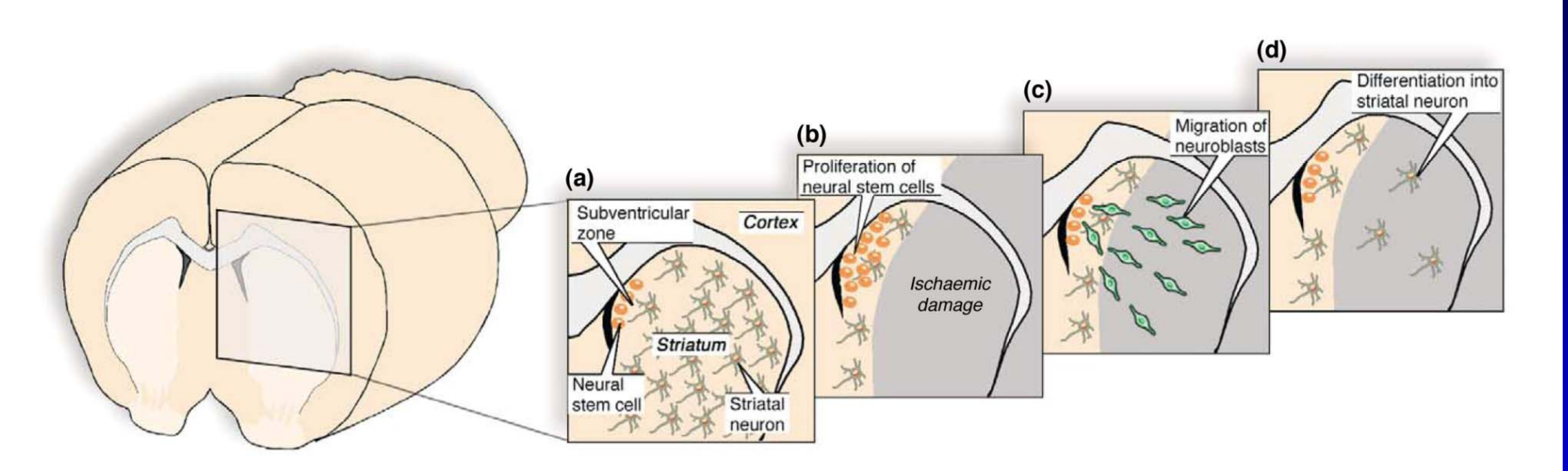
Summary of transcriptional events in the perilesional area of young and aged rats after stroke



Pilot studies aimed at improving recovery of function in aged rats after stroke

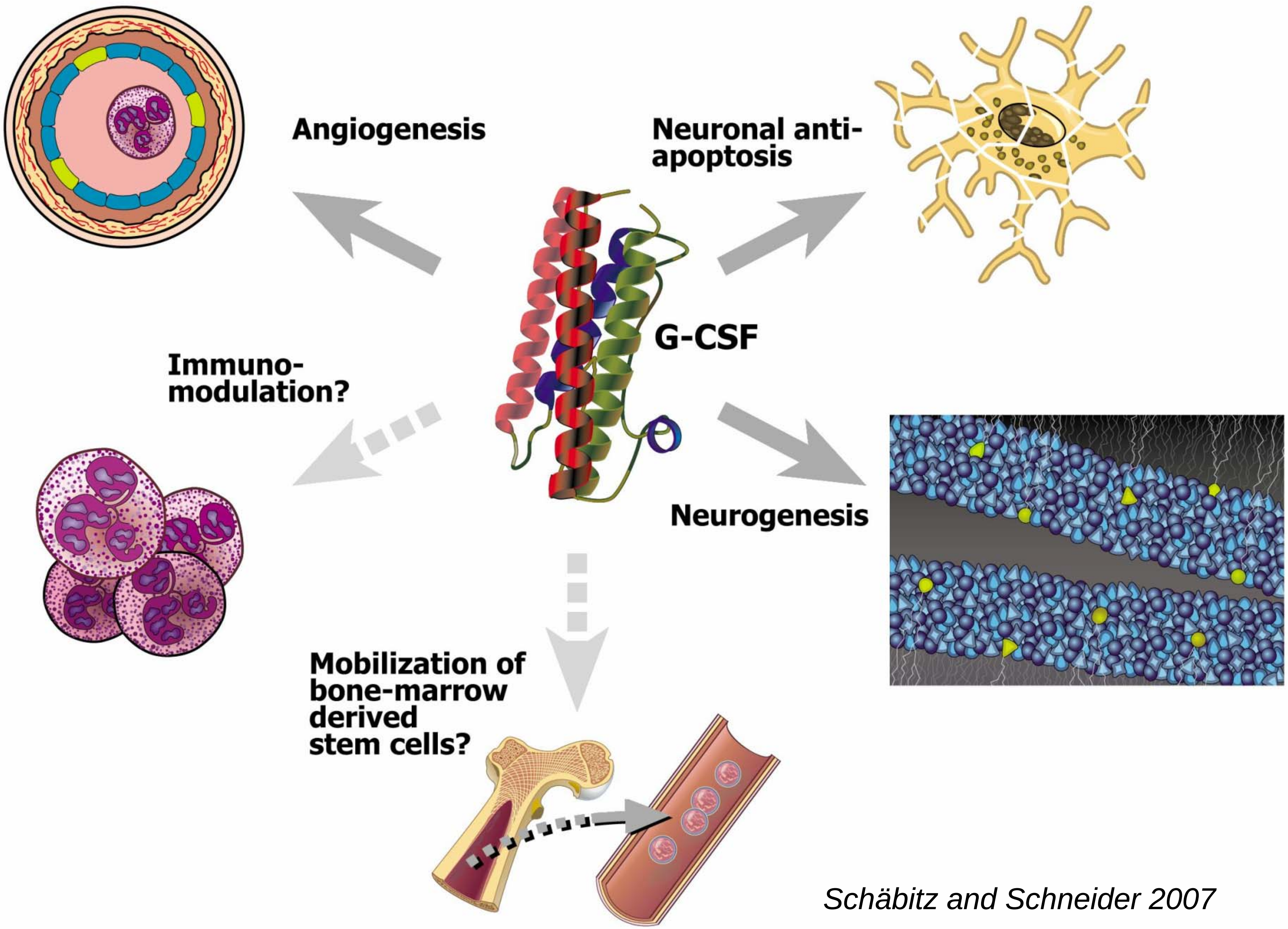
Stimulation of endogenous neurogenesis Indirectly using Chemoattractants

