gene expression

UEFISCDI
UNITATEA EXECUTIVA PENTRU FINANTAREA INVATAMANTULUI SUPERIOR, A CERCETARII, DEZVOLTARII SI INOVARII
INOVARE SI CREATIVITATE

IDENTIFICATION OF AXONAL GROWTH-RELEVANT GENE IN THE AGED BRAIN AFTER STROKE

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ROMANIA

Aging brain and age-related disease

- decrease in volume,
- cognitive decline,
- high risk of neurological disorders- cerebral ischemia and dementia)

gene expression

Biochemistry

Genetics

Cell biology

Demography

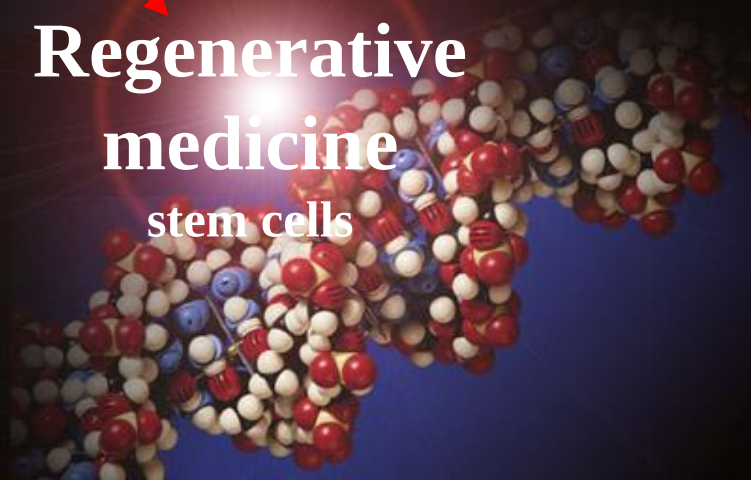
Ageing vs Developmental changes

Ageing-related disease

Stroke, cardiovascular disease, Alzheimer's, cancer, diabetes, etc

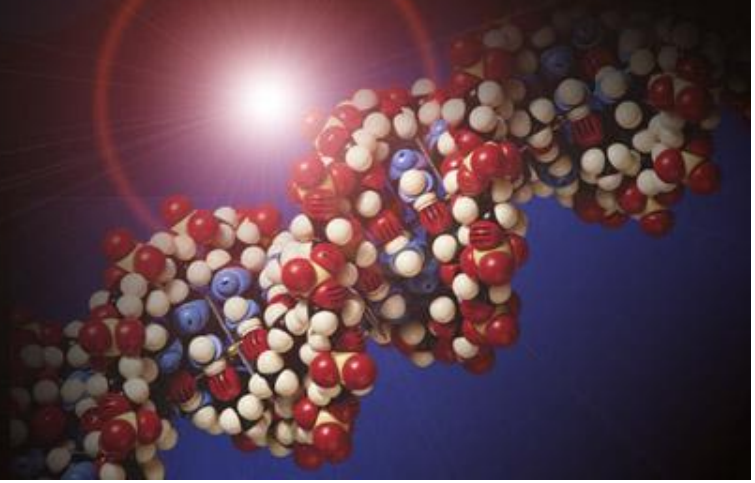
Regenerative medicine

stem cells



Biological Ageing

1. When biological aging occur in life and why?
2. What are the molecular basis of aging?
3. What is the molecular course of aging?
4. What are the intervention?



When biological aging occur in life?

- For Humans:

Maximum lifespan: 122 years

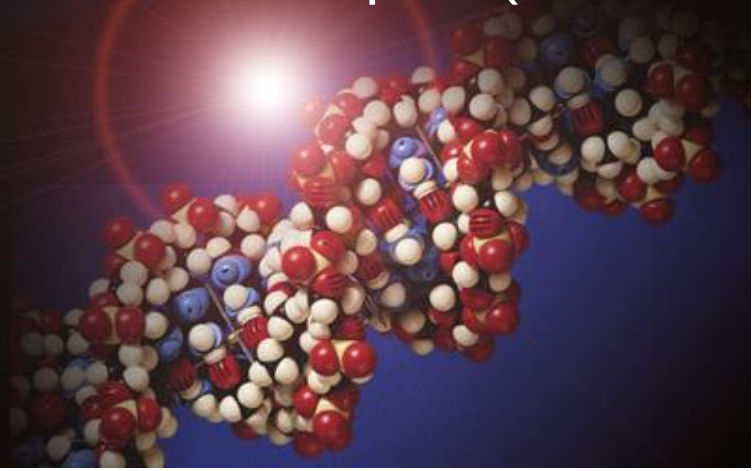
Average lifespan: 70 - 80 years

Essential lifespan: 40-46 years

When biological aging occur in life?

Biologically: ageing happens after essential lifespan

Psychologically: ageing “stops” after essential lifespan (about 40-50 years)



We are not programmed to age and die

- There are no gerontogenes with the only function to cause ageing and eventual death

- Genes determine essential lifespan of a species (Longevity genes)
- Genes that affect the longevity and the qualityof life (Potential gerontogenes)

Survival is a permanent challenge

- Oxigen metabolites
- Biochemical errors
- Nutritional metabolites

External and Internal Sources of damage

Maintenance and repair

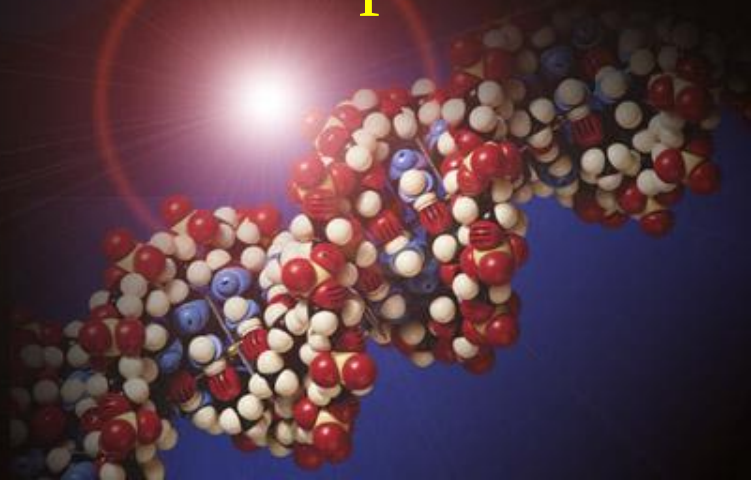
Genomis stability
Protein stability
Oxidative stress protection
Tissue repair after lesion

Aging = progresive decrease of our ability to repair these errors

Molecular Reasons of Ageing?

Ageing occurs at all biological levels.

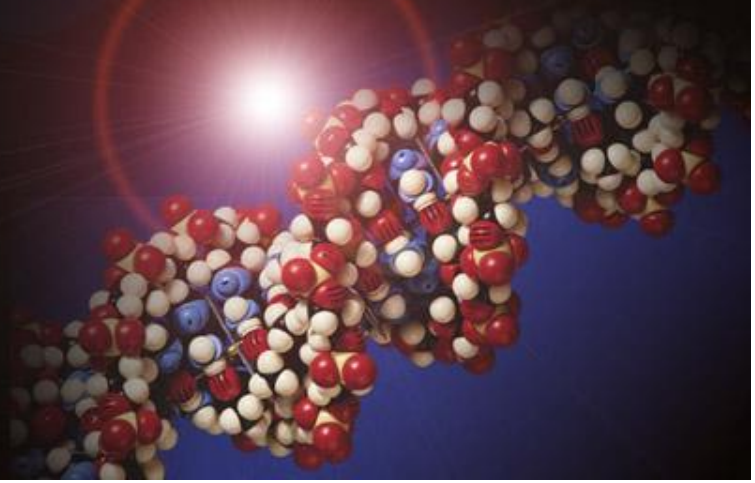
1. Accumulation of molecular damage: DNA and RNA damage, protein damage, tissue damage.
2. Imperfection of Maintenance and Repair systems



What to be established?

