

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

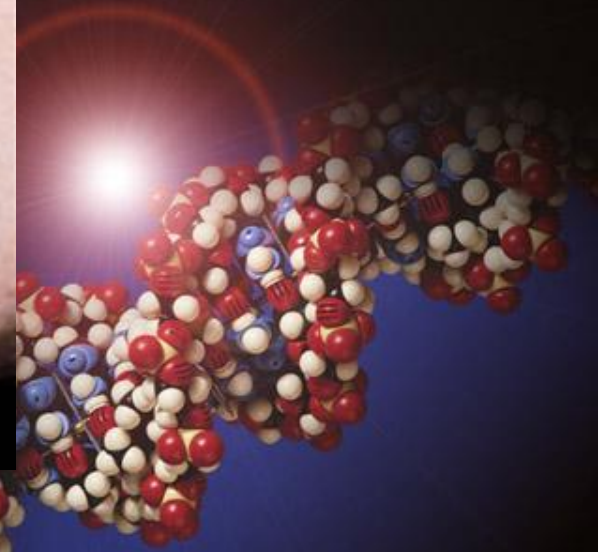


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Aging brain and age-related disease

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



Genetics

Biochemistry

Cell biology

Demography

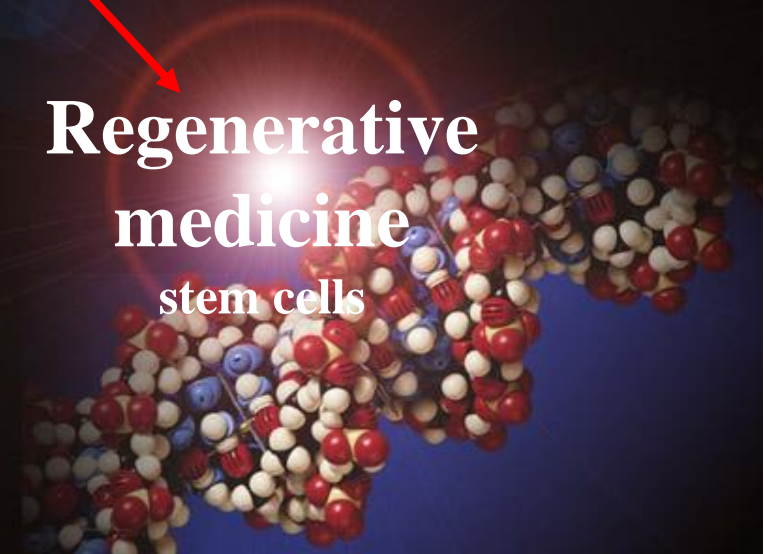
**Ageing vs
Developmental changes**

**Ageing-related
disease**

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

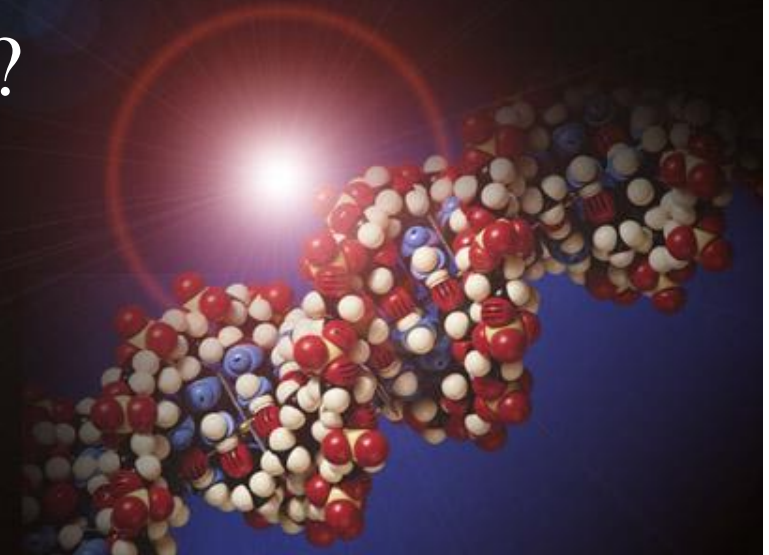
**Regenerative
medicine**

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

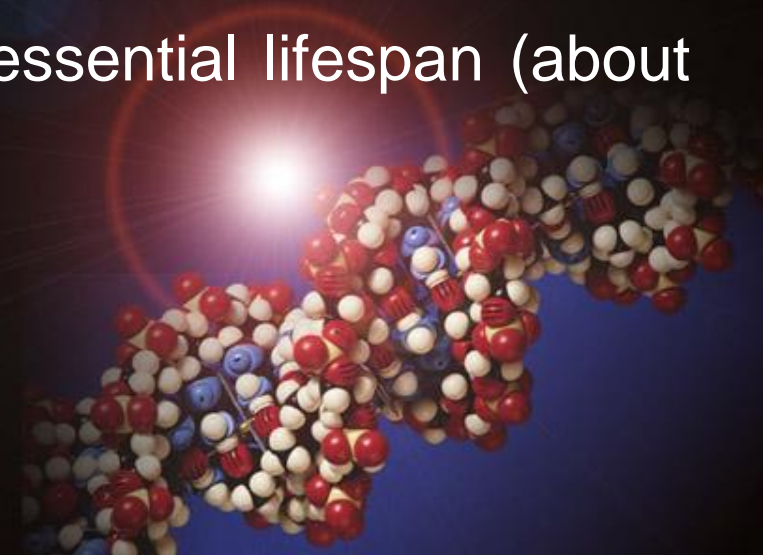
Essential lifespan: 40-46 years



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 quality of life (**Potential gerontogenes**)



