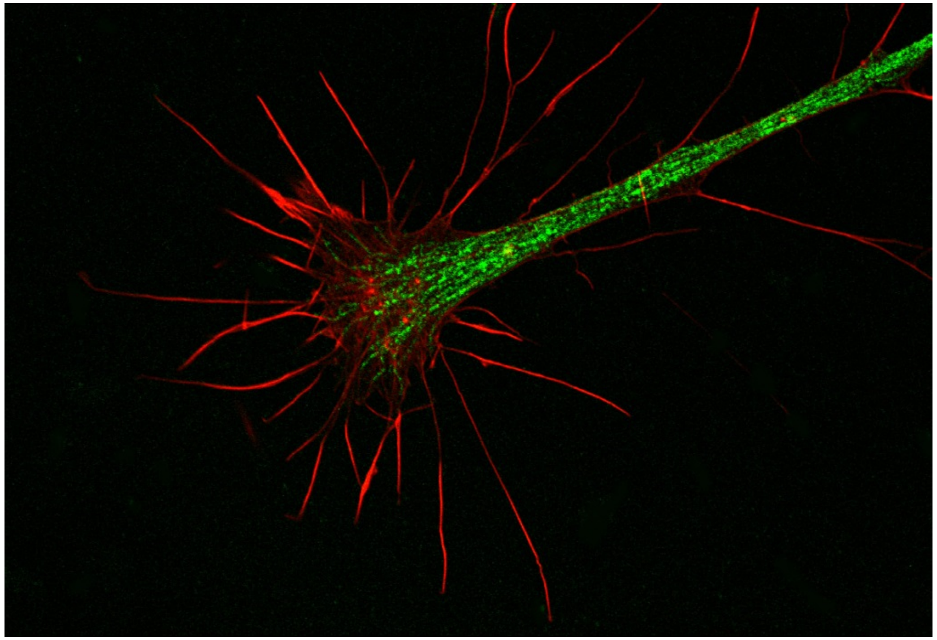
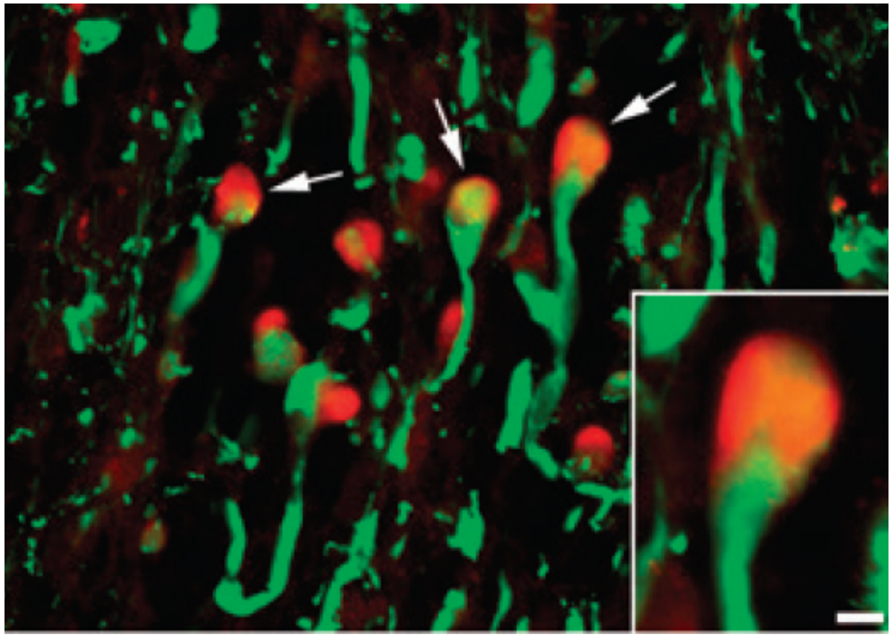
AXON GROWTH AND REGROWTH