Neurogenesis is fully functional in the subventricular zone of the adult brain



Kokaia & Lindvall, Current Opinion in Neurobiology 2003

G-CSF, multimodale Mechanismen in der Schlaganfalltherapie

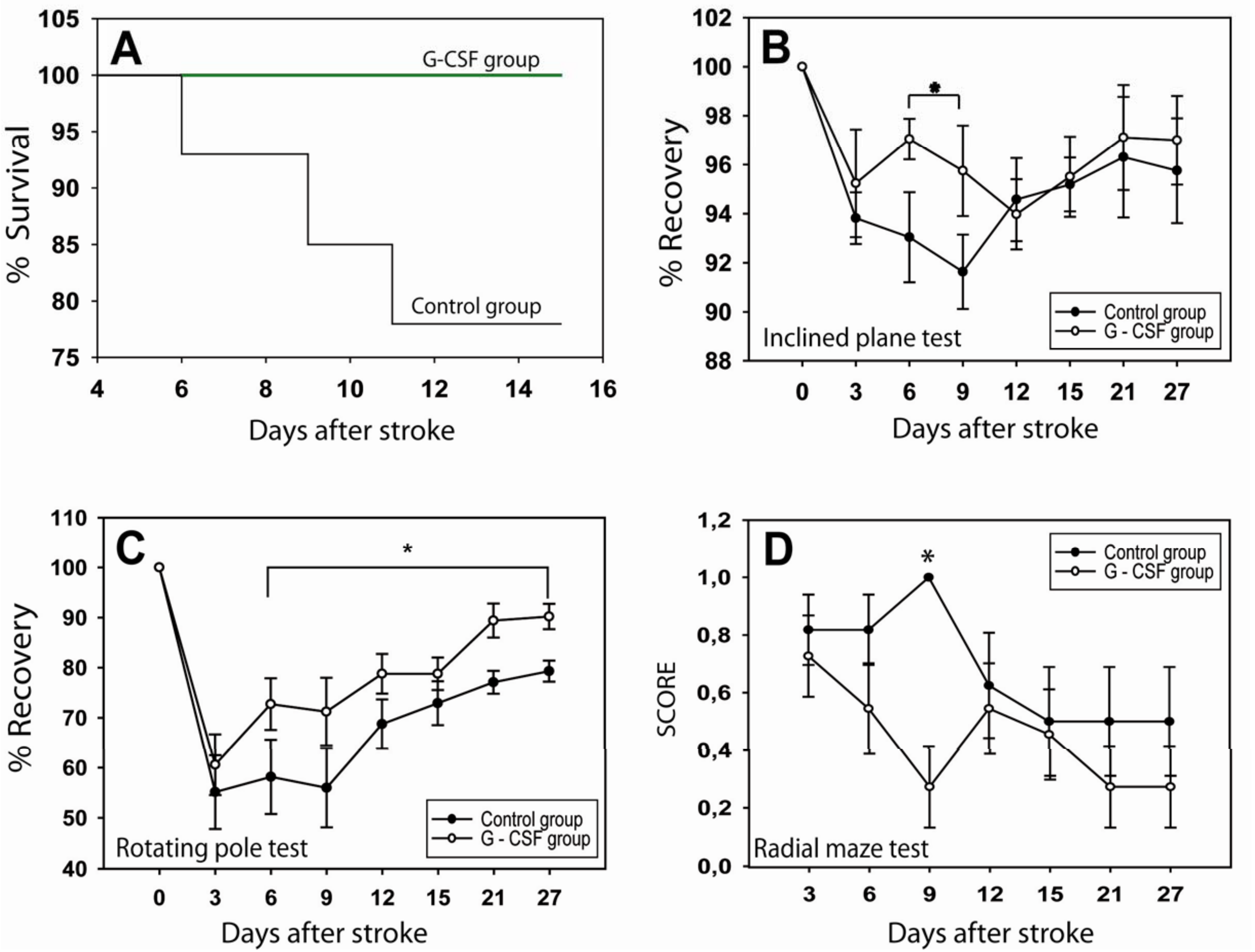


Schäbitz and Schneider 2007

Pilot studies aimed at improving recovery of function in aged rats after stroke

Daily treatment with G-CSF of aged rats after stroke significantly reduces the mortality rate

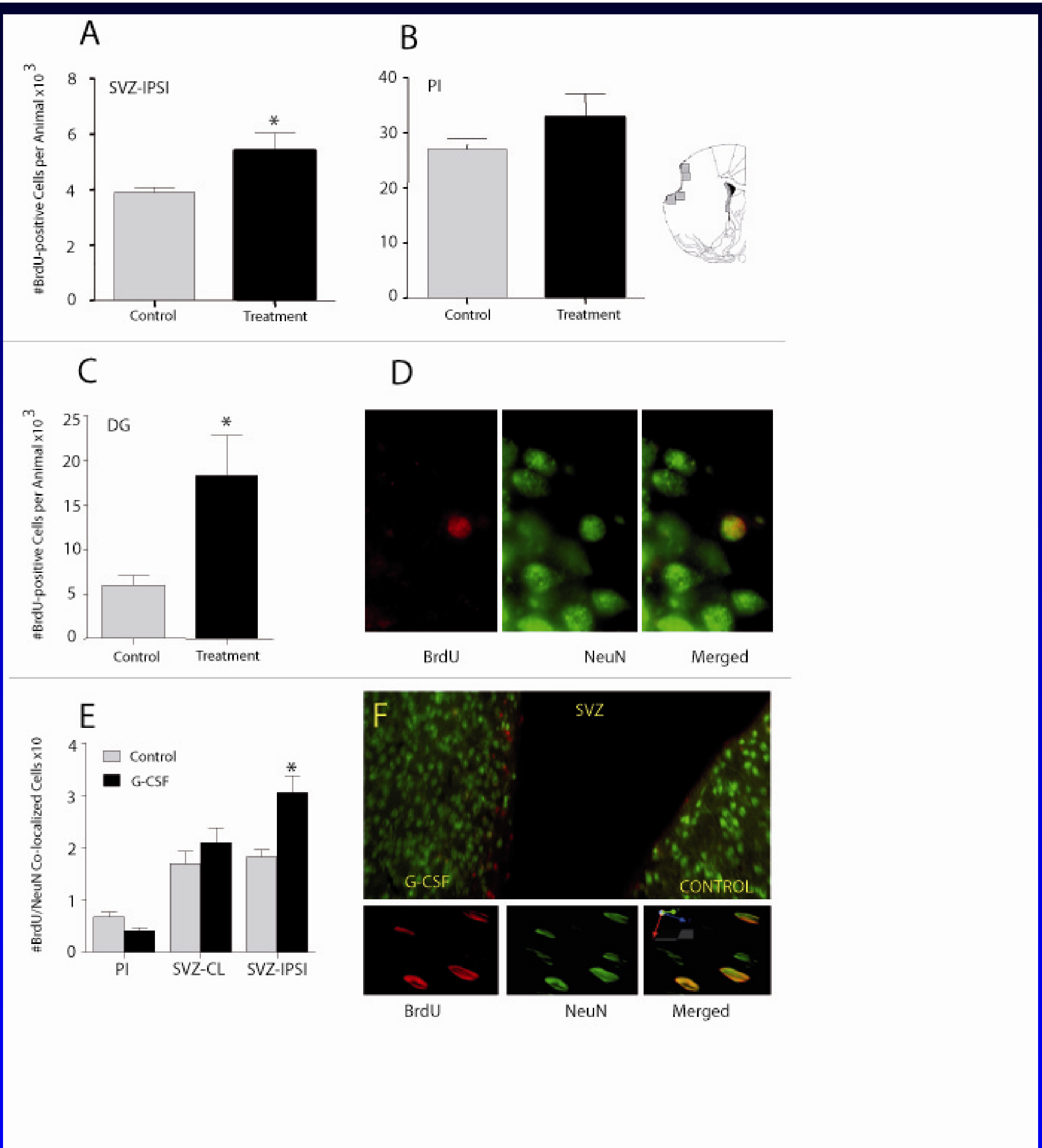
G-CSF treatment after stroke significantly improved mortality rate and performance in several but <u>not all</u> functional tests.



Pilot studies aimed at improving recovery of function in aged rats after stroke

Daily treatment with G-CSF of aged rats after stroke significantly reduces the mortality rate

Effect of the G-CSF treatment on cellular proliferation and neurogenesis.