Survival is a permanent challenge

- Oxygen metabolites
- Biochemical errors
- Nutritional metabolites

External and Internal Sources of damage

Aging = progressive decrease of our ability to repair these errors

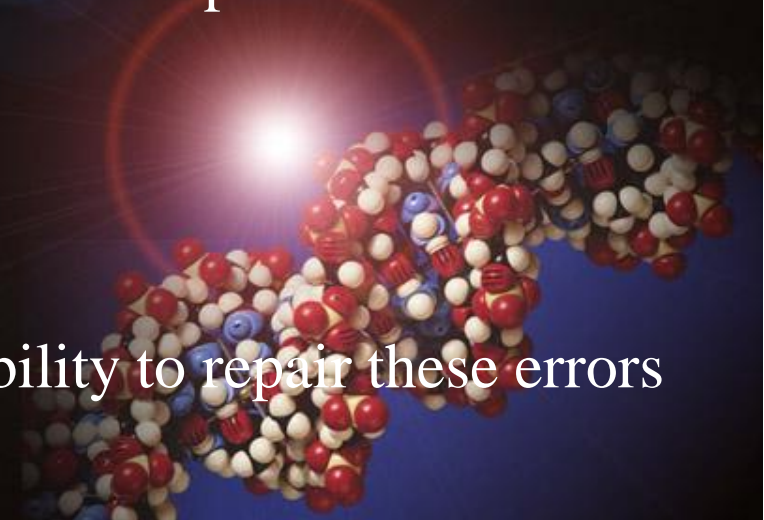
Maintenance and repair

Genomic stability

Protein stability

Oxidative stress protection

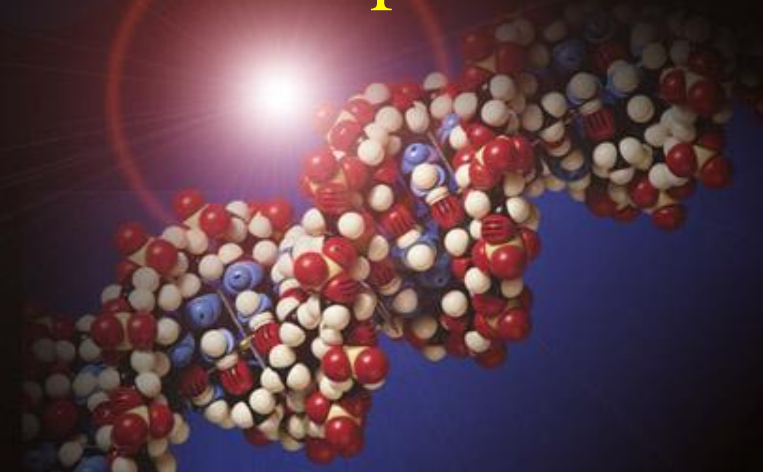
Tissue repair after lesion



Molecular Reasons of Ageing?

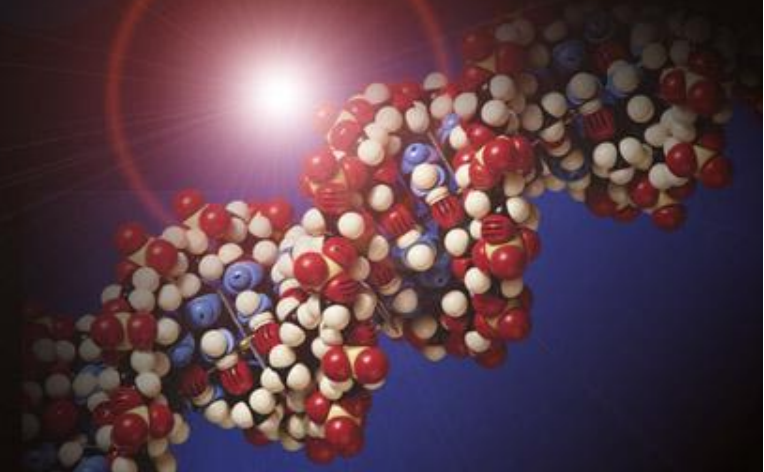
Ageing occurs at all biological levels.

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**



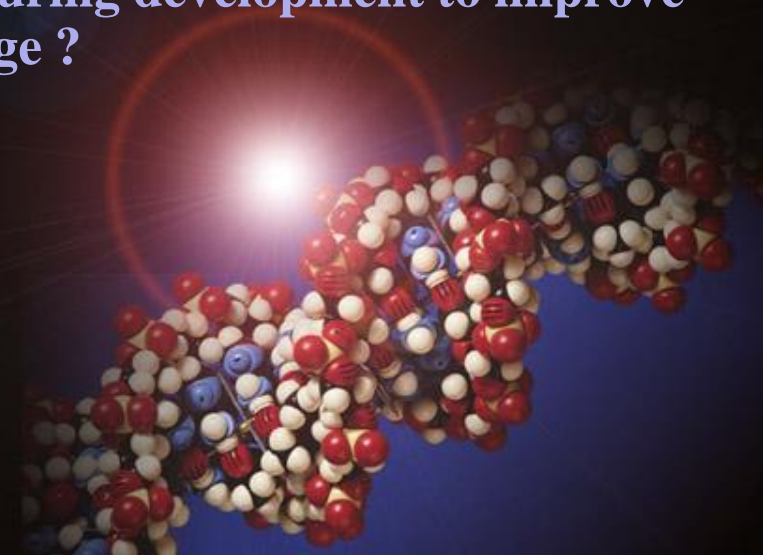
Questions

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 ?



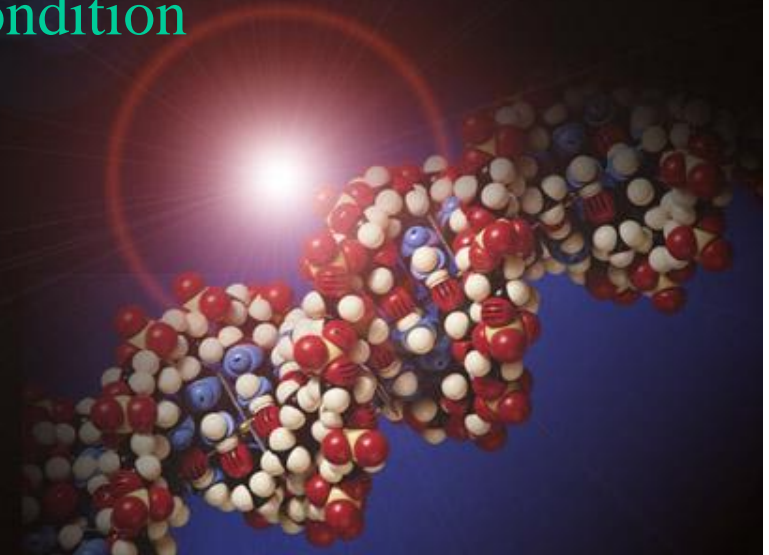
Overall Goal and Hypothesis in Genomics of Ageing