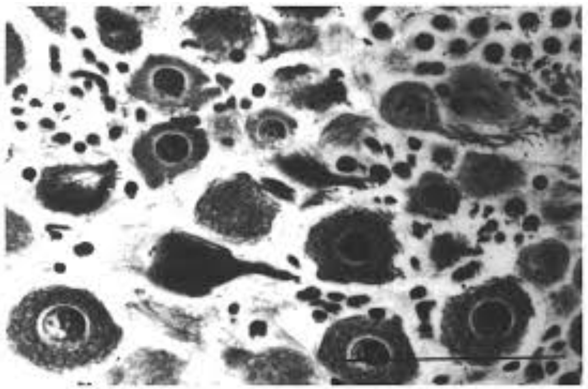
e-mail: matilde.ghibaudi@unito.it

Matilde Ghibaudi
PhD student

TWO OPPOSITE VIEWS

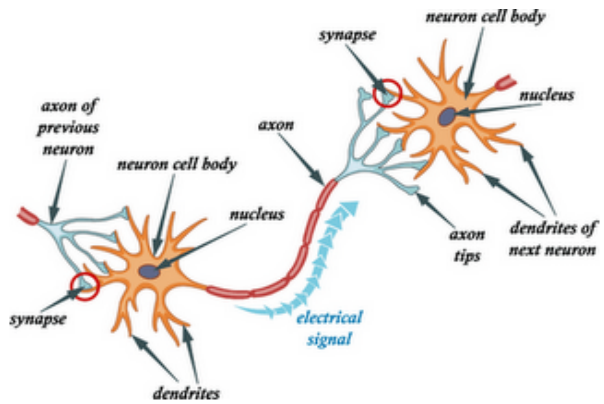
"RETICULARISTIC THEORY"

CONTINUOS SYSTEM OF NERVE CELLS
ANASTOMOSED
INTO A SINGLE NETWORK

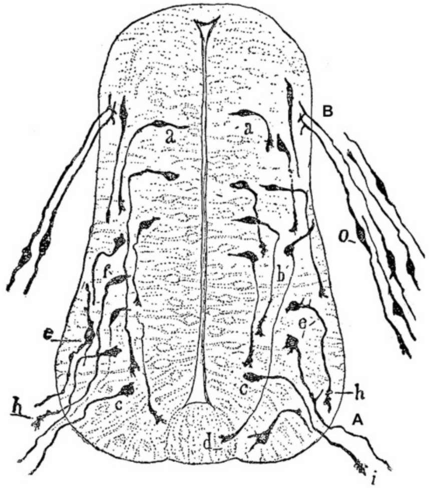


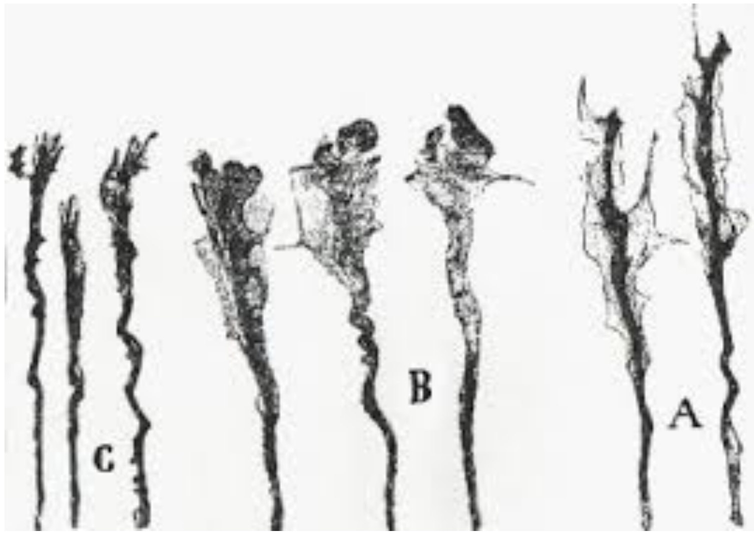
"NEURONISTIC THEORY"