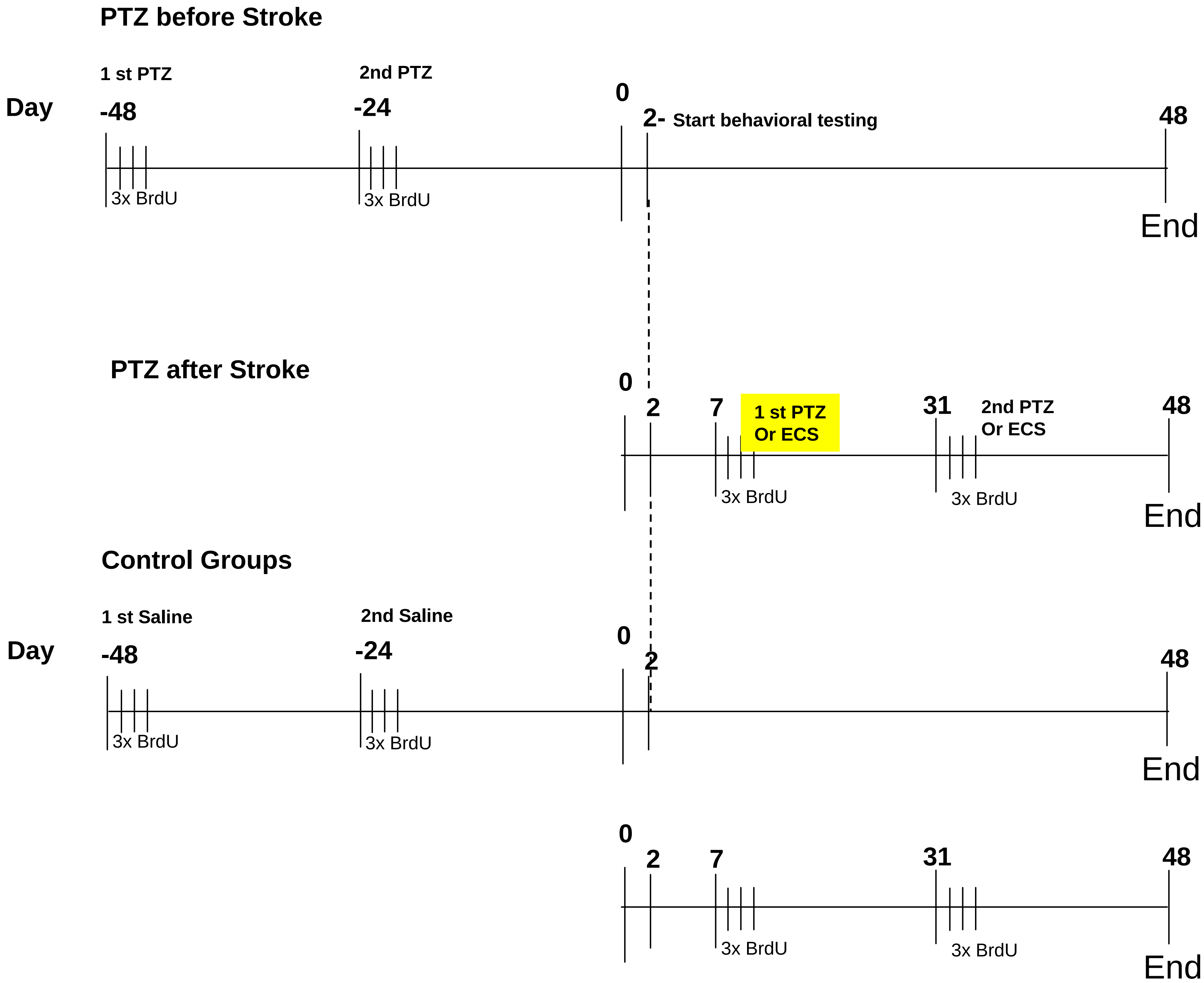


Take home messages (I):

Our results suggests that the G-CSF treatment in aged rats has a survival enhancing capacity and a beneficial effect on functional outcome most likely via supportive cellular processes such as neurogenesis

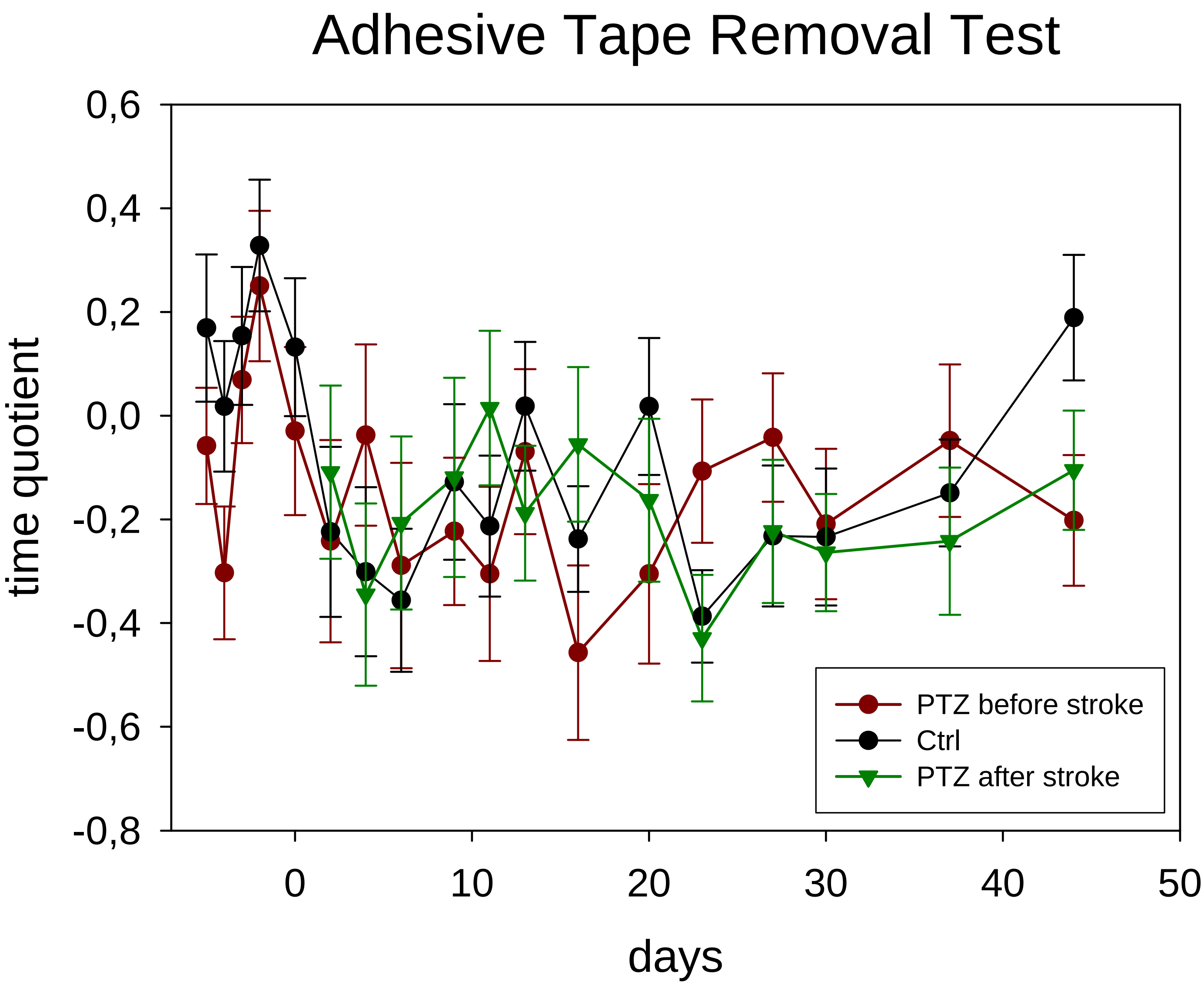
Pilot studies aimed at improving recovery of function in aged rats after stroke

Stimulation of endogeneous neurogenesis by chemical and by small electrical currents

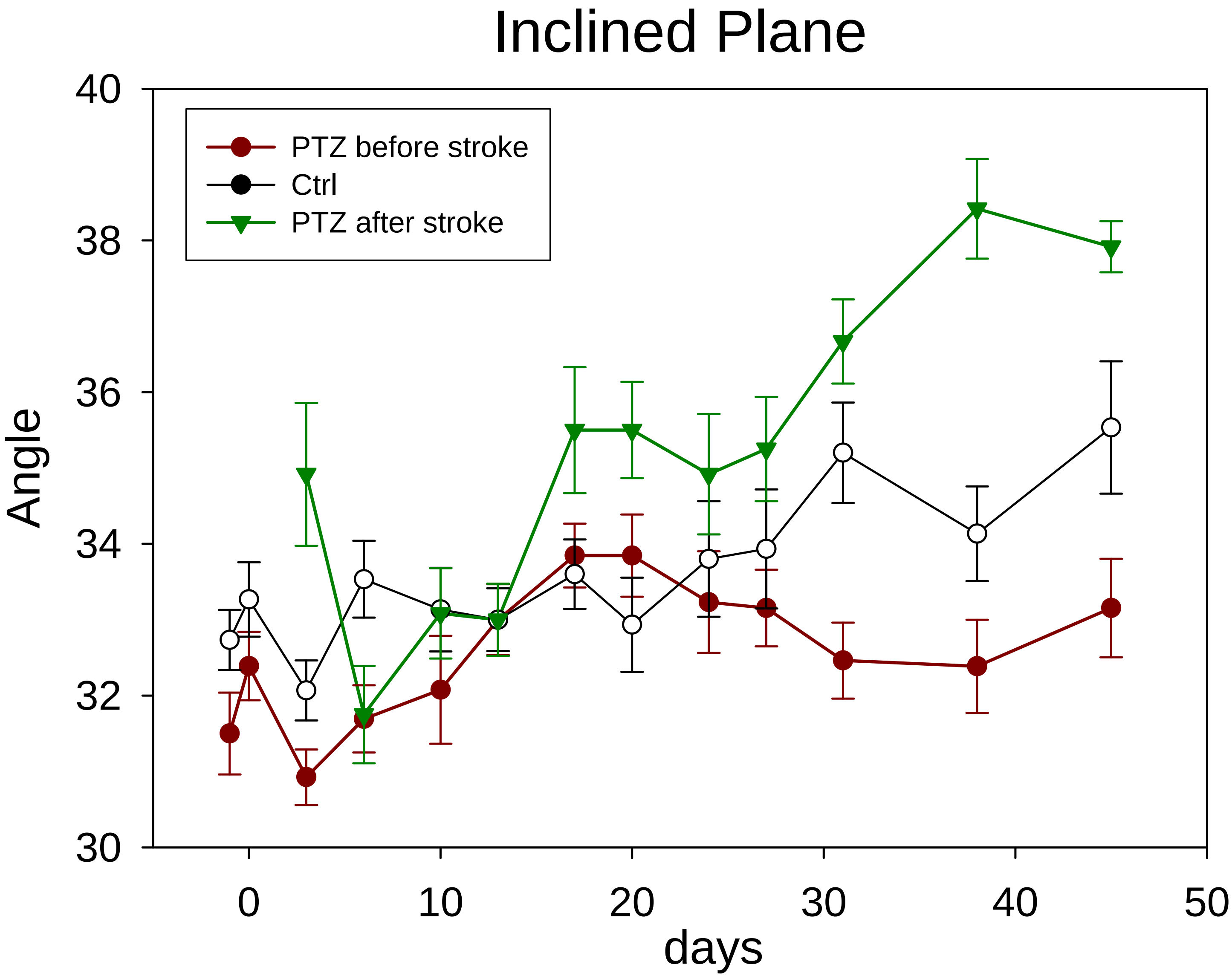


PTZ before Stroke
1 st PTZ
2nd PTZ
Day
-48
-24
0
2- Start behavioral testing
48
3x BrdU
3x BrdU
End
PTZ after Stroke
0
2
7
1 st PTZ
Or ECS
31
2nd PTZ
Or ECS
48
3x BrdU
3x BrdU
End
Control Groups
1 st Saline
2nd Saline
Day
-48
-24
0
2
48
3x BrdU
3x BrdU
End
0
2
7
31
48
3x BrdU
3x BrdU
End