- **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

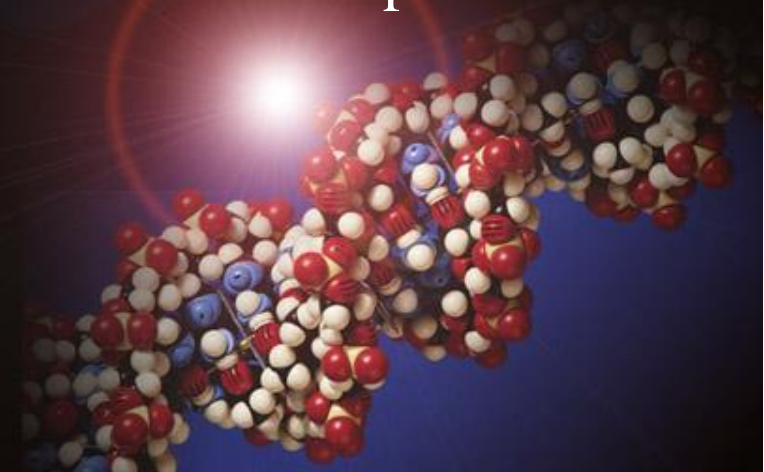
Ideal Disease Model

- 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 ?



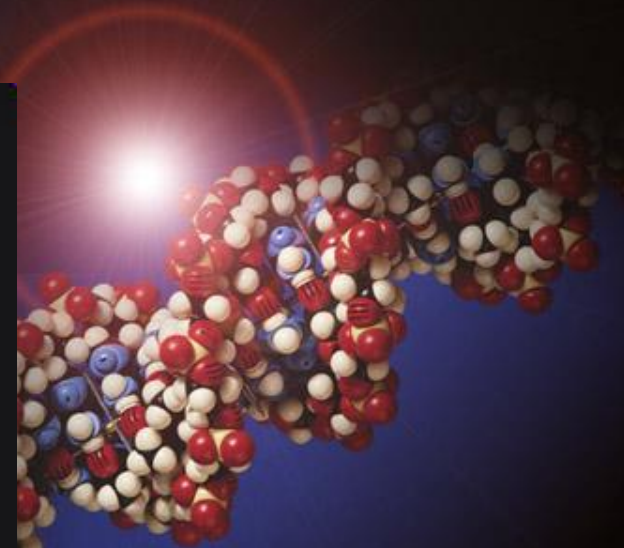
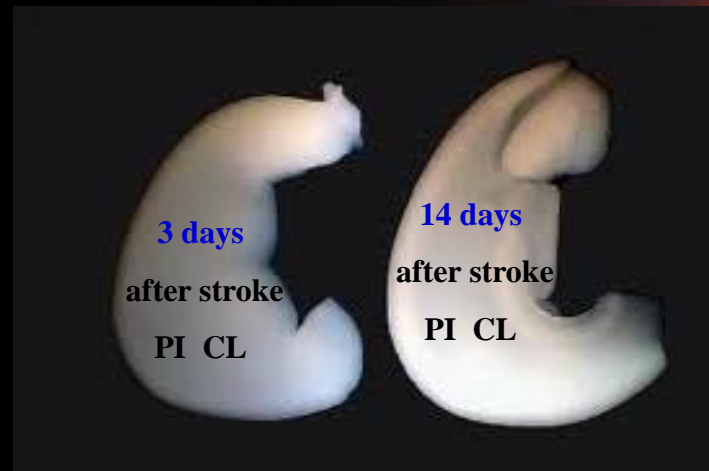
Postnatal 3d



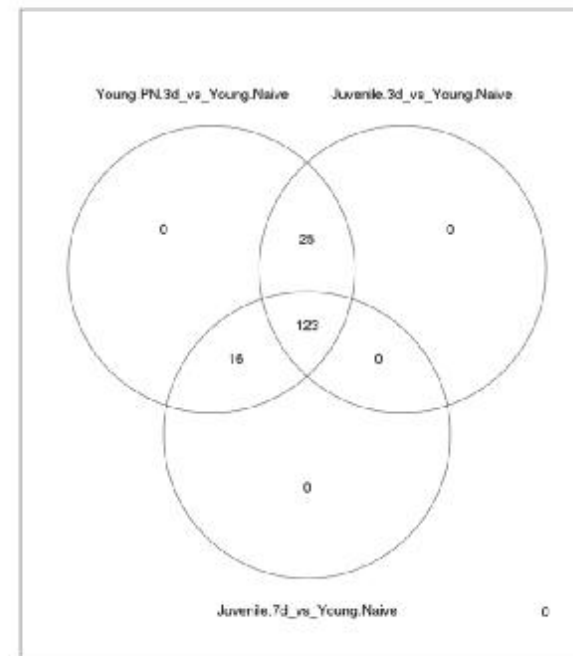
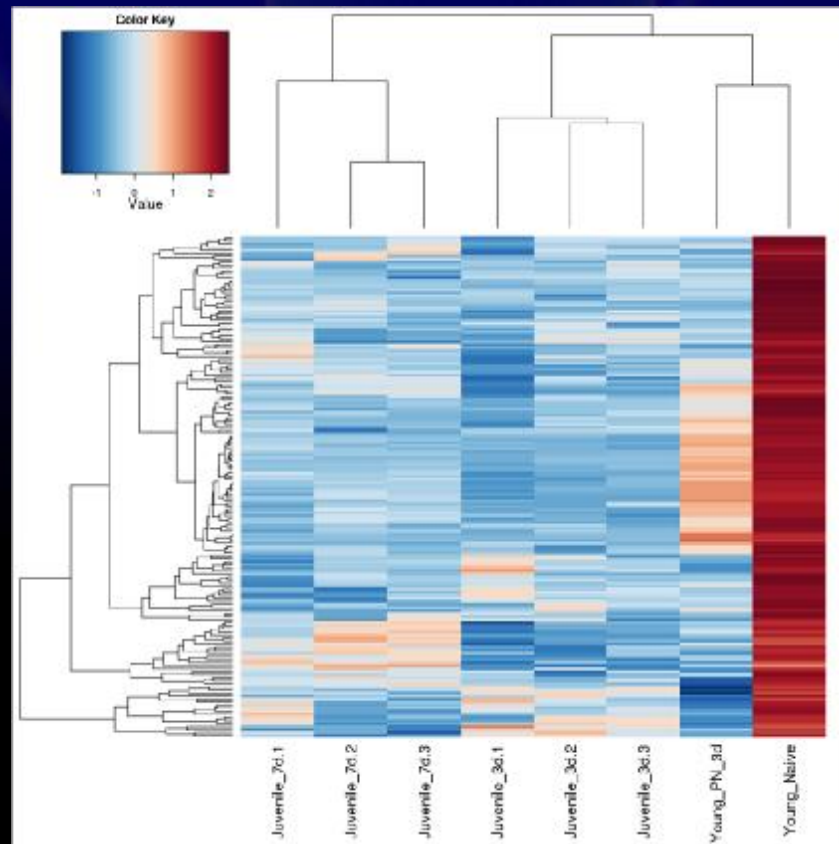
Postnatal 7d