1. The significance of different levels of molecular damage - prevention/reversion

1. Nature of young and old healthy and diseases molecular mechanisms – remodeling/adaptation



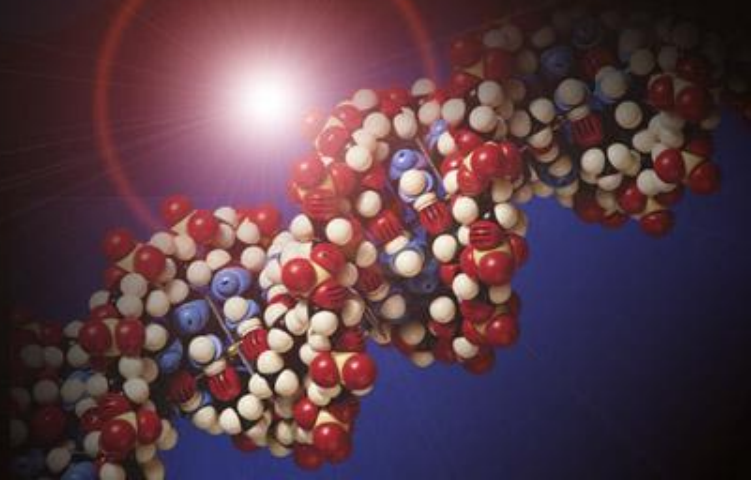
Questions

Genetics?

Are ageing controlled by the genome, and if so what are this changes?

How does changes give rise to ageing-related disease like Stroke and can be this controlled to increase the recovery?

Can we reactivate pathways that are active during development to improve the functional recovery and to limit damage ?



gene expression

Overall Goal and Hypothesis in Genomics of Ageing

- Our Hypothesis: changes in gene expression level are related with differentiation of tissue involved in development and are associated with functional decline in aging
- regenerating tissues can restart developmental signalling pathways that were active during ontogenesis

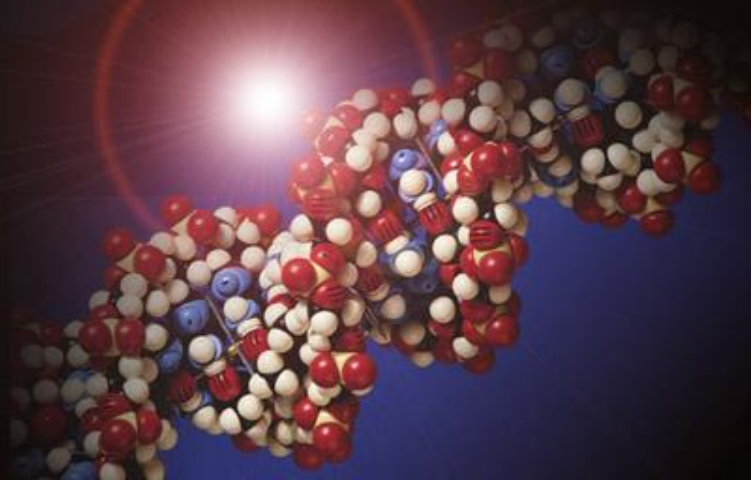
- Goal:
- creating an age-specific database using genome wide analysis of regeneration associated genes to identify genetic pathways associated with axonal growth/inhibition in response to injuries to the aged central nervous system.

- identification of molecular and cellular mechanisms leading to failure of axonal regeneration after a brain injury in aged animals.

Animal models needed to study physiopathology / therapies of stroke

Ideal Disease Model

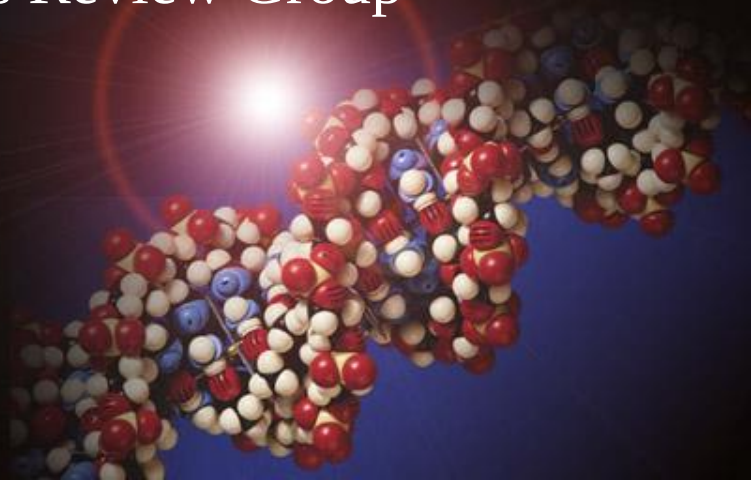
- Robust Phenotype
- Rapid disease onset/progression
- Well defined behavioral abnormalities (quantification)
- Neuropathology mirrors human condition



Understanding the cellular and genetic response to stroke in aged subjects is required for developing efficient therapies

- In this light, we consider that the aged post-acute animal model is clinically most relevant to stroke rehabilitation and cellular studies

- A recommendation issued by the STAIR committee and more recently by the Stroke Progress Review Group



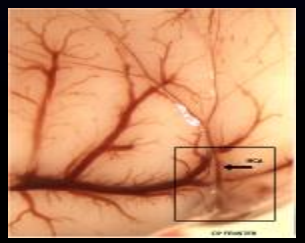
Mammalian Model for Aging

Aged-related changes in the transcriptional activity in the brains could be one factor contributing to reduced functional recovery ? If yes can be that changed ?

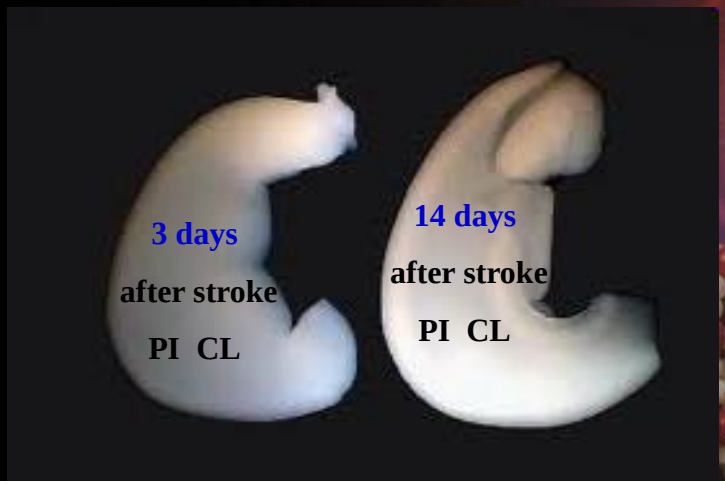
Postnatal 3d

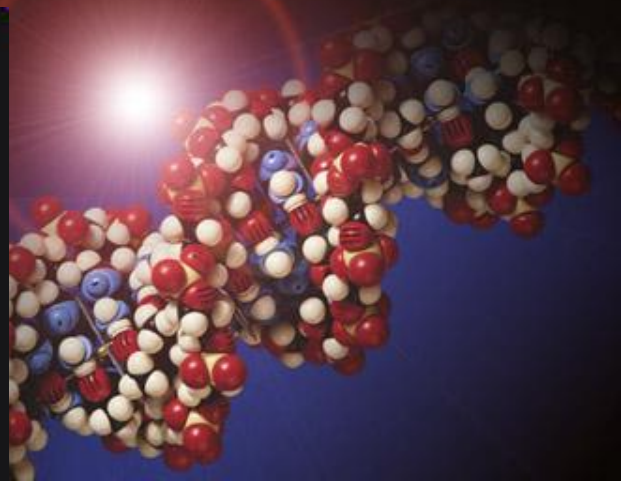
?