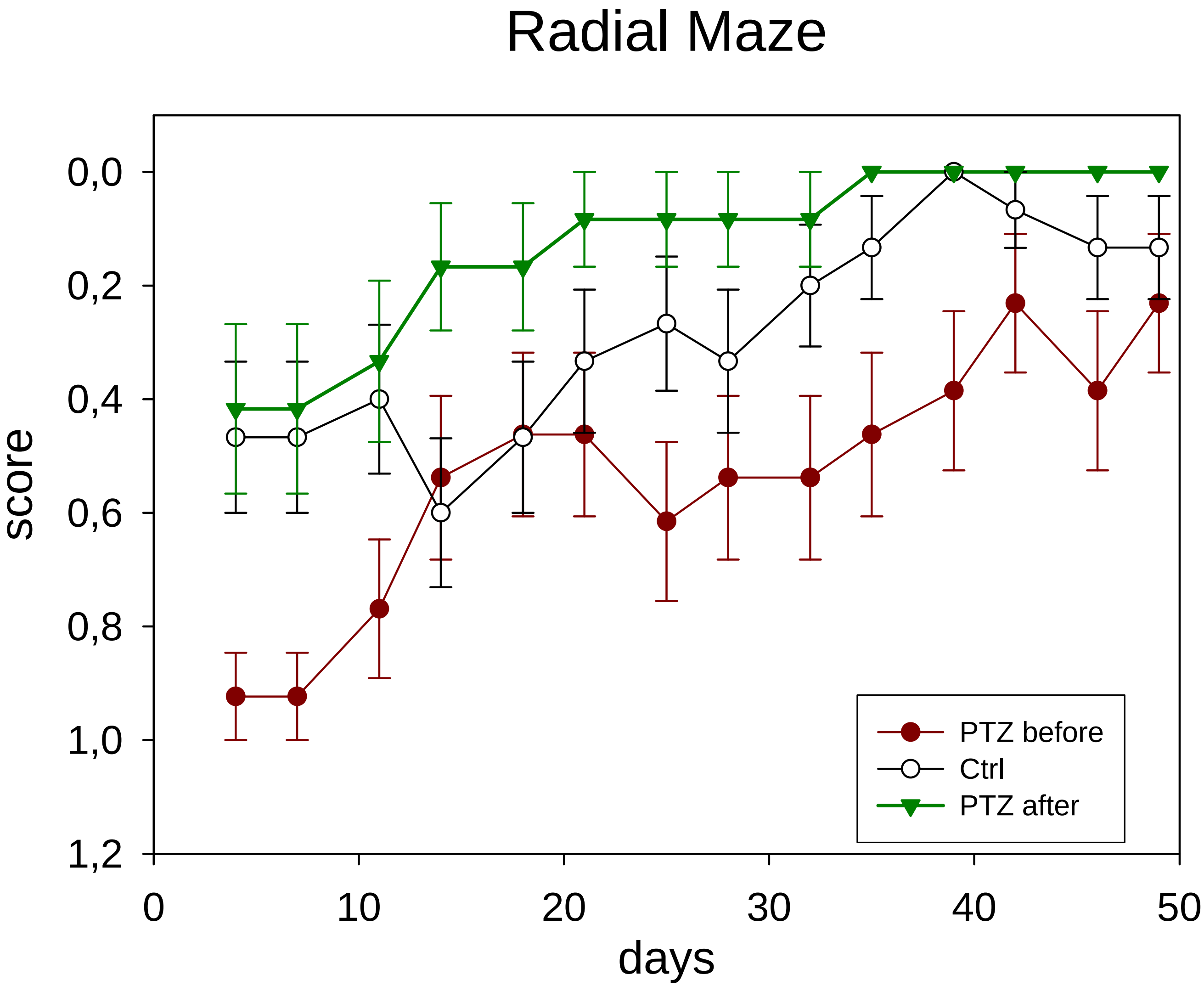
Neurogenesis does not significantly improved performance in



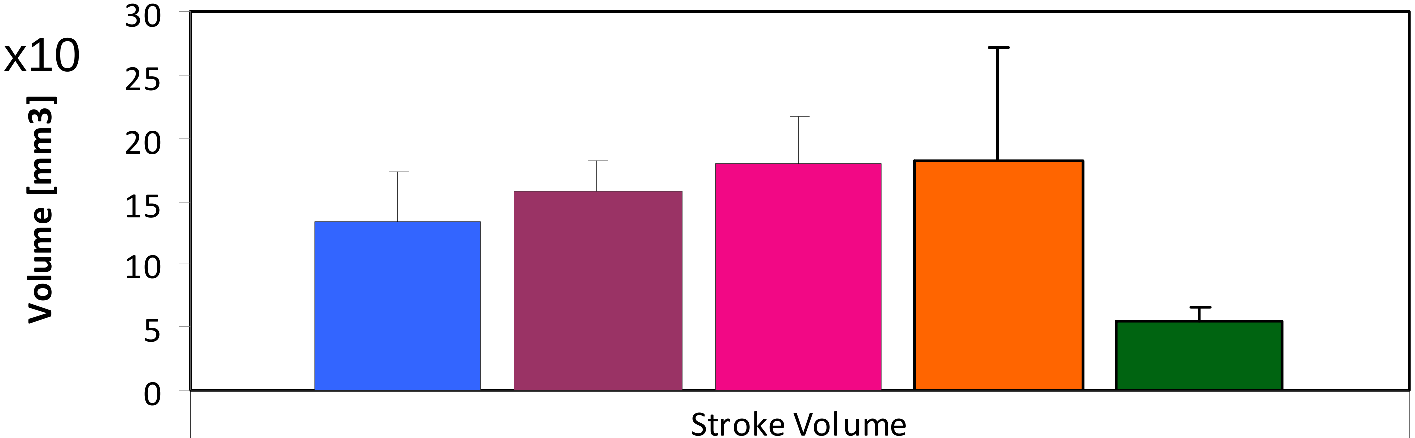
Neurogenesis after stroke significantly improved performance on the



Neurogenesis after stroke significantly improved performance in

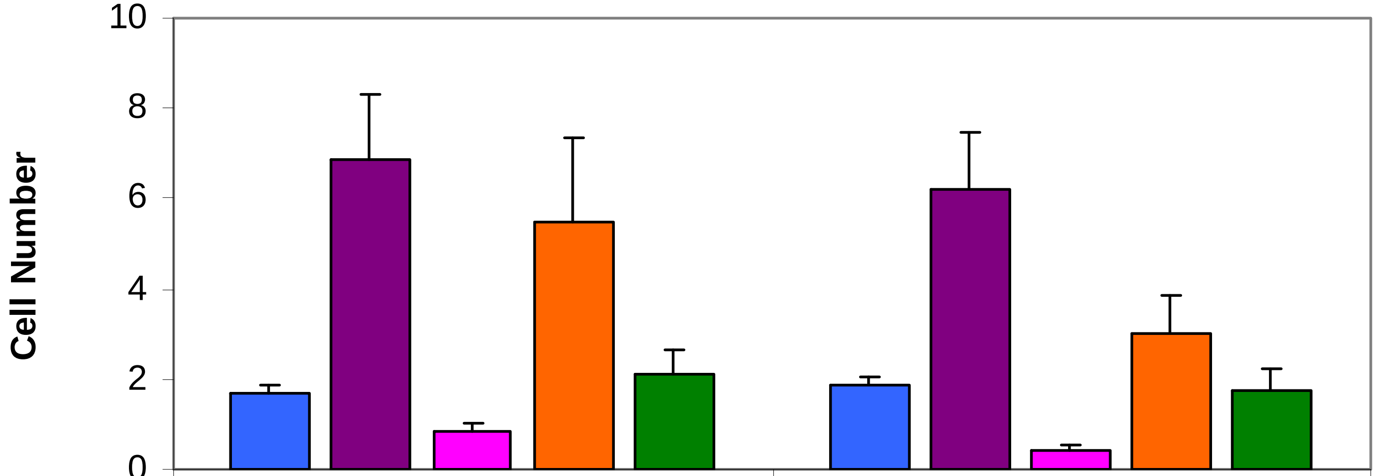


Stroke Volume of all Groups in the NGS Project



	Stroke Volume
Control	13,38
PTZ before Stroke	15,69
PTZ after Stroke	17,86
ES after Stroke	18,16
Sham	5,49