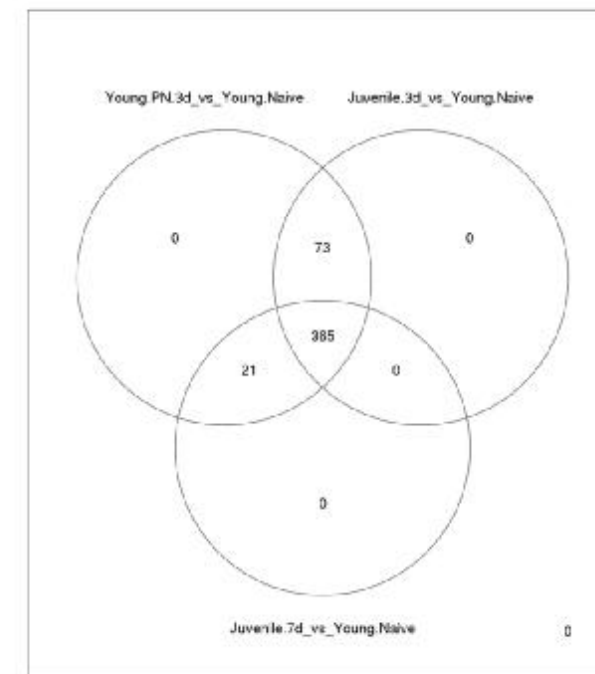
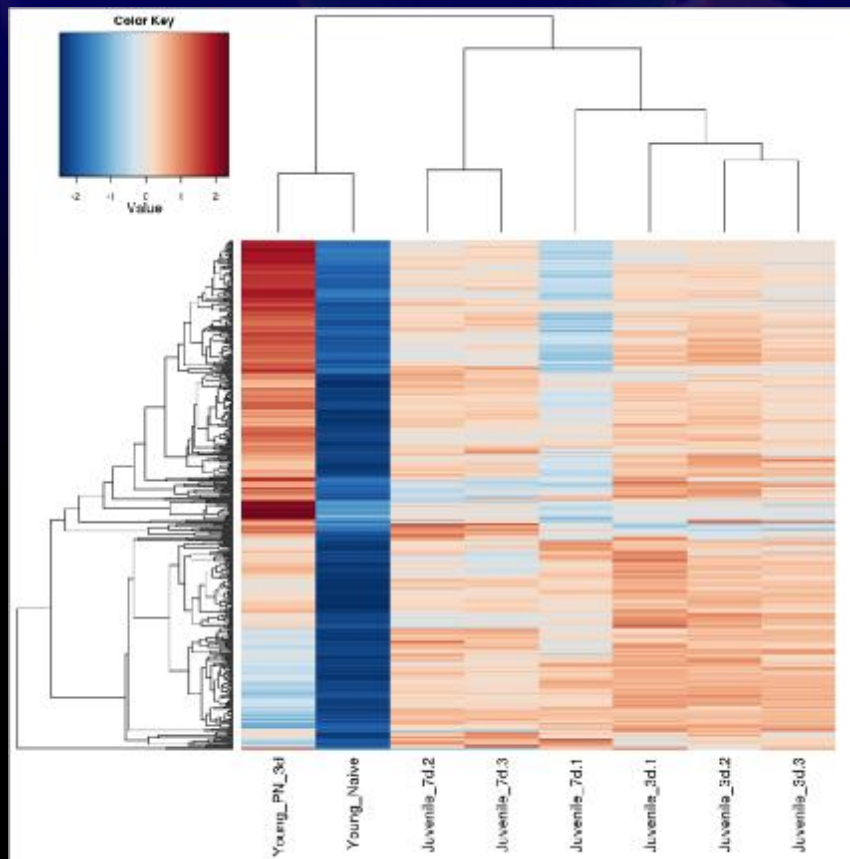
- Reversible occlusion of the middle cerebral artery (MCAO) with a tungsten hook for 90 min.



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



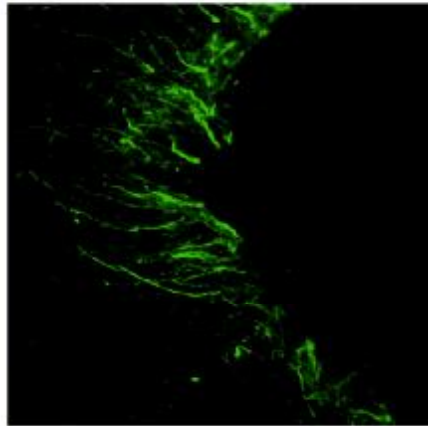
Relative expression values clearly distinguish naive rats from post-stroke groups and juvenile group