Postnatal 7d

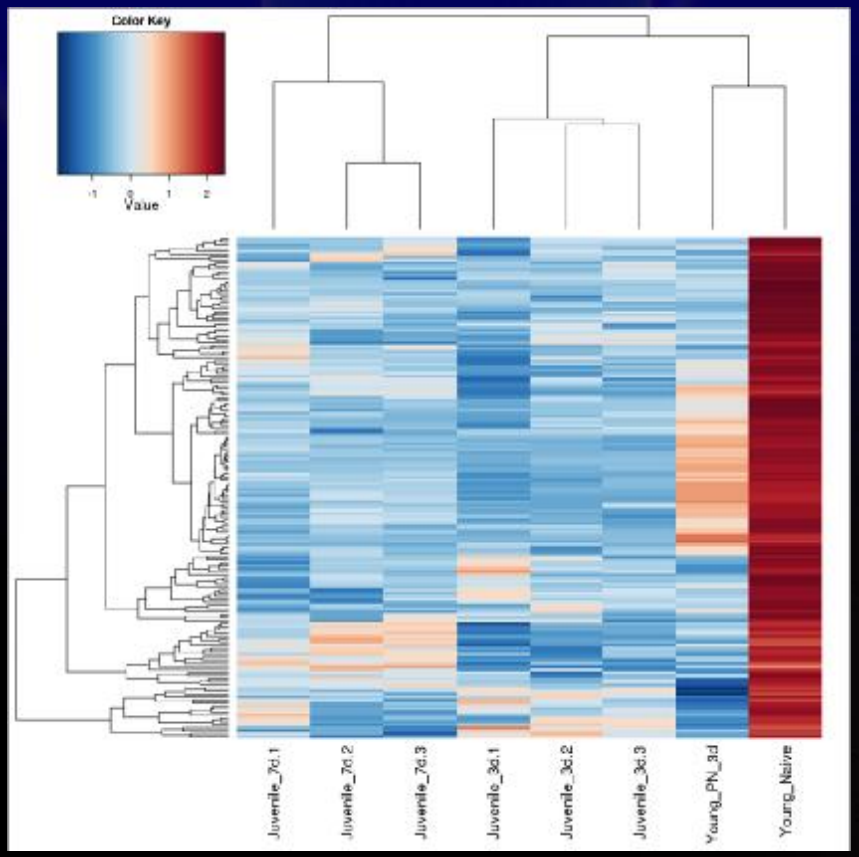


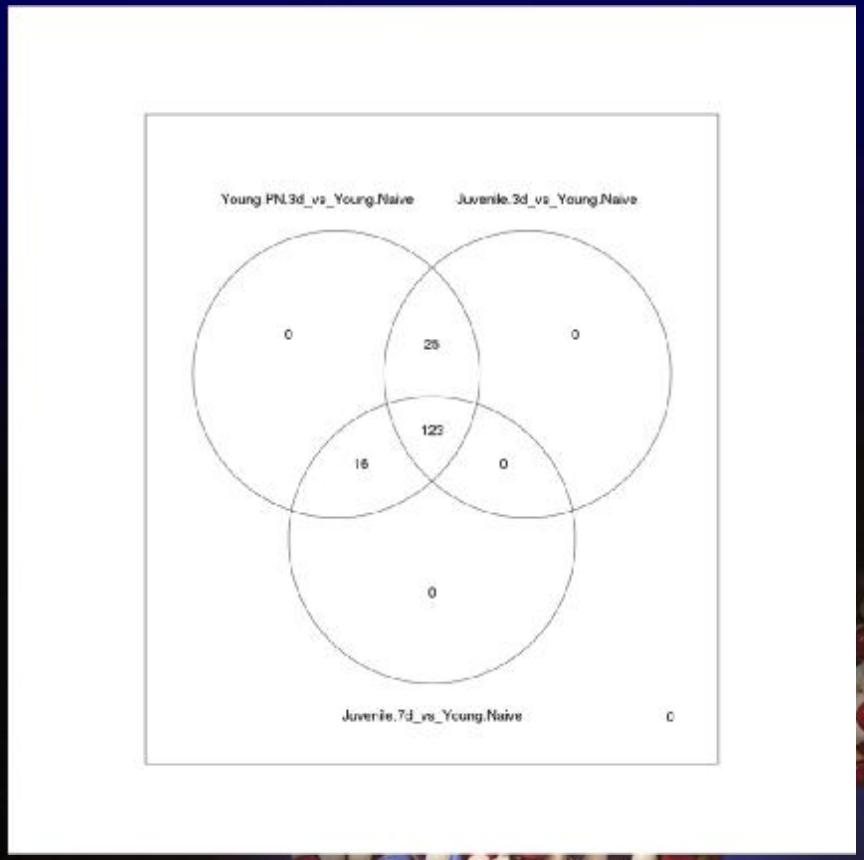
•Reversible occlusion of the middle cerebral artery (MCAO) with a tungsten hook for 90 min.