AXONS GROWTH OUT FROM
NEURONAL CELL BODIES
AND REACH OTHER NEURONS

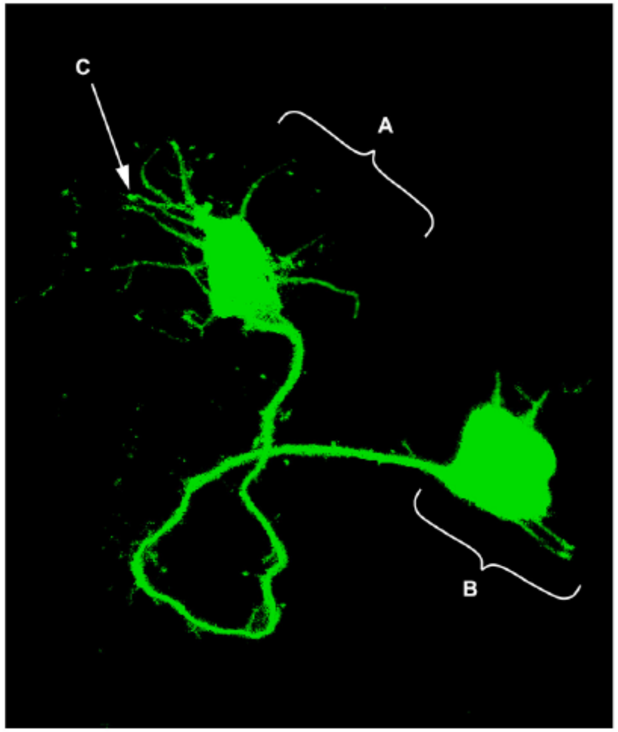


''CONE LIKE LUMP WITH A PERIPHERAL BASE DECORATED BY TRIANGULAR OR SHORT THORNY PROCESSES''