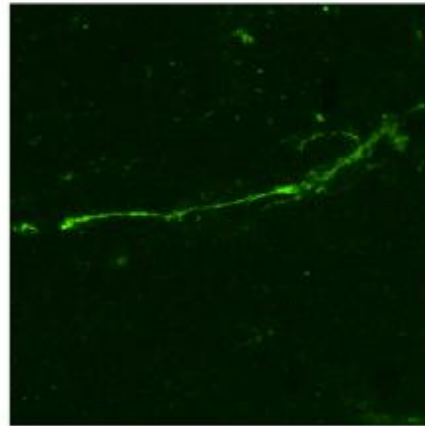
Sequence Code	Gene Symbol	Gene name	Young PN 3d vs Young Naive				Young PN 14d vs Young Naive				Juvenile 5d vs Young Naive				Juvenile 7d vs Young Naive			
			Array logFC	Array FC	RT-PCR FC	SD	Array FC	PDR	RT-PCR FC	SD	Array logFC	Array FC	RT-PCR FC	SD	Array logFC	Array FC	RT-PCR FC	SD
GO:0032268--regulation of cellular protein metabolic process																		
10526482	Trim1		4.92	36.19	67.48	3.47	5.21	8.73E-10	7.17	1.25	1.53	2.80	3.02	0.15	1.51	2.84	3.26	0.34
10849737	Bub1		3.36	16.28	23.13	2.83	1.16	9.60E-01	7.1	0.5	2.86	7.28	3.02	0.16	2.59	6.01	24.76	2.42
10515437	Dnmt1*		0.91	1.87	-1.37	0.03	-1.07	9.55E-01	-1.17	0.07	1.57	2.97	8.32	0.72	1.38	2.68	8.77	0.85
GO:0042127--regulation of cell proliferation																		
10515437	Dnmt1*		0.91	1.87	-1.37	0.03	-1.07	9.55E-01	-1.17	0.07	1.57	2.97	9.30	0.72	1.38	2.68	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	3.58	11.73	21	0.00	2.70	6.48	14.34	1.53
10725155	Ybx1		1.25	2.89	2.86	0.2	1.43	9.52E-01	6.36	0.56	2.11	4.82	10.08	1.5	2.17	4.49	13.26	0.76
10856814	Myc		2.03	4.08	4.01	0.11	1.20	9.11E-01	8.57	0.77	2.84	7.14	444.84	39.14	2.68	6.48	2310.81	227.97
10823852	Ccna2		1.18	5.12	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	2.86	1.88	3.68	8.62	0.18
GO:0008285--negative regulation of cell proliferation																		
10755652	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
10817301	s100b11		4.56	23.61	35.75	1.26	4.49	6.26E-08	8.03	0.62	1.36	6.13	7.42	0.75	2.34	6.06	7.83	0.18
GO:0006259--DNA metabolic process																		
10755652	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		2.03	4.08	4.01	0.11	1.20	9.11E-01	8.57	0.77	2.84	7.14	444.84	39.14	2.68	6.48	2310.81	227.97
10515437	Dnmt1*		0.91	1.87	-1.37	0.03	-1.07	9.55E-01	-1.17	0.07	1.57	2.97	8.32	0.72	1.38	2.68	8.77	0.85
10726408	Mxd7		4.20	16.33	8.03	0.52	-1.35	9.57E-01	3.34	0.32	3.33	6.73	5.5	0.01	2.99	7.94	4.27	0.48
10883503	Rrm2		4.15	17.70	47.98	1.85	2.01	2.45E-01	6.93	0.15	2.20	4.60	16.23	1.41	2.03	4.19	14.11	0.57
10862584	Ubr1		2.46	5.47	9.39	0.31	1.42	8.45E-01	4.41	1.07	2.40	6.30	2.08	0.36	2.20	4.61	2.18	0.07
GO:0051726--regulation of cell cycle																		
10755652	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*		2.03	4.08	4.01	0.11	1.20	9.11E-01	8.57	0.77	2.84	7.14	444.84	39.14	2.68	6.48	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	2.86	1.88	3.68	8.62	0.18
GO:0006997--nucleus organization																		
10755652	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*		2.03	4.08	4.01	0.11	1.20	9.11E-01	8.57	0.77	2.84	7.14	444.84	39.14	2.68	6.48	2310.81	227.97
GO:0006974--response to DNA damage stimulus																		
10755652	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
10781346	Pik		1.20	9.18	29.15	2.79	1.65	6.70E-01	7.14	0.43	1.80	3.74	4.82	0.56	1.97	3.93	1.76	0.01
GO:0007017--microtubule-based process																		
10764821	Tubb2b		1.26	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	6.55	5.13	0.5
10849568	Spca5		2.22	4.64	5.70	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
10813048	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																		
10754824	Tubb2b		1.26	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	6.55	5.13	0.5
10755293	Hirt1hb		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	Aldh1l2		0.94	1.92	16.13	1.02	-	-	54.3	1.23	1.36	16.25	185.15	10.08	2.74	6.89	98.62	6.36
GO:000194--extracellular matrix organization																		
10873638	Mfp2		0.96	1.94	2.08	0.33	3.40	3.81E-05	9.71	0.93	2.88	7.34	15.65	1.37	2.42	6.35	7.59	1.5
10815169	Peain		1.13	6.74	8.28	0.45	1.67	5.47E-01	6.67	1.55	1.68	5.19	3.45	0.87	2.11	4.51	-1.76	0.23
10904163	Sir		1.68	6.42	8.88	0.42	2.42	2.79E-02	27.41	0.85	4.79	27.88	59.88	5.52	4.58	22.70	45.71	2.47
GO:0007165--signal transduction																		
1053814	Mek1		1.49	2.80	3.24	0.33	-	-	14.58	1.15	1.76	9.61	10.18	1.75	3.81	14.26	15.12	0.74
10813096	Clic1		1.41	16.60	9.58	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
10817383	s100b11		4.56	23.61	35.75	1.26	4.49	6.26E-08	8.03	0.62	1.36	6.13	7.42	0.75	2.34	6.06	7.83	0.18
10782533	Stb47		0.89	1.86	1.16	0.12	1.34	8.59E-01	3	0.24	1.51	2.84	2.75	0.46	1.21	2.91	5.13	0.26
10817098	Clim4		-0.94	-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	-8.7	0.02
10819548	Depdc1		2.52	5.72	-1.85	0.04	1.03	9.47E-01	2.2	0.17	1.14	4.41	1.12	0.04	2.09	4.27	-1.01	0.03
10882146	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	-6.88	-24.58	0.00
10816873	Itpka		-1.03	-2.13	-4.24	0.02	-1.62	6.89E-01	-3.82	0.01	-3.21	-9.25	-195.88	0.00	-2.62	-5.06	-45.32	0.00
GO:0016477--cell migration																		
10843559	Vav2																	
GO:0007049--cell cycle																		
10849568	Spca5		2.22	4.64	5.70	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		2.71	4.64	3.84	0.13	-1.04	9.48E-01	1.97	0.09	1.77	4.83	15.77	0.81	1.98	3.97	23.17	1.65
10849737	Bub1		3.36	16.28	23.13	2.83	1.15	9.60E-01	7.1	0.5	2.86	7.28	27.6	0.78	2.59	6.01	24.76	2.42
10812852	Ccna2		1.18	5.12	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	2.86	1.88	3.68	8.62	0.18
10813048	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
10823188	Sgo2		2.86	7.28	13.86	0.8	-	-	6.67	0.58	2.05	4.13	3.84	0.47	1.96	3.89	1.48	0.61
GO:0000280--nuclear division																		
10849568	Spca5		2.22	4.64	5.70	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
10828745	Casr2		3.45	16.91	-30.67	0	1.72	4.91E-01	4.65	0.4	1.76	6.88	2.7	0.91	2.72	6.61	2.11	0.95
GO:000902--cell morphogenesis																		
10823558	Irf2		1.05	6.27	20.57	2.72	1.34	8.88E-01	12.69	1.84	1.13	4.39	9.15	0.76	2.03	4.05	7.04	1.48
GO:0036811--ion transport																		
10882145	Gabrd		-1.18	-2.27	-4.74	0.04	-1.55											