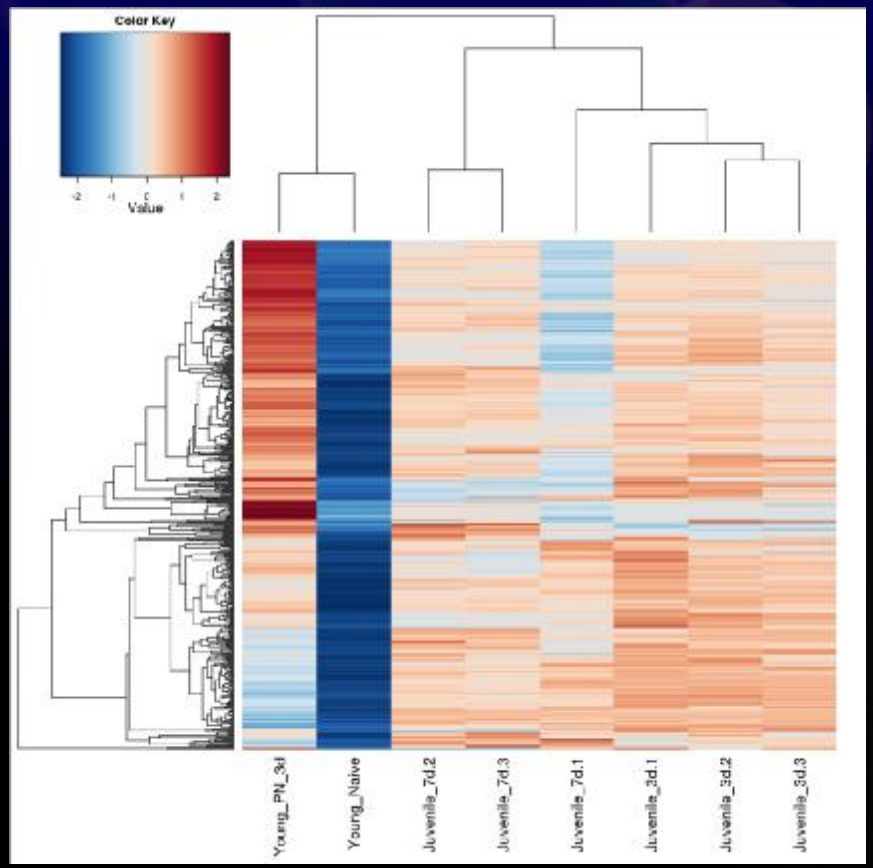


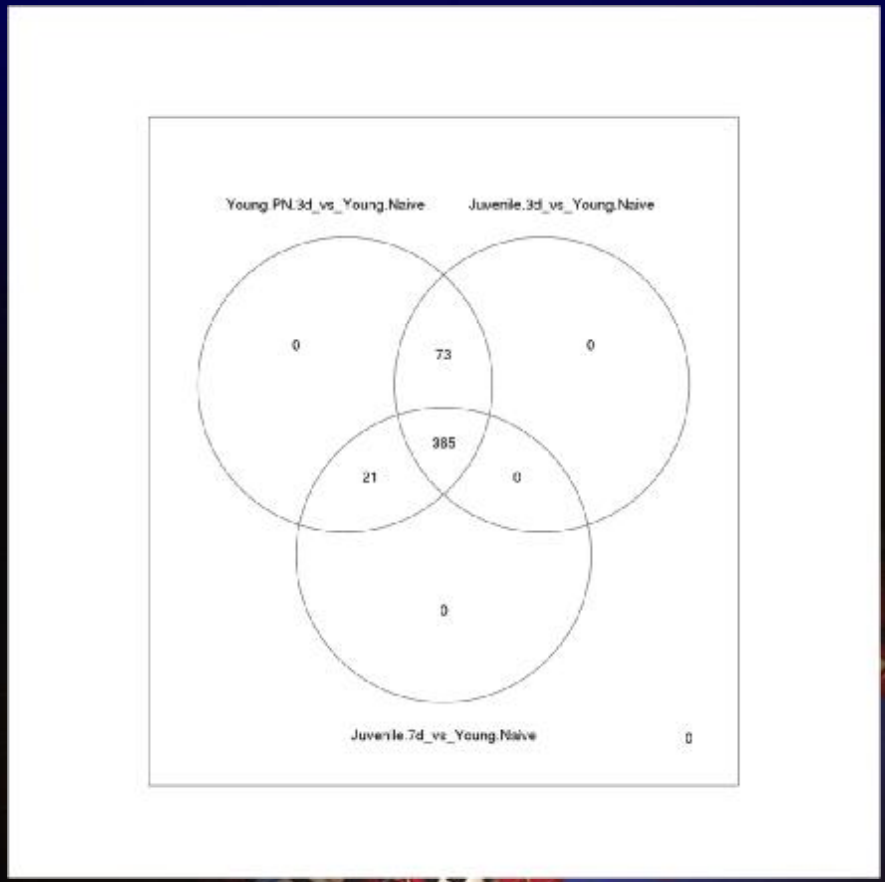
Relative expression values clearly distinguish naive rats from post-stroke groups and juvenile group





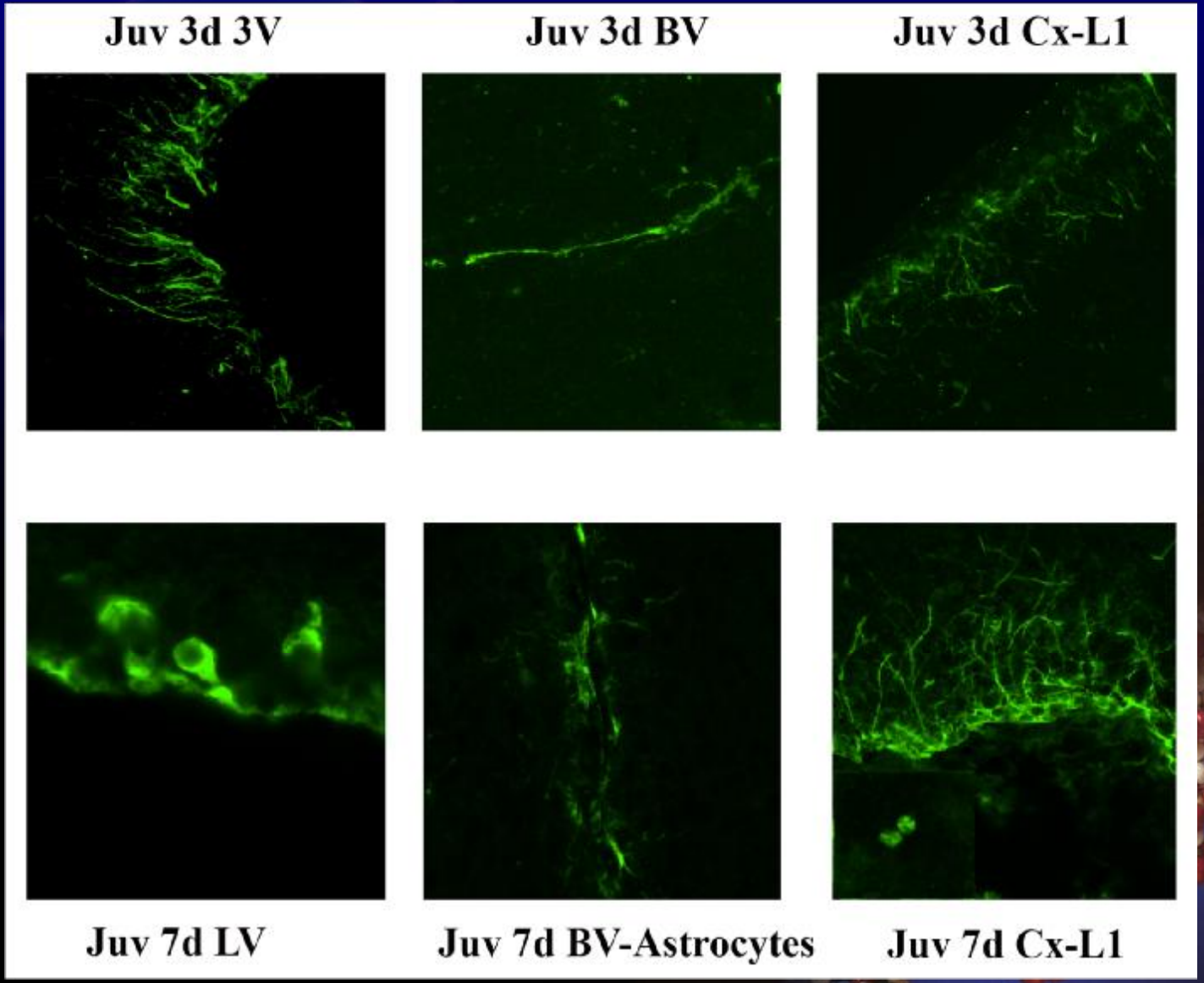
Relative expression values clearly distinguish naive rats from post-stroke groups and juvenile group