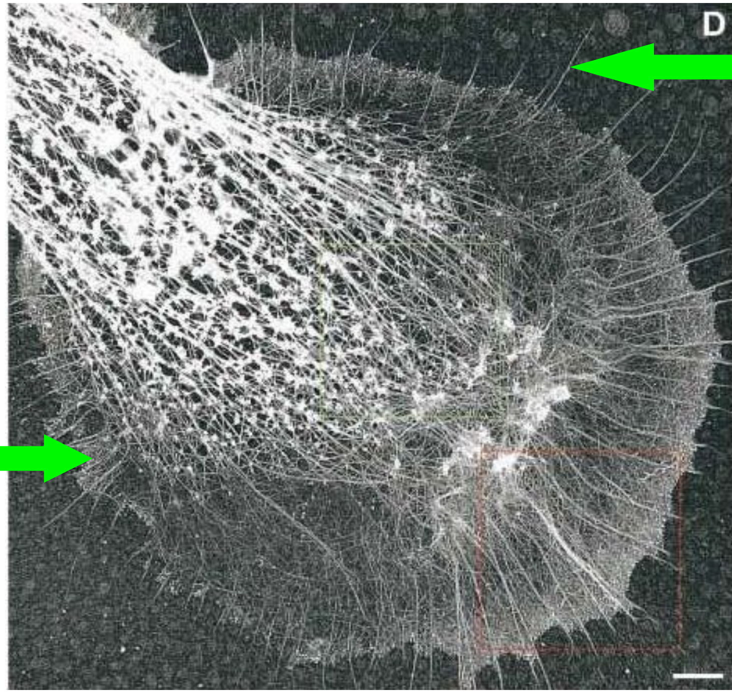




STRUCTURE OF THE GROWTH CONE



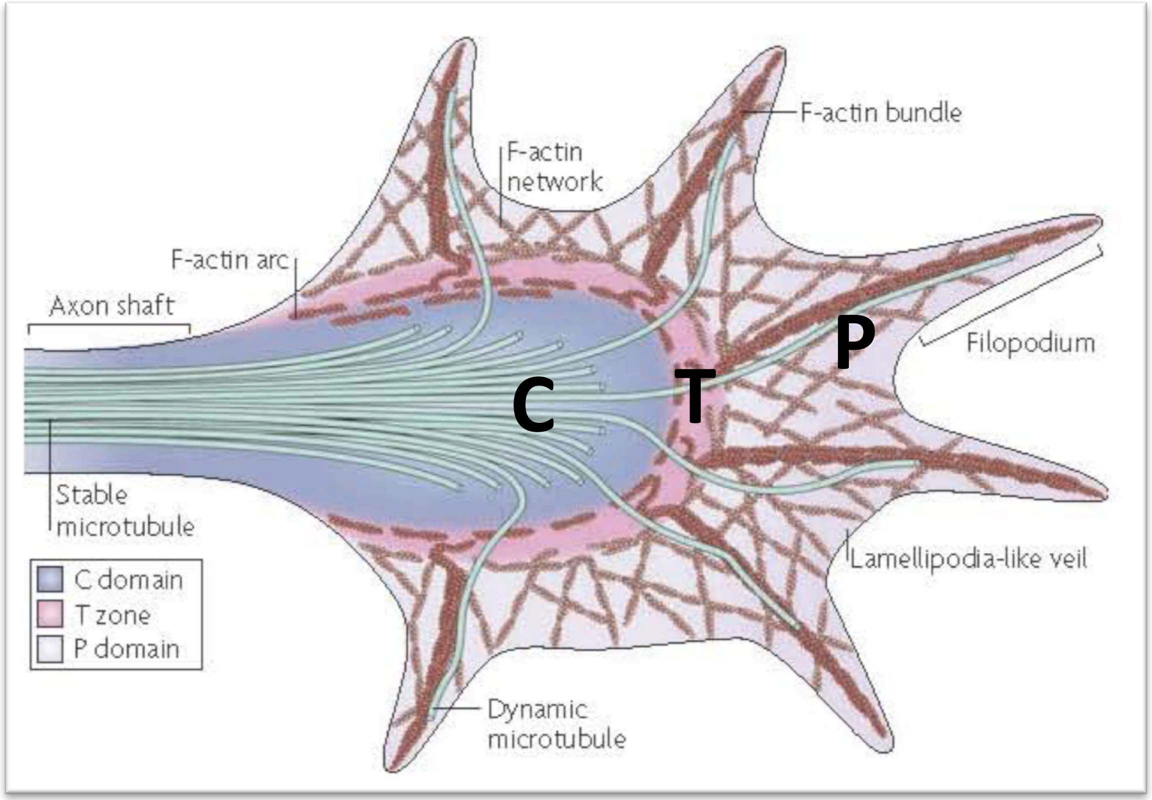
A GROWH CONE