Kinesin superfamily member 4 is expressed in juvenile brain brain

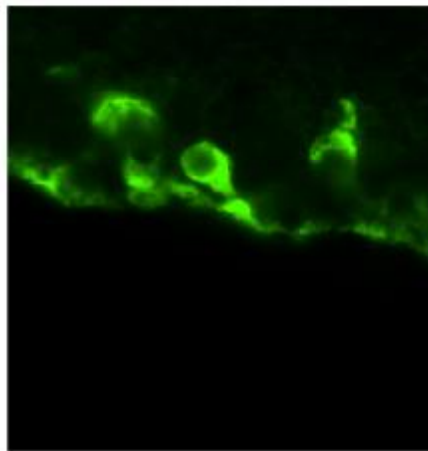
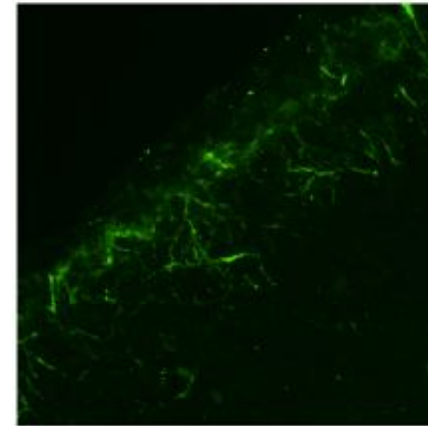
Juv 3d 3V



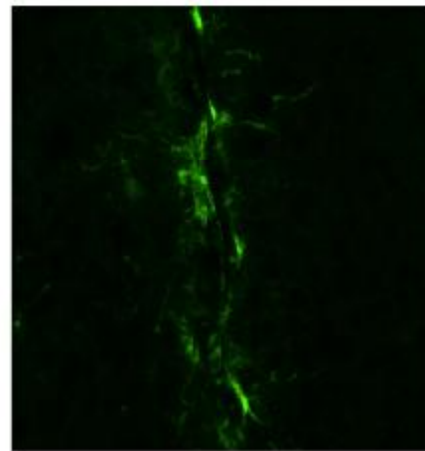
Juv 3d BV



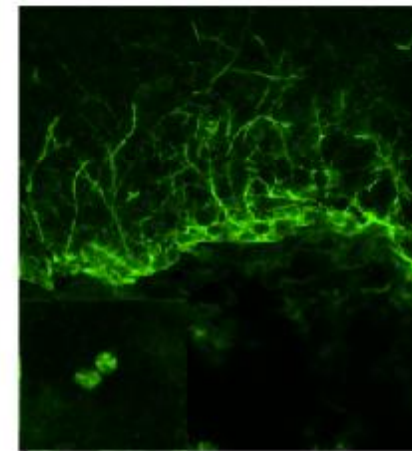
Juv 3d Cx-L1



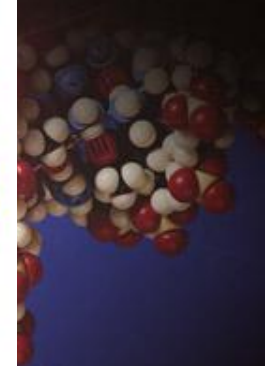
Juv 7d LV



Juv 7d BV-Astrocytes

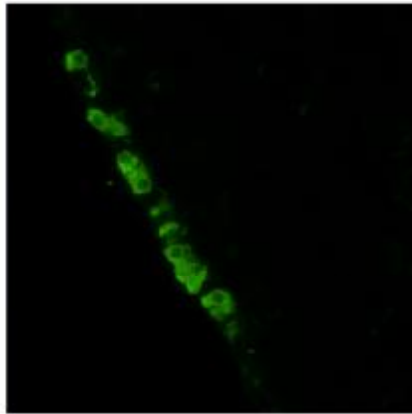


Juv 7d Cx-L1

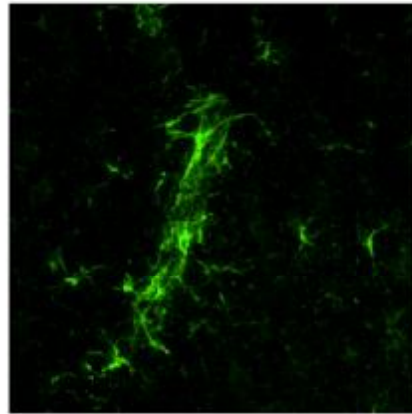


and reactivated after stroke in adult brain

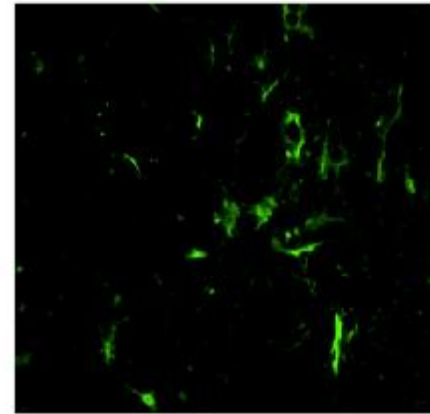
Y3d SVZ



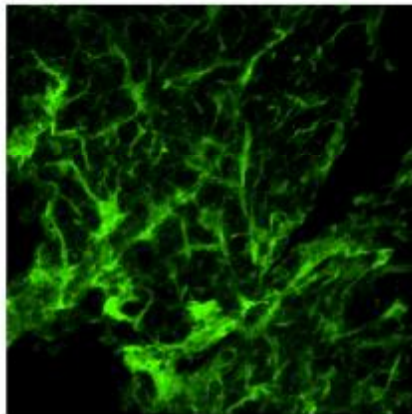
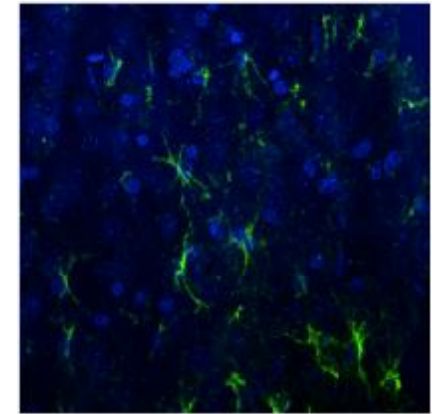
Y3d BV