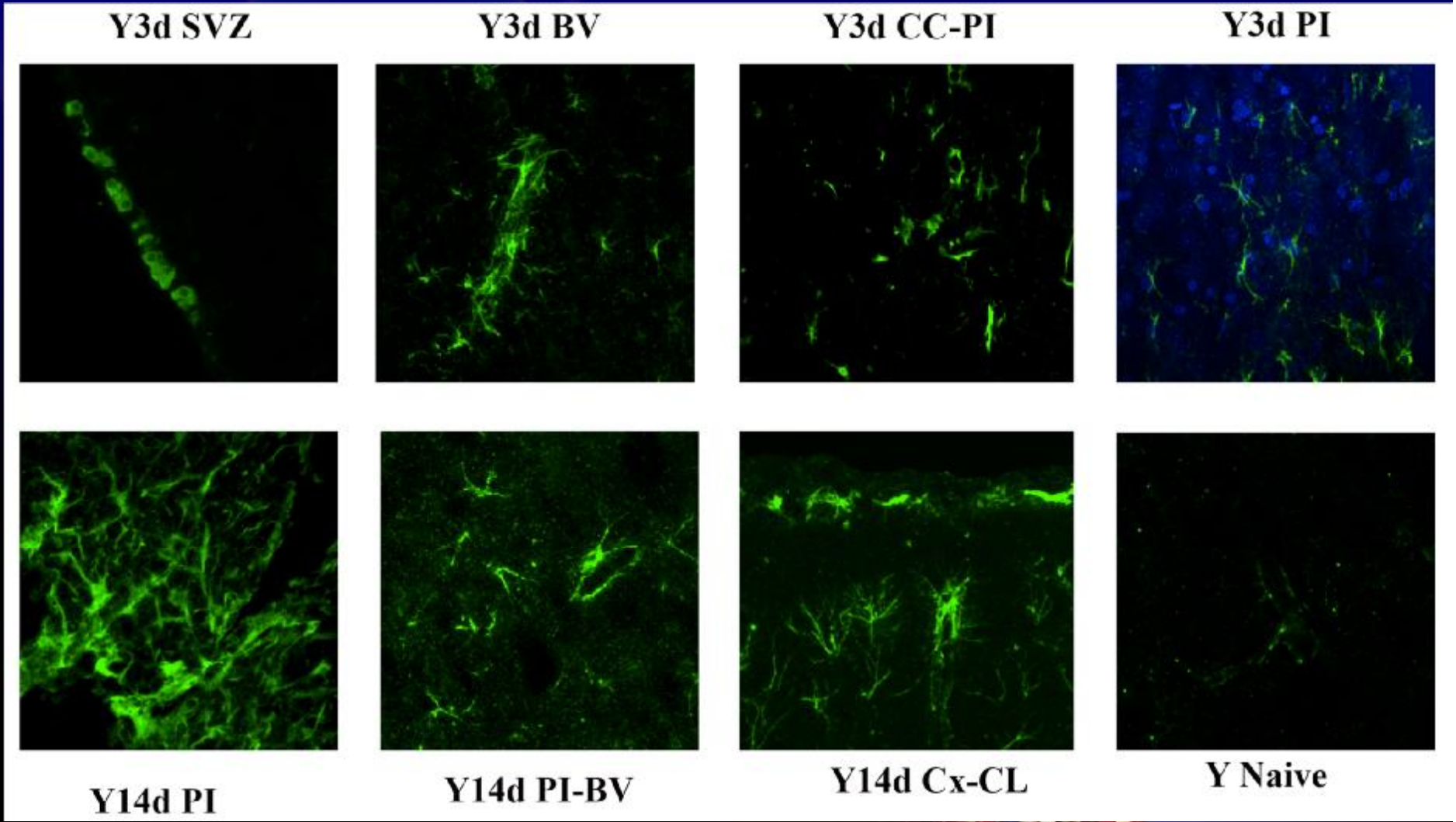


Sequence Code	Gene Symbol	Gene name	Young PN 3d vs Young Naive				Young PN 14d vs Young Naive				Juvenile 3d vs Young Naive				Juvenile 7d vs Young Naive			
			Array logFC	Array FC	RT-PCR FC	SD	Array FC	FDR	RT-PCR FC	SD	Array logFC	Array FC	RT-PCR FC	SD	Array logFC	Array FC	RT-PCR FC	SD
GO:0032268~regulation of cellular protein metabolic process																		
10836482	Timp 1		4,92	30,19	67,48	3,47	8,21	8,73E-10	7,17	1,23	1,53	2,90	3,02	0,15	1,51	2,84	3,26	0,34
10849737	Bub1		3,36	10,26	23,13	2,83	1,16	9,60E-01	7,1	0,5	2,86	7,26	27,5	0,18	2,59	6,01	24,76	2,42
10915437	Dnmt1*		0,91	1,87	-1,37	0,03	-1,07	9,55E-01	-1,17	0,07	1,57	2,97	9,32	0,72	1,38	2,60	8,77	0,85
GO:0042127~regulation of cell proliferation																		
10915437	Dnmt1*		0,91	1,87	-1,37	0,03	-1,07	9,55E-01	-1,17	0,07	1,57	2,97	9,32	0,72	1,38	2,60	8,77	0,85
GO:0008284~positive regulation of cell proliferation																		
10754567	Sox4		0,90	1,87	1,16	0,16	-	-	5,29	0,51	3,56	11,73	21	0,00	2,70	6,48	14,04	1,53
10725155	Ybx1		1,25	2,38	2,88	0,2	1,43	9,52E-01	6,36	0,56	2,11	4,32	10,08	1,5	2,17	4,49	13,26	0,76
10856814	Myc		3,03	4,09	4,01	0,11	1,20	9,11E-01	8,57	0,77	2,84	7,14	444,84	39,14	2,68	6,40	2310,81	227,97
10823853	Ccna2		3,19	9,12	30,48	0,36	1,16	9,30E-01	6,53	0,55	2,20	4,59	26,57	1,09	2,02	4,05	21,13	1
10770649	Cenpf		2,66	6,32	10,1	0,62	1,04	9,54E-01	24,56	3,08	2,08	4,22	14	2,66	1,88	3,68	8,62	0,18
GO:0008285 ~negative regulation of cell proliferation																		
10759652	Casp3		1,07	2,10	1,8	0,1	1,27	8,70E-01	3,88	0,43	2,87	7,29	7,01	0,21	2,89	7,41	8,51	0,08
10877183	s100a11		4,56	23,61	35,75	1,56	4,49	6,26E-08	8,63	0,62	3,36	5,13	7,43	0,76	2,34	5,06	7,83	0,18
GO:0006259~DNA metabolic process																		
10759652	Casp3		1,07	2,10	1,8	0,1	1,27	8,70E-01	3,88	0,43	2,87	7,29	7,01	0,21	2,89	7,41	8,51	0,08
10856814	Myc		3,03	4,09	4,01	0,11	1,20	9,11E-01	8,57	0,77	2,84	7,14	444,84	39,14	2,68	6,40	2310,81	227,97
10915437	Dnmt1*		0,91	1,87	-1,37	0,03	-1,07	9,55E-01	-1,17	0,07	1,57	2,97	9,32	0,72	1,38	2,60	8,77	0,85
10726408	Mki67		4,20	18,33	8,03	0,52	-1,35	9,57E-01	3,34	0,32	3,13	8,73	5,5	0,01	2,99	7,94	4,27	0,48
10883903	Rrm2		4,15	17,70	47,90	1,85	2,01	2,45E-01	6,93	0,15	2,20	4,60	16,22	1,41	2,03	4,10	14,11	0,57
10860584	Dbf4		2,45	5,47	5,39	0,31			4,41	1,07	2,40	5,30	2,08	0,36	2,20	4,61	2,18	0,07
GO:0051726~regulation of cell cycle																		
10759652	Casp3		1,07	2,10	1,8	0,1	1,27	8,70E-01	3,88	0,43	2,87	7,29	7,01	0,21	2,89	7,41	8,51	0,08
10856814	Myc*		3,03	4,09	4,01	0,11	1,20	9,11E-01	8,57	0,77	2,84	7,14	444,84	39,14	2,68	6,40	2310,81	227,97
10770649	Cenpf		2,66	6,32	10,1	0,62	1,04	9,54E-01	24,56	3,08	2,08	4,22	14	2,66	1,88	3,68	8,62	0,18
GO:0006997~nucleus organization																		
10759652	Casp3		1,07	2,10	1,8	0,1	1,27	8,70E-01	3,88	0,43	2,87	7,29	7,01	0,21	2,89	7,41	8,51	0,08
10856814	Myc*		3,03	4,09	4,01	0,11	1,20	9,11E-01	8,57	0,77	2,84	7,14	444,84	39,14	2,68	6,40	2310,81	227,97
GO:0006974~response to DNA damage stimulus																		
10759652	Casp3		1,07	2,10	1,8	0,1	1,27	8,70E-01	3,88	0,43	2,87	7,29	7,01	0,21	2,89	7,41	8,51	0,08
10781146	Pbk		3,20	9,18	29,19	2,79	1,65	6,70E-01	7,14	0,43	1,90	3,74	4,82	0,56	1,97	3,93	1,76	0,01
GO:0007017~microtubule-based process																		
10794824	Tubb2b		1,28	2,44	1,17	0,09	1,03	9,65E-01	4,42	0,28	2,85	7,23	5,72	0,39	2,80	6,95	5,13	0,5
10849568	Spc25		2,22	4,64	5,79	0,46	1,16	9,31E-01	1,6	0,05	2,04	4,12	46,22	4,6	2,09	4,26	71,04	8,18
10830488	Ndc80		2,97	7,84	10,51	1,12	1,42	8,45E-01	3,94	0,15	2,08	4,22	2,01	1,42	2,05	4,14	5,48	0,32