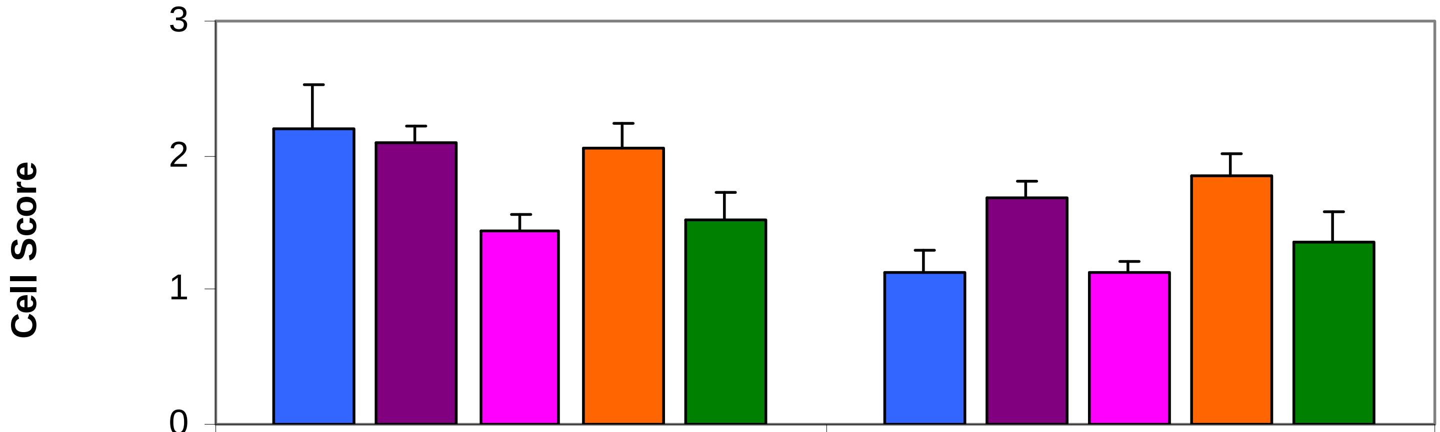
Number of Doublecortin positive Cells in Hippocampus



	Hippocampus ipsilateral	Hippocampus contralateral
Control	1,68	1,85
PTZ before Stroke	6,89	6,21
PTZ after Stroke	0,85	0,42
ES after Stroke	5,48	3,00
Sham	2,13	1,72

Tissue

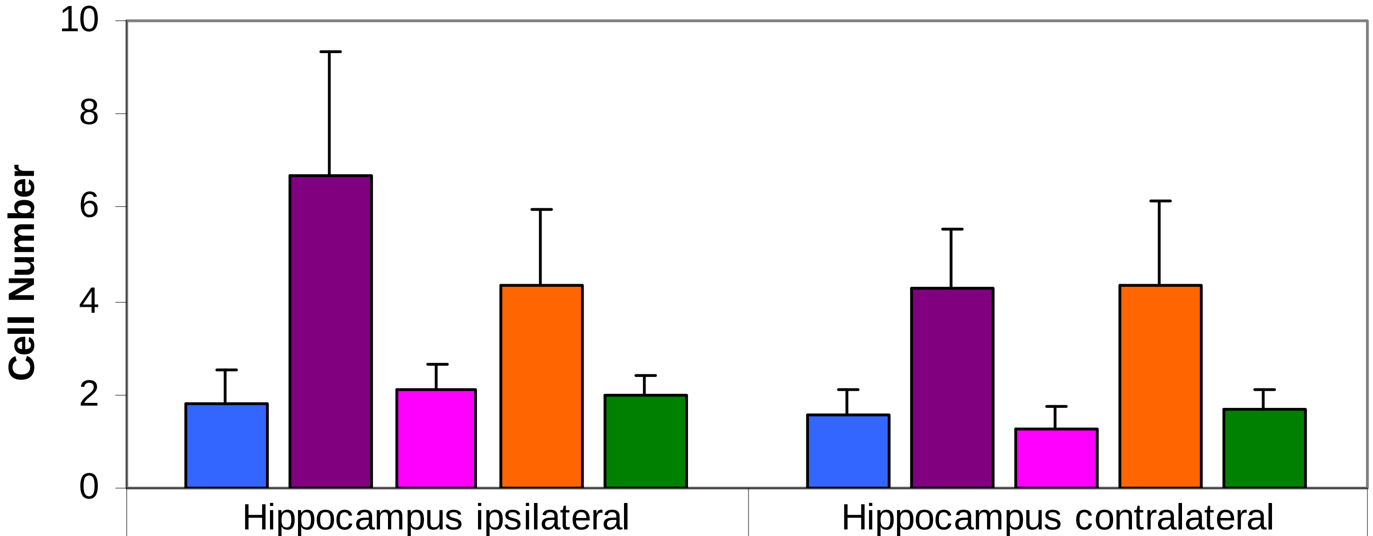
Semiquantitative Evaluation of Doublecortin positive Cells in Subventricular Zone



	Subventricular Zone ipsilateral	Subventricular Zone contralateral
Control	2,19	1,12
PTZ before Stroke	2,09	1,69
PTZ after Stroke	1,43	1,12
ES after Stroke	2,06	1,84
Sham	1,52	1,36

Tissue

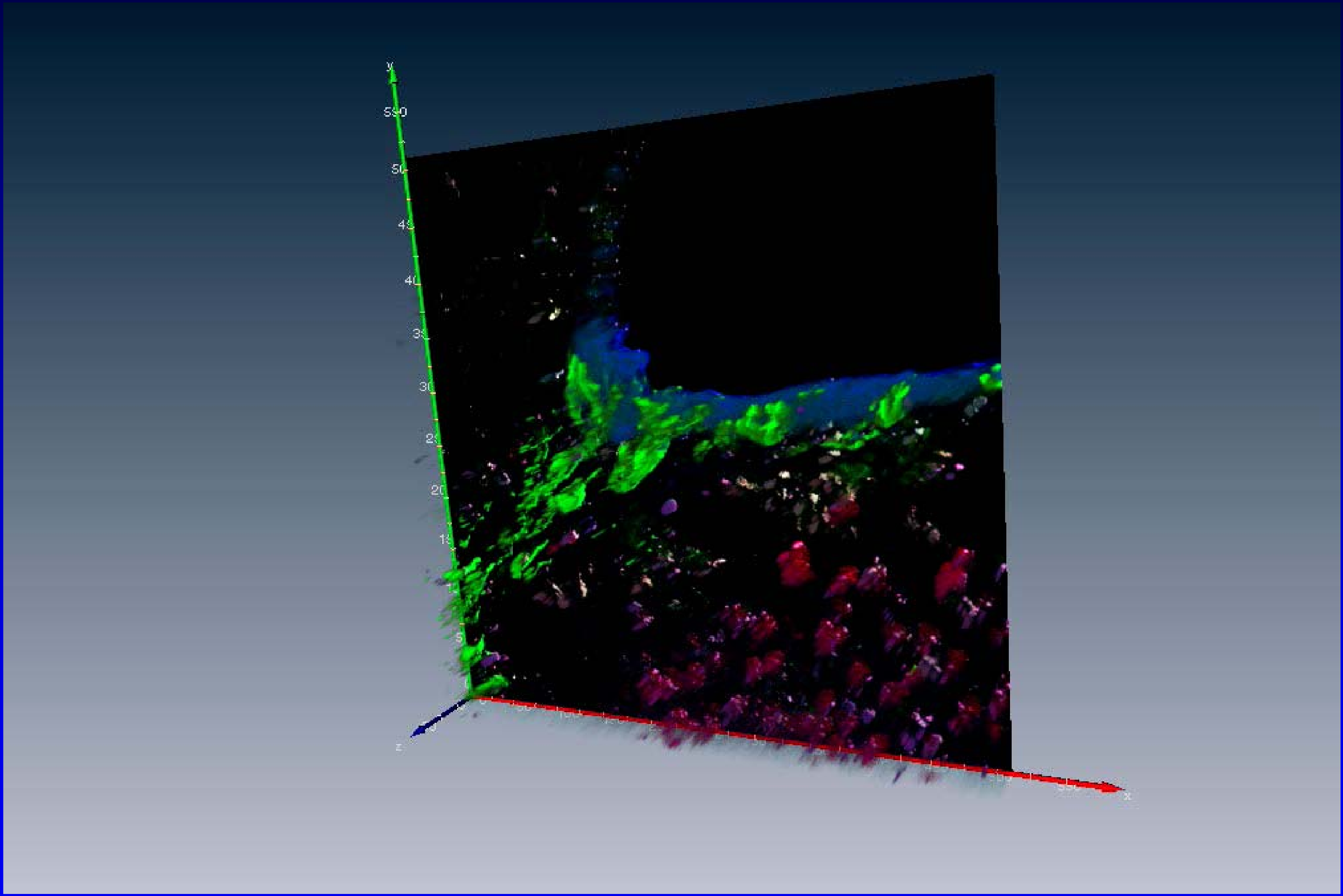
Number of PSA-NCAM positive cells in Hippocampus