B CELL BODY
C FILOPODIA



FILIPODIA

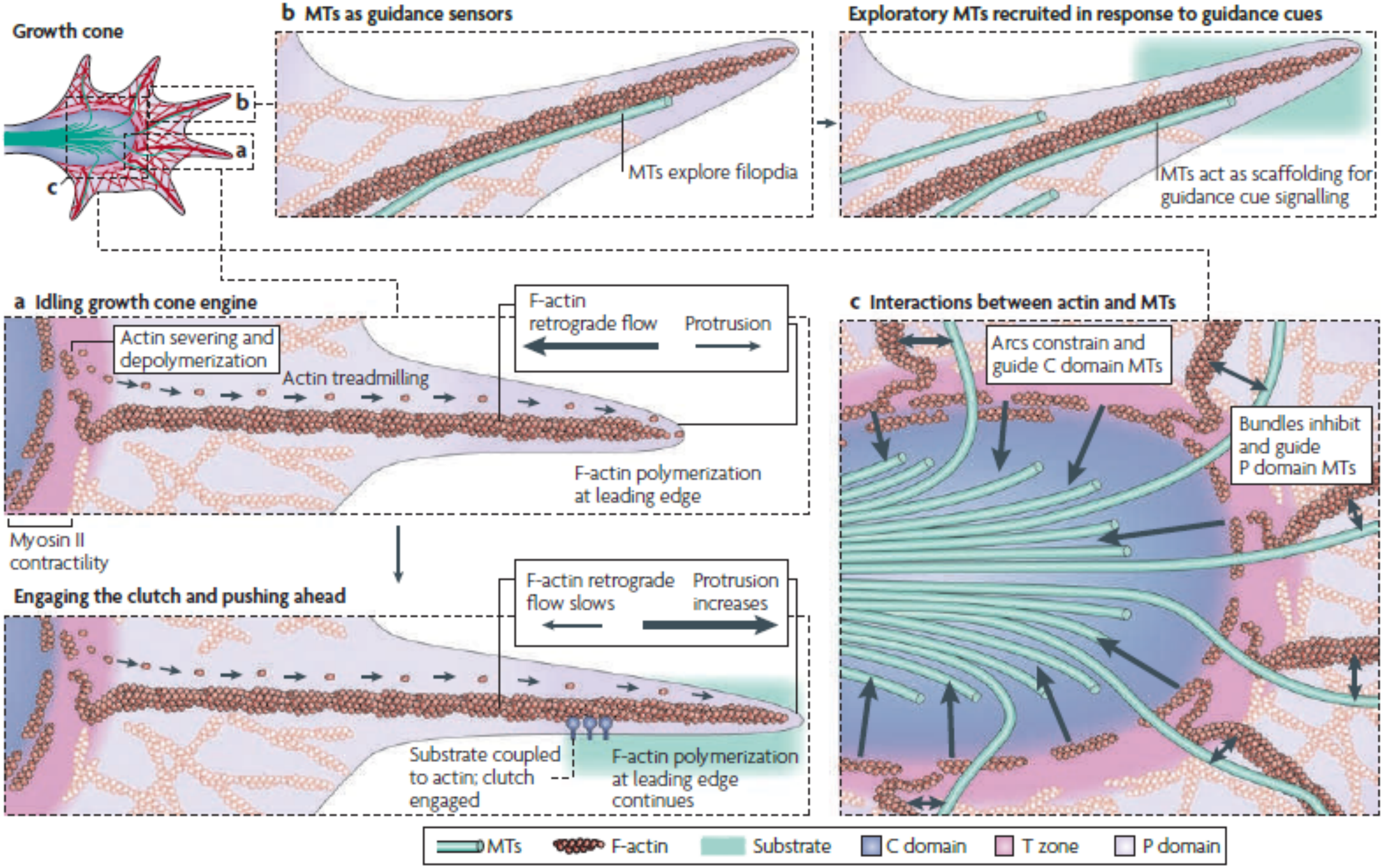
LAMELLIPODIA

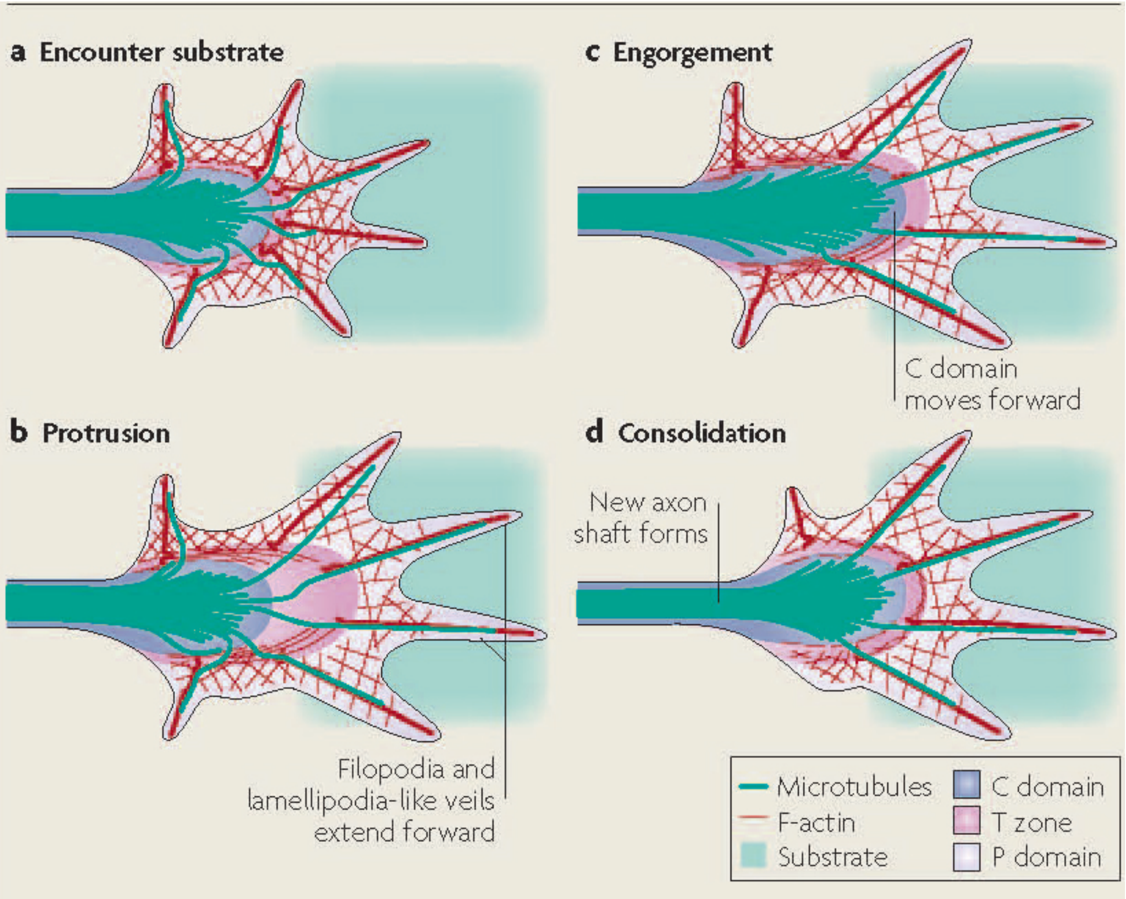
STRUCTURE OF THE GROWTH CONE



C CENTRAL
P PERIPHERAL
T TRANSITION

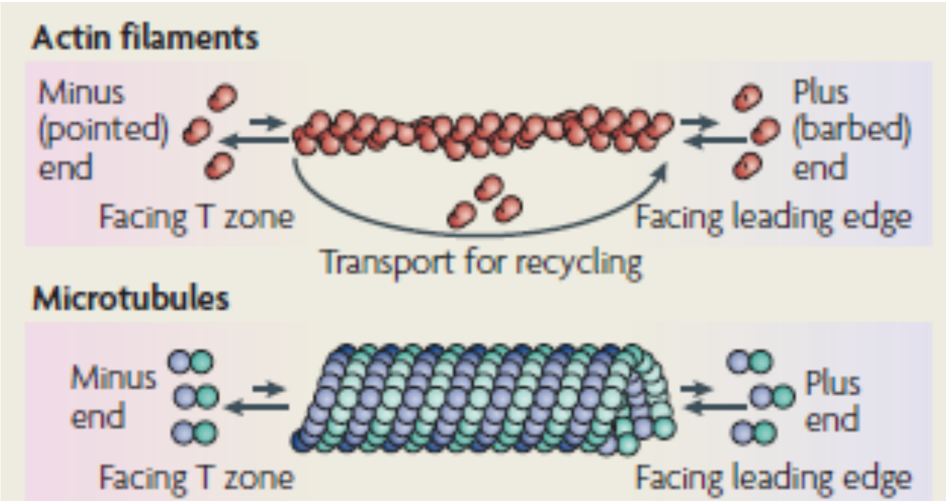
HOW DOES IT MOVE?