GO:0034622~cellular macromolecular complex assembly																		
10794824	Tubb2b		1,29	2,44	1,17	0,09	1,03	9,65E-01	4,42	0,28	2,85	7,23	5,72	0,39	2,80	6,95	5,13	0,5
10755201	Hist1h1b		2,68	6,40	12,88	1,26	1,17	9,11E-01	5,53	0,29	2,03	4,08	4,43	0,44	1,55	2,92	-1,06	0,11
GO:0009058 biosynthetic process																		
10854582	Aldh 1l2		0,94	1,92	16,13	1,02	-	-	54,3	1,23	3,36	16,25	185,15	10,08	2,74	6,69	90,62	6,36
GO:0090198~extracellular matrix organization																		
10873639	Mfap2		0,96	1,94	2,08	0,33	3,40	3,81E-05	9,71	0,93	2,88	7,34	15,85	1,32	2,42	5,35	7,59	1,5
10815369	Postn		3,13	8,74	8,26	0,45	1,67	5,47E-01	6,67	1,55	1,68	3,19	3,45	0,67	2,11	4,31	-1,76	0,23
10904161	Ste		2,68	6,42	8,88	0,42	2,42	2,79E-02	27,41	0,65	4,79	27,88	59,85	5,52	4,58	22,78	45,71	2,47
GO:0007165 - signal transduction																		
10853819	Mcf		1,48	2,80	3,24	0,33	-	-	14,58	1,15	3,76	9,61	10,18	1,75	3,83	14,26	15,12	0,74
10833090	Clic1		3,41	10,60	9,55	1,29	2,33	5,20E-02	2,61	0,2	0,80	1,76	1,67	0,21	0,92	1,69	3,34	0,04
10817183	s100a11		4,56	23,61	35,75	1,56	4,49	6,26E-08	8,63	0,62	3,36	5,13	7,43	0,76	2,34	5,06	7,83	0,18
10762533	Slc4a7		0,89	1,86	1,16	0,12	1,34	8,59E-01	3	0,24	1,51	2,84	2,73	0,46	1,21	2,31	5,13	0,26
10833698	Chrm4		-0,96	-1,95	-2,42	0,03	-1,55	6,82E-01	-1,53	0,13	-1,79	-2,44	-10,77	0,01	-0,88	-1,84	-9,7	0,02
10819548	Depdc1		2,52	5,72	-1,85	0,04	1,03	9,47E-01	2,2	0,17	2,14	4,41	1,12	0,04	2,09	4,27	-1,01	0,03
10882145	Gabrd		-1,18	-2,27	-4,74	0,04	-1,55	3,18E-01	-7,77	0,01	-2,96	-7,78	-42,57	0,00	-2,72	-6,58	-24,58	0,00
10838870	Itpka		-1,09	-2,13	-4,24	0,02	-1,62	6,89E-01	-3,62	0,01	-3,21	-9,25	-185,68	0,00	-2,63	-6,06	-45,32	0,00
GO:0016477~cell migration																		
10843558	Vav2																	
GO:0007049~cell cycle																		
10849568	Spc25		2,22	4,64	5,79	0,46	1,16	9,31E-01	1,6	0,05	2,04	4,12	46,22	4,6	2,09	4,26	71,04	8,18
10878032	Cks1b		2,21	4,64	3,84	0,13	-1,04	9,48E-01	1,97	0,06	2,27	4,83	15,77	0,81	1,99	3,97	23,17	1,65
10849737	Bub1		3,36	10,26	23,13	2,83	1,15	9,60E-01	7,1	0,5	2,86	7,26	27,5	0,18	2,59	6,01	24,76	2,42
10823853	Ccna2		3,19	9,12	30,48	0,36	1,16	9,30E-01	6,53	0,55	2,20	4,59	26,57	1,09	2,02	4,05	21,13	1
10770649	Cenpf		2,66	6,32	10,1	0,62	1,04	9,54E-01	24,56	3,08	2,08	4,22	14	2,66	1,88	3,68	8,62	0,18
10830488	Ndc80		2,97	7,84	10,51	1,12	1,42	8,45E-01	3,94	0,15	2,08	4,22	2,01	1,42	2,05	4,14	5,48	0,32
10823383	Sgol2		2,86	7,28	13,66	0,8	-	-	5,67	0,58	2,05	4,13	3,84	0,47	1,96	3,89	1,48	0,61
GO:0000280~nuclear division																		
10849568	Spc25		2,22	4,64	5,79	0,46	1,16	9,31E-01	1,6	0,05	2,04	4,12	46,22	4,6	2,09	4,26	71,04	8,18
10838745	Casc5		3,45	10,91	-30,67	0	1,72	4,91E-01	4,65	0,4	2,74	6,66	2,7	0,91	2,73	6,61	2,11	0,95
GO:0030902~cell morphogenesis																		
10823568	Ect2		3,05	8,27	20,57	2,72	1,34	8,88E-01	12,69	1,84	2,13	4,39	9,15	0,76	2,03	4,05	7,04	1,48
GO:0006811 ion transport																		
10882145	Gabrd		-1,18	-2,27	-4,74	0,04	-1,55	3,18E-01	-7,77	0,01	-2,96	-7,78	-42,57	0,00	-2,72	-6,58	-24,58	0,00
GO:0004952 dopamine neurotransmitter receptor activity																		
10794155	Drd1a		-1,23	-2,34	-1,19	0,05	1,04	9,50E-01	-5,49	0,06	-1,18	-2,26	-5,66	0,07	-1,36	-2,57	-3,86	0,01
Axon guidance																		
10854238	Kif4	Axon guidance;Immune System; MHC class II antigen presentat	3,03	8,18	10,54	0,71	-	-	3,47	0,51	2,10	4,29	4	0,21	1,99	3,98	2,62	0,57
Unclasified																		
10813452	Cotl1	No GC for biological process	1,96	3,90	6,05	0,24	2,12	1,60E-01	3,21	0,24	0,90	1,86	3,40	0,28	0,84	1,79	3,03	0,08