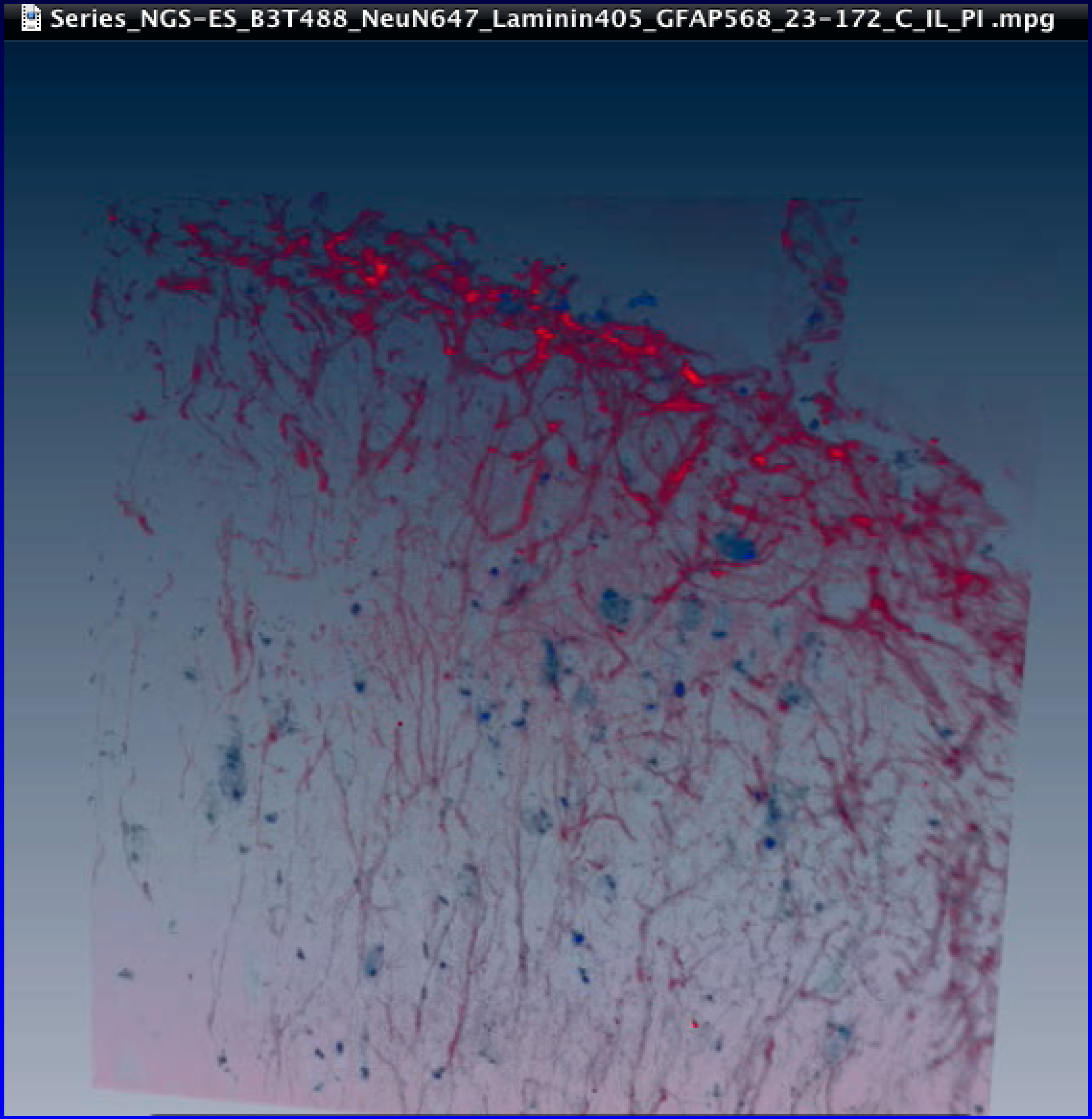
	Hippocampus ipsilateral	Hippocampus contralateral
Control	1,84	1,58
PTZ before Stroke	6,71	4,29
PTZ after Stroke	2,10	1,28
ES after Stroke	4,31	4,31
Sham	2,00	1,68

Tissue

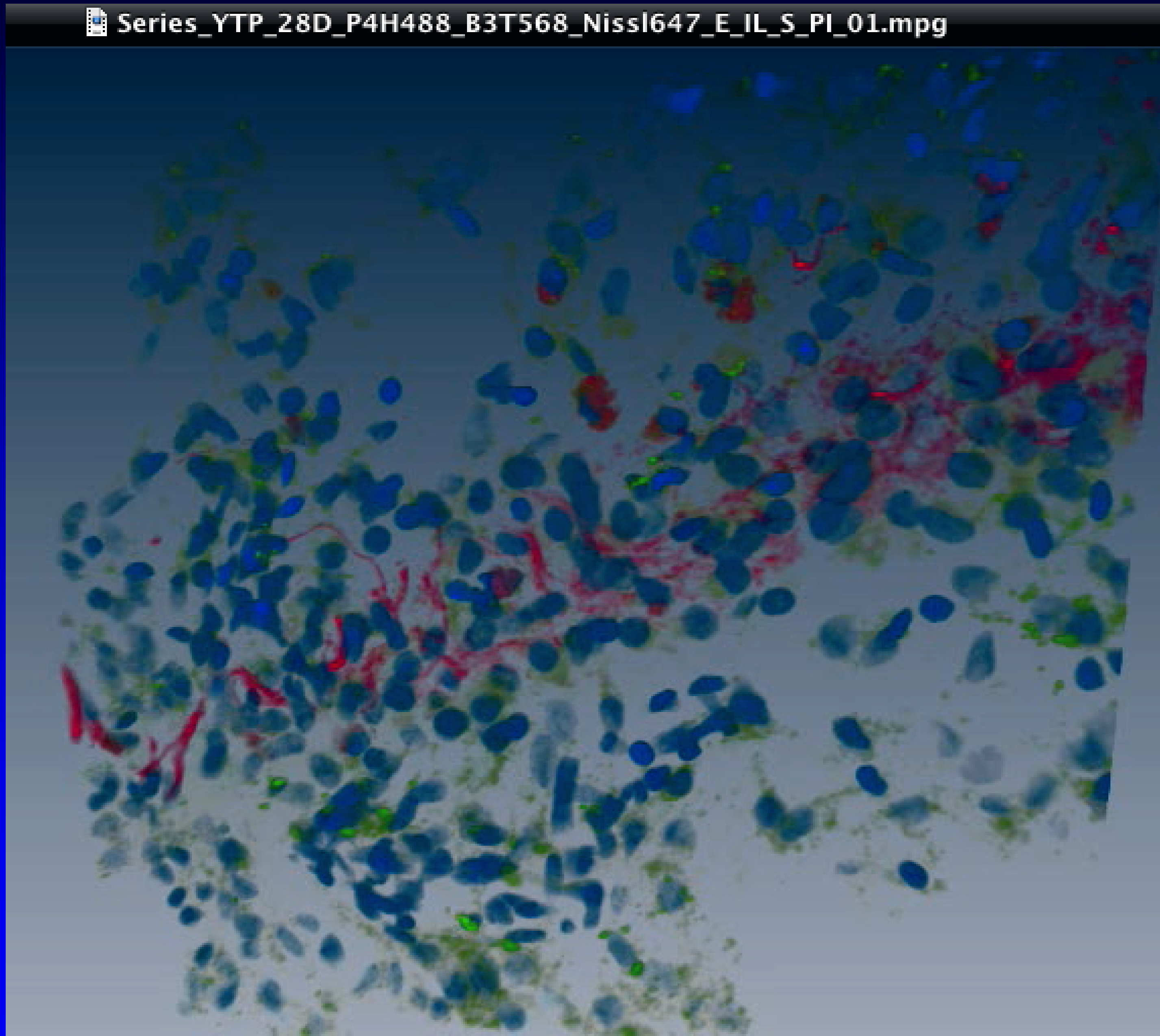
Electric Stimulation is efficient in increasing the number of early markers of neurogenesis like *Doublecortin* (shown in green) in the subventricular zone