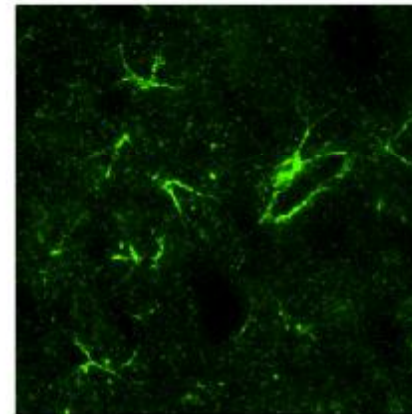
Y3d CC-PI



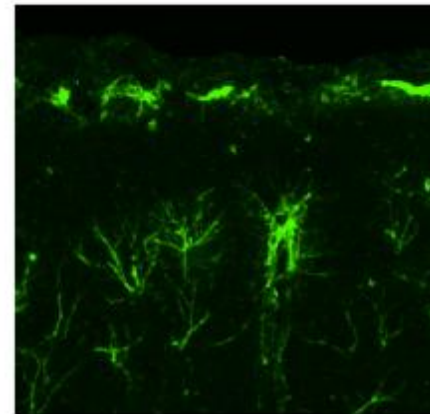
Y3d PI



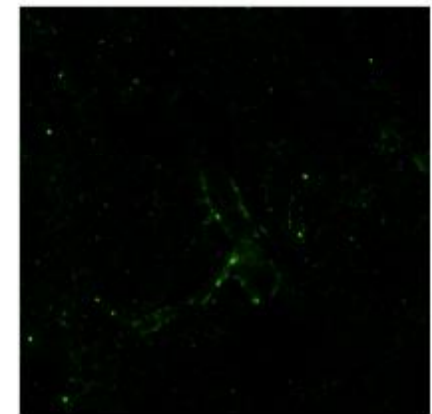
Y14d PI



Y14d PI-BV

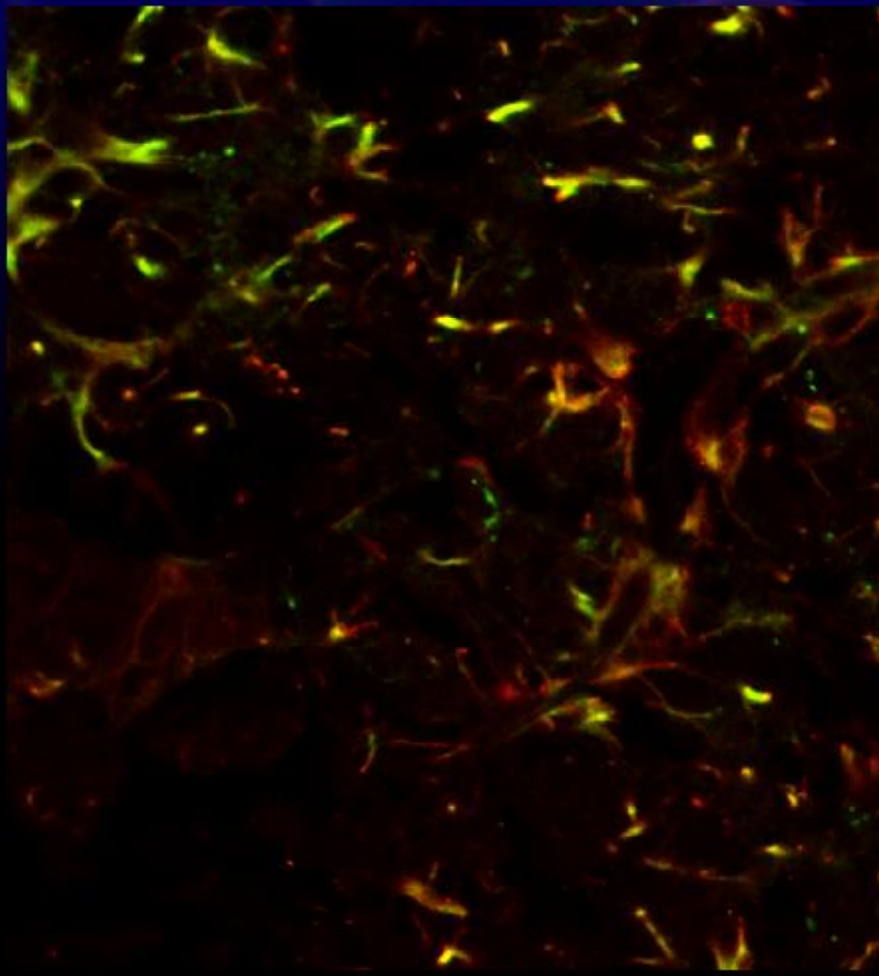


Y14d Cx-CL

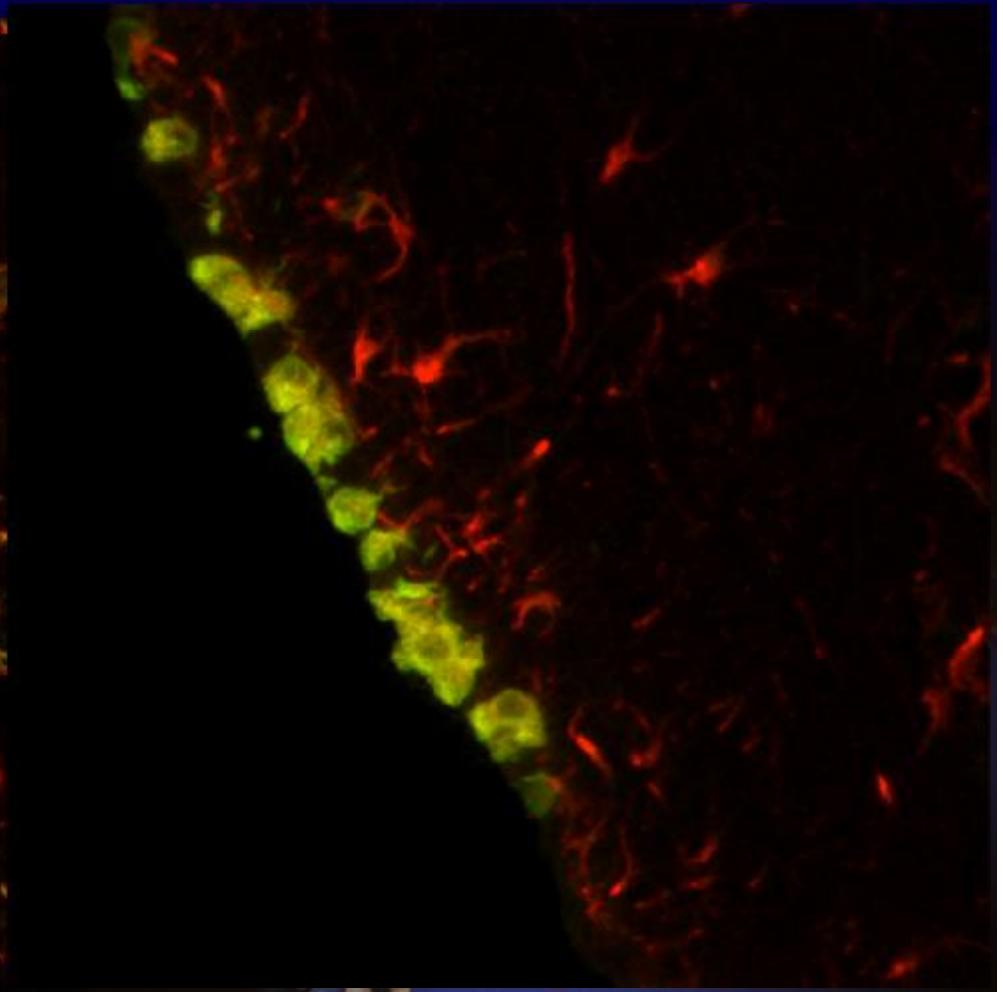