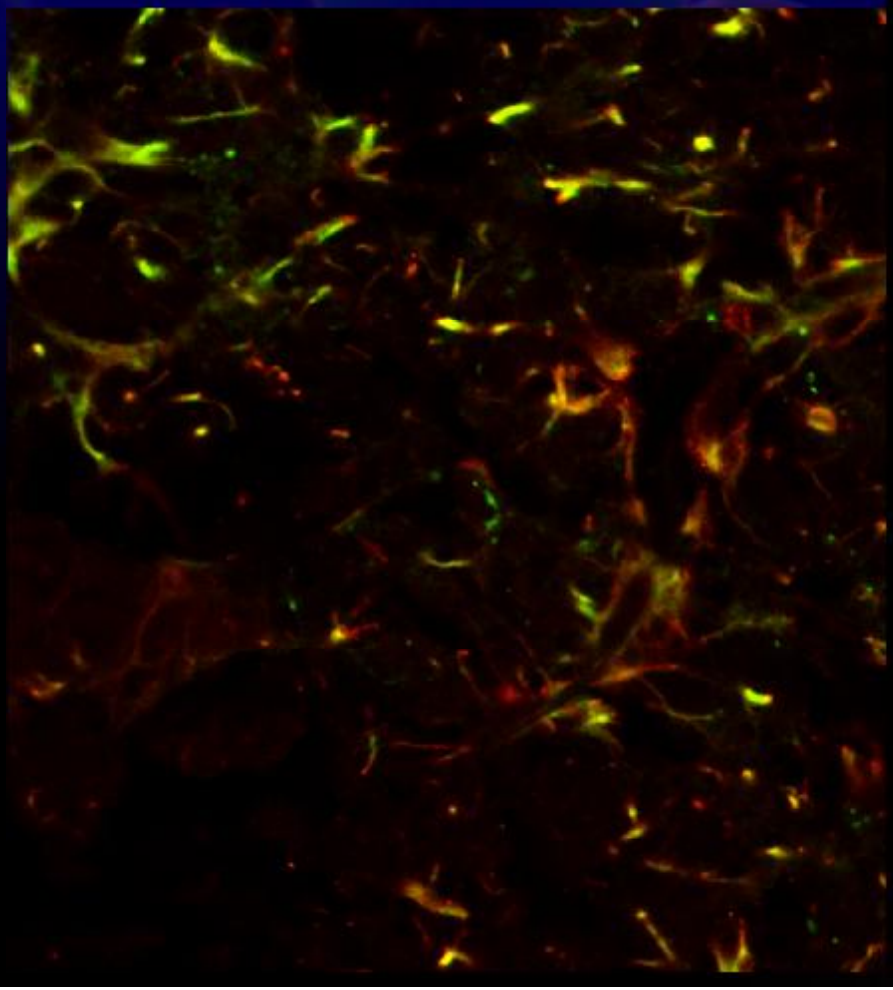
Kinesin superfamily member 4 is expressed in juvenile brain brain



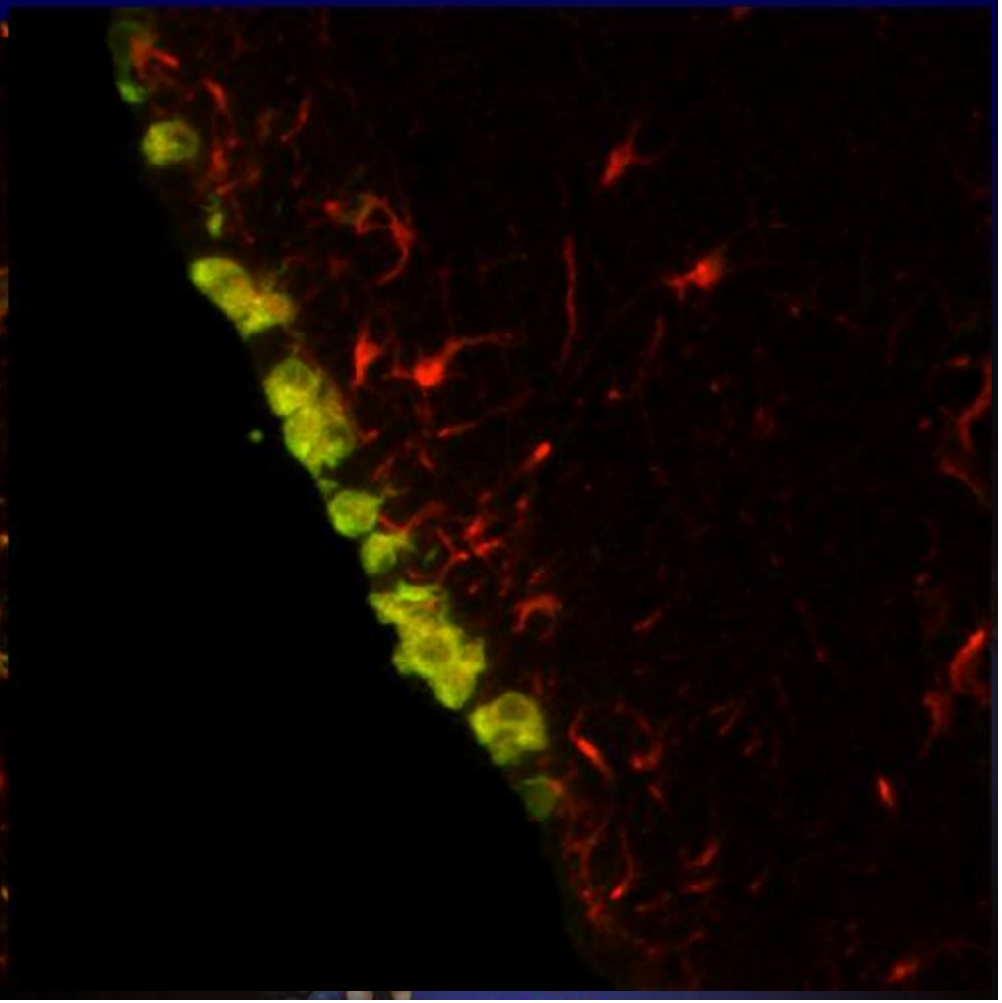
and reactivated after stroke in adult brain



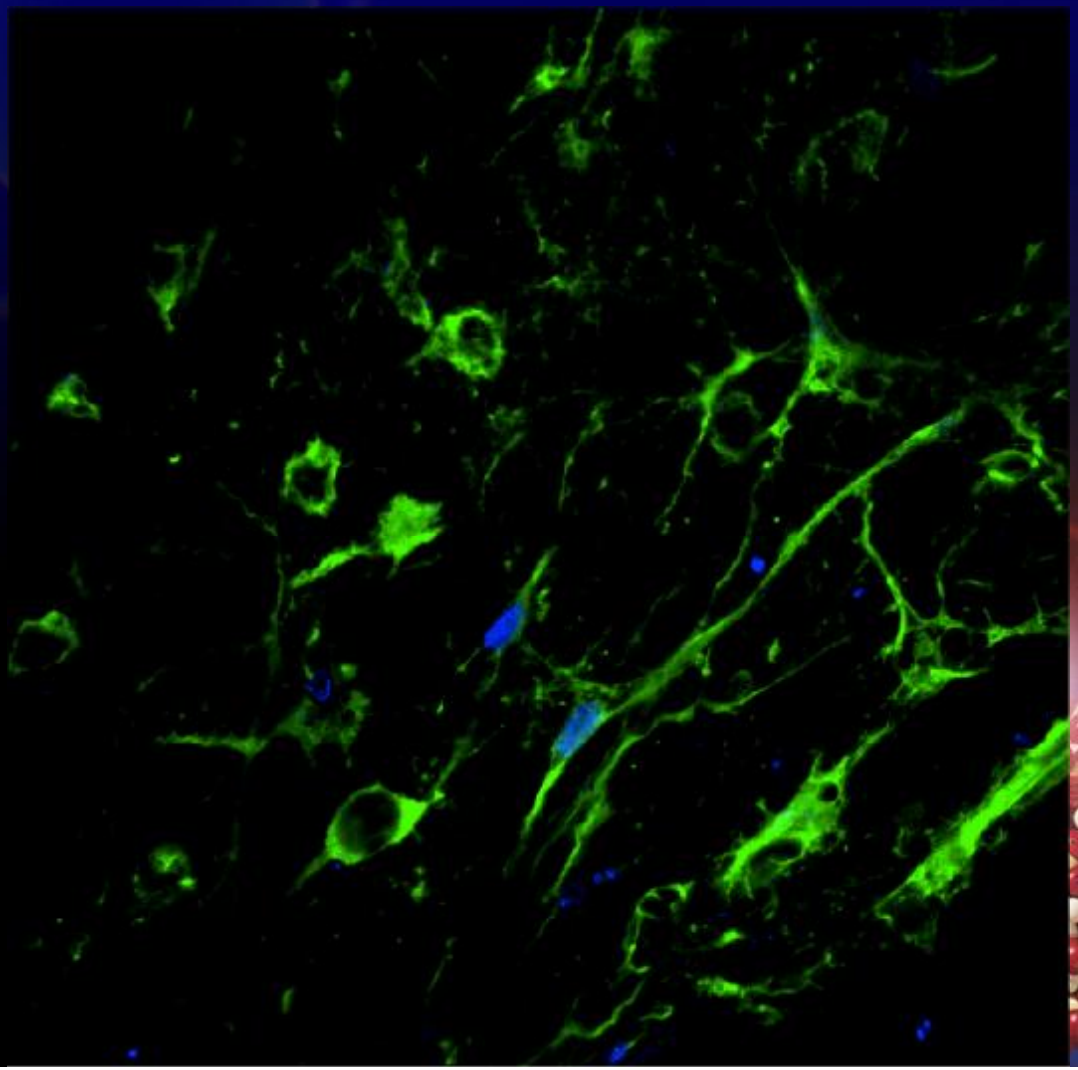
Colocalization
KIF 4_ GFAP in infarct area



Duble staining
KIF 4_ GFAP in SVZ



KIF4 colocalisation with BrdU in infarct area



CONCLUSION

mRNA expression pattern of KIFs reverted to the juvenile pattern during regeneration

KIF4, both of which is expressed abundantly in juvenile brain, increase significantly during regeneration in adult brain

aging brain can have a regenerative potential, one key factor involved in this process is Kif4, a novel-microtubule associated protein that can be associated with axonal grow and regeneration after injury in young brain.

The present study addresses these gaps in knowledge by assessing gene expression profiles in the aging brain after injury compare with developmental stage of the brain.

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INOVARE SI CREATIVITATE

gene expression

NEUROBIOLOGY OF AGING YOUNG RESEARCH TEAMS - PN-II-RU-TE-2014- 4-0705

The impact of microglia phagocytosis of live neurons on the efficacy of stem cell therapy of stroke

Postdoc: Dr Sandu Raluca –neurologist

PhD student: Dr Madalina Filfan

PhD student: Dr Roxana Surugiu

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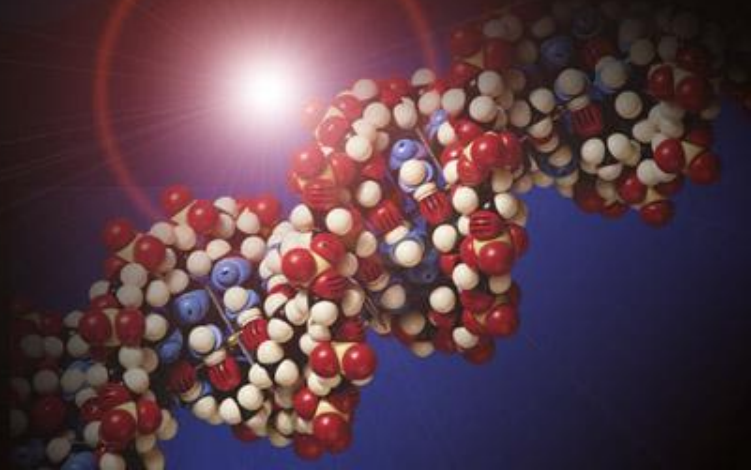

Colaboration:

Prof. Emil Toescu – Birmingham University, UK

Prof. Aurel Popa-Wagner – Rostock University, Germany

Prof. Hannah Monyer – University of Heidelberg, Germany

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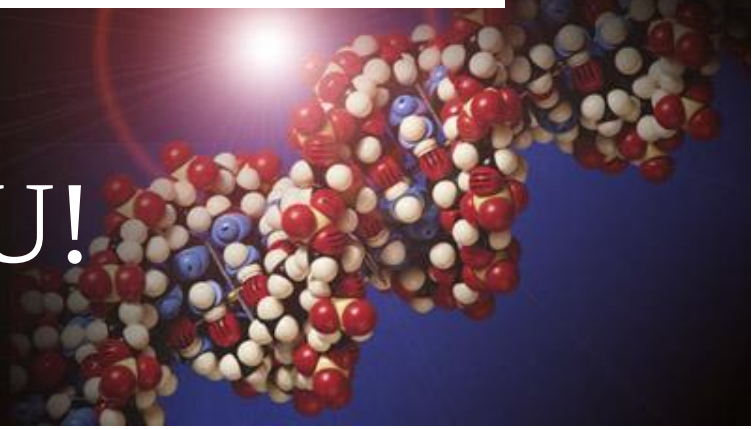

THANK YOU!