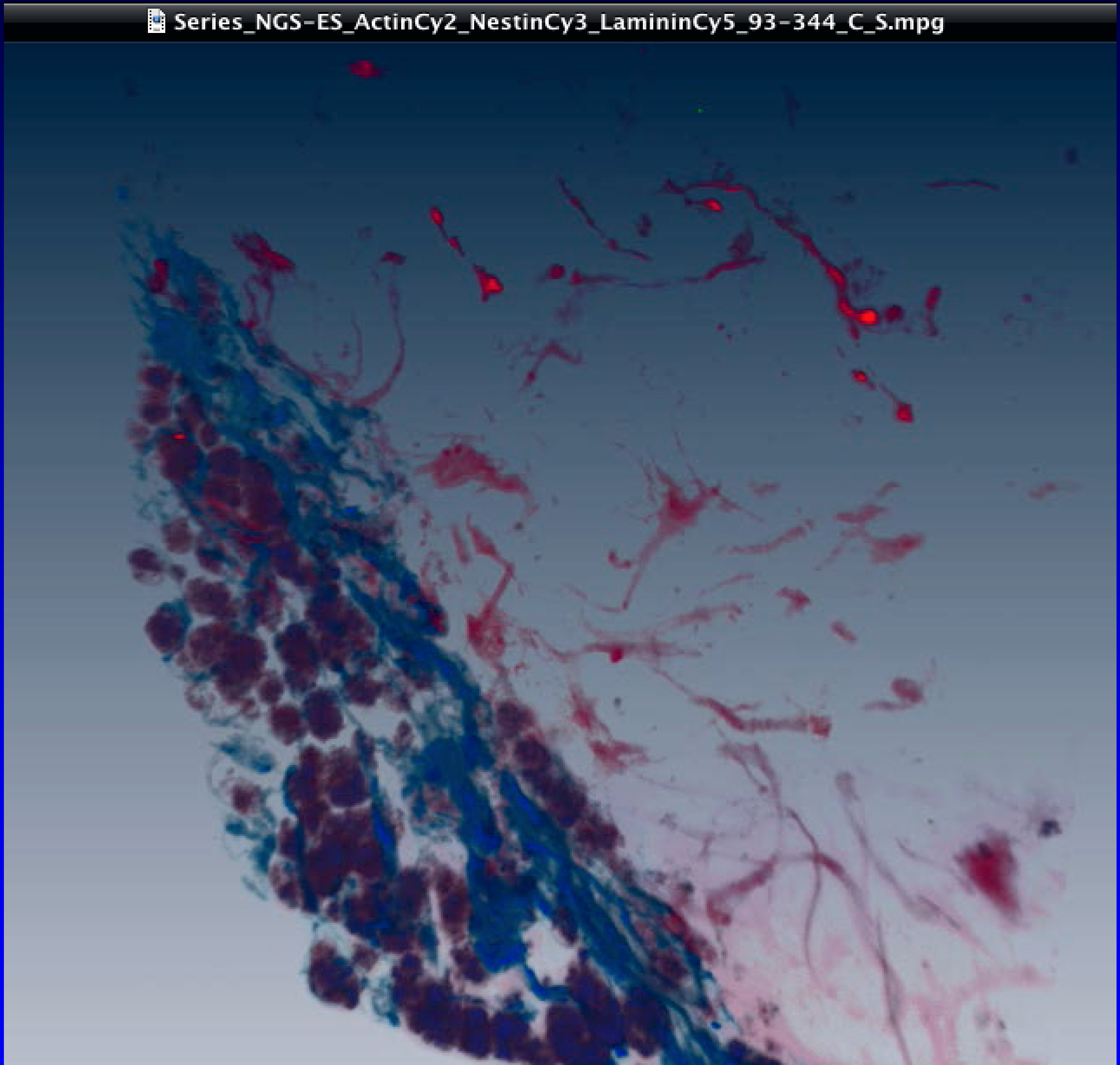
Eight weeks after stroke, beneath the glial scar (RED) scattered neurons are visible (Blue)cc



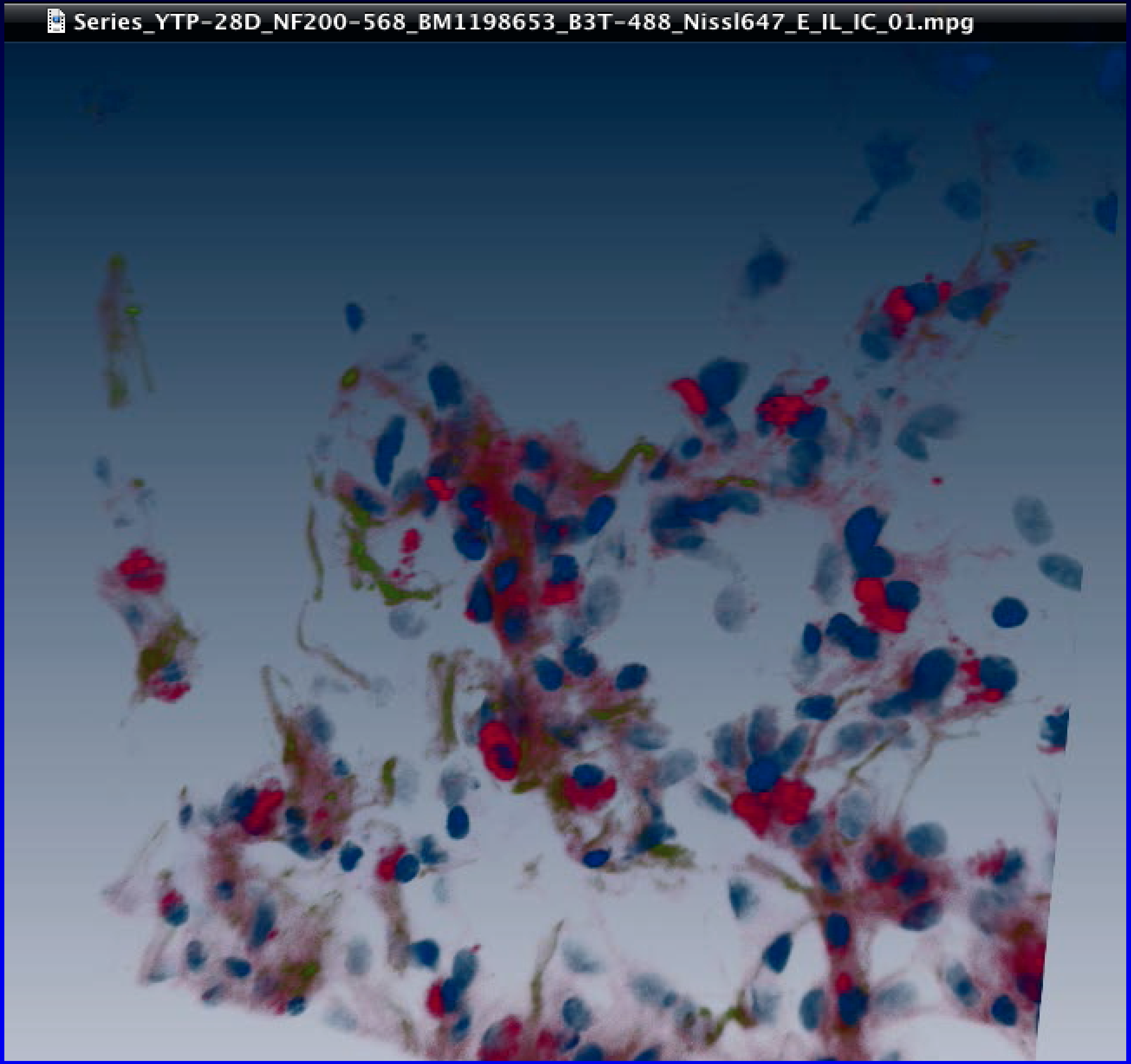
Eight weeks after stroke the glial scar (RED) region is heavely populated with cells of unknown origin (Blue)