Y Naive

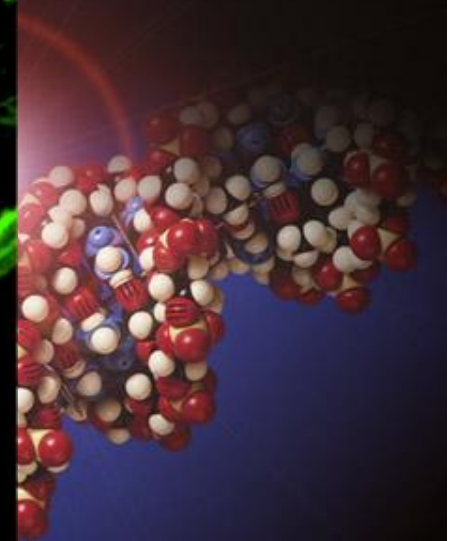
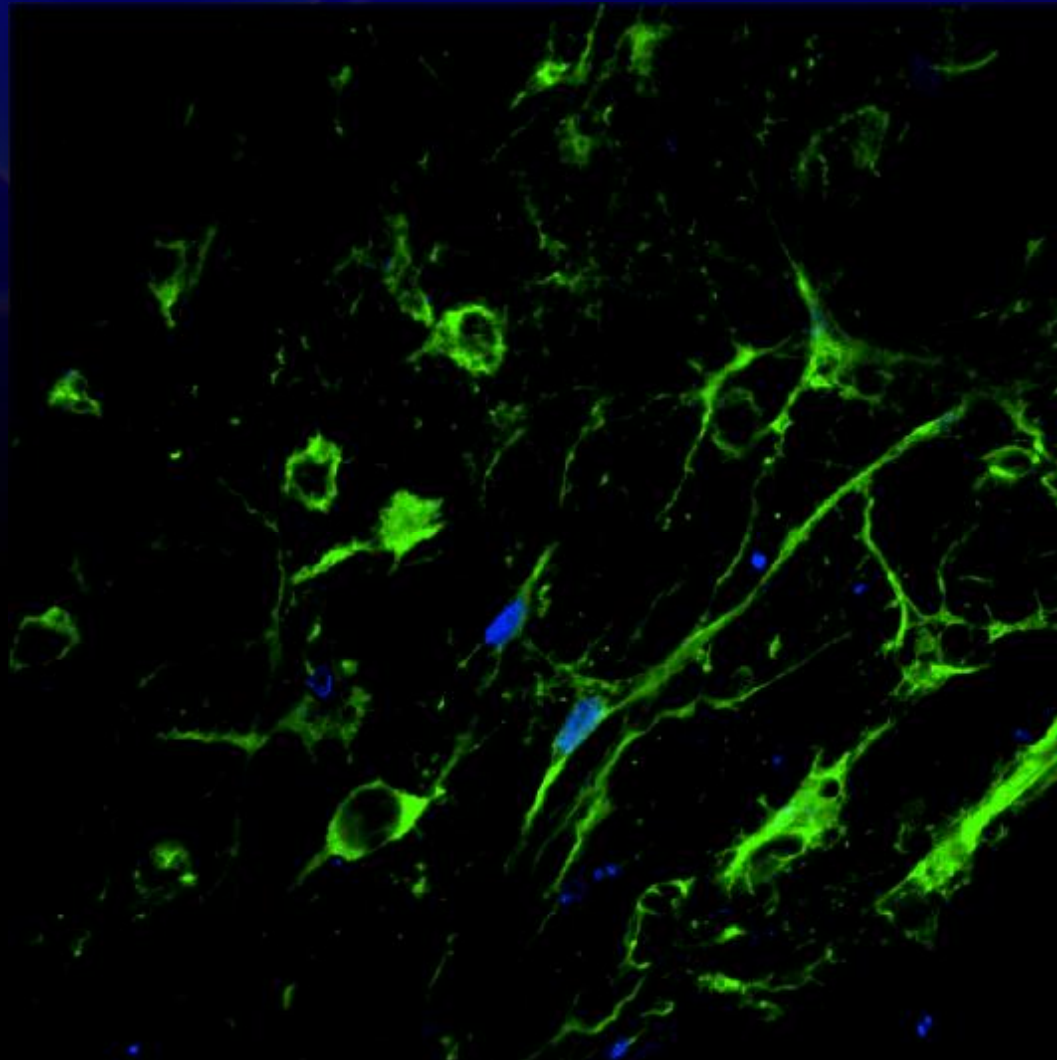
Colocalization
KIF 4_ GFAP in infarct area



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.

THANK YOU!

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