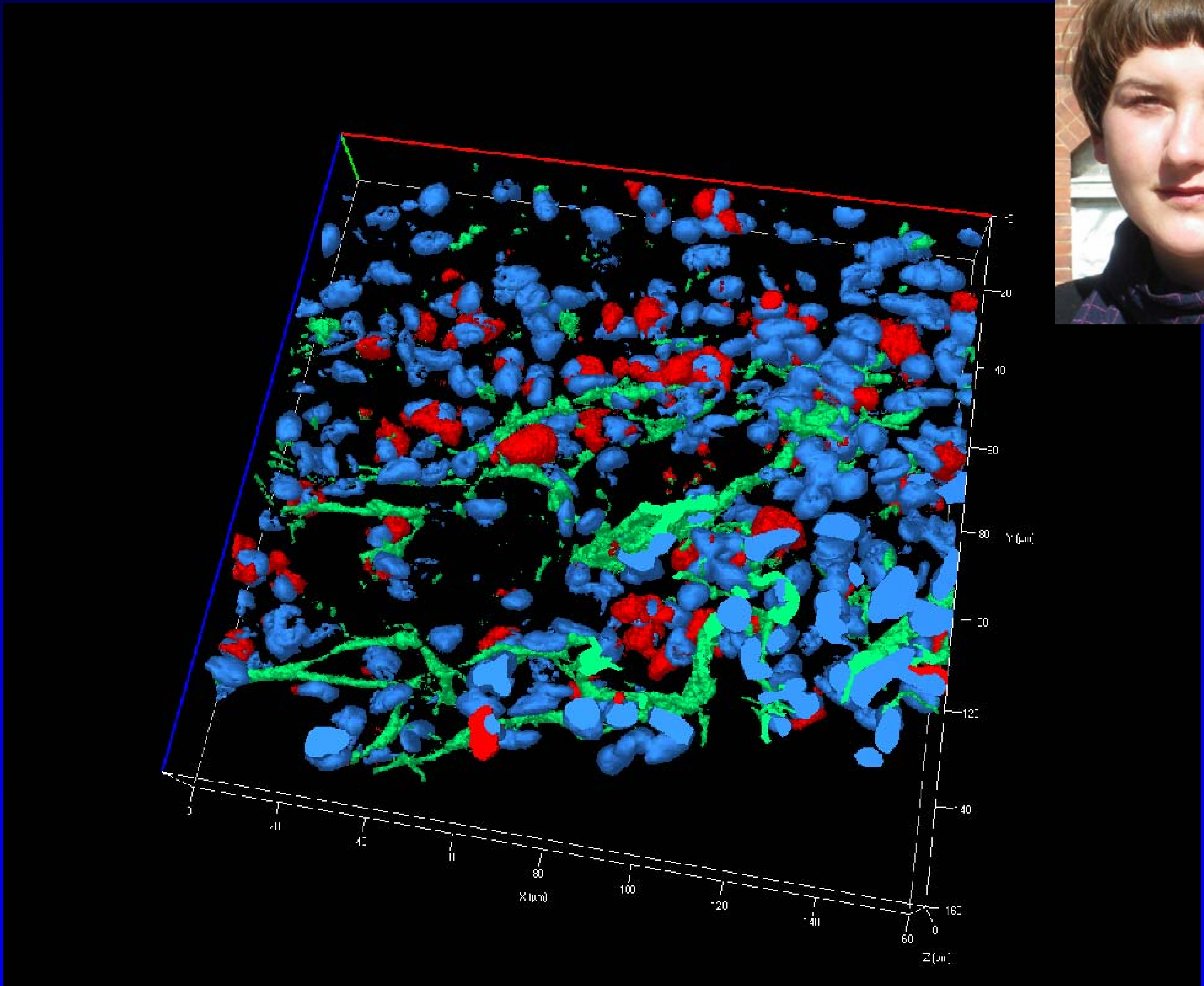
Eight weeks after stroke, vasculogenesis is well underway (laminin, BLUE) while few nestin-positive cells are detected (RED)



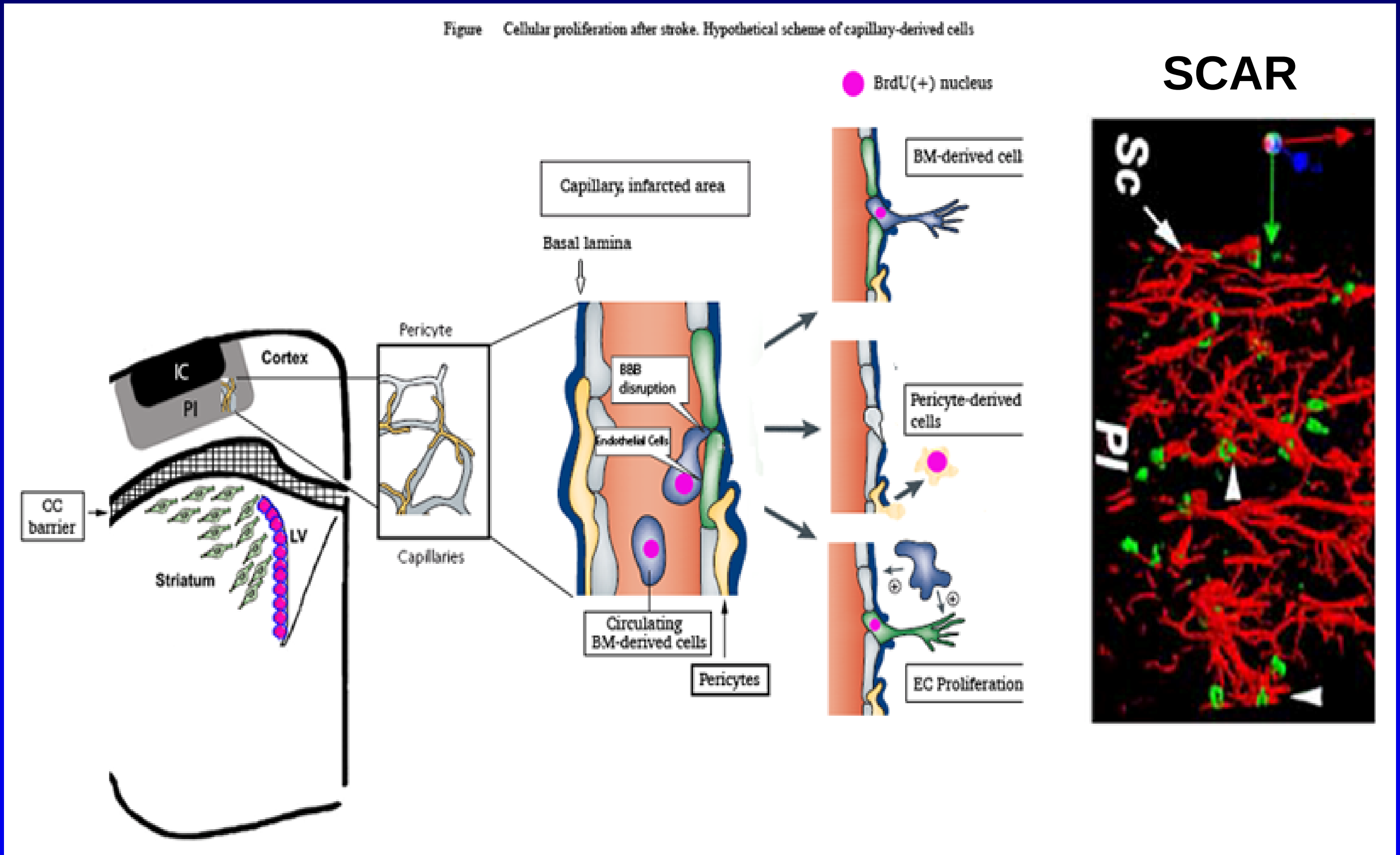
Eight weeks after stroke beneath the glial scar (RED) scattered neurons are visible (Blue)cc



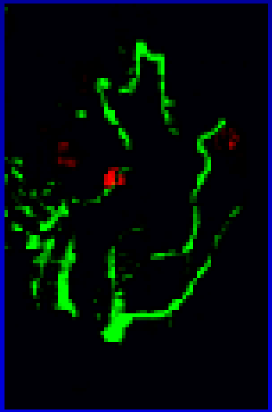
Increased number of axons in the perilesional area of aged rats following neurogenesis enhancement



Early formation of the growth-inhibiting SCAR and late outgrowth of axons after stroke in aged subjects



AXONS



Take home messages (I):

Our findings indicate that the aged brain has still the capability to mount a neurogenic response to stroke but this needs to be stimulated for therapeutic purposes.


Kiel
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Berlin
Hannover
Potsdam
Magdeburg
Düsseldorf
Erfurt
Dresden
Wiesbaden
Mainz
Saarbrücken
Stuttgart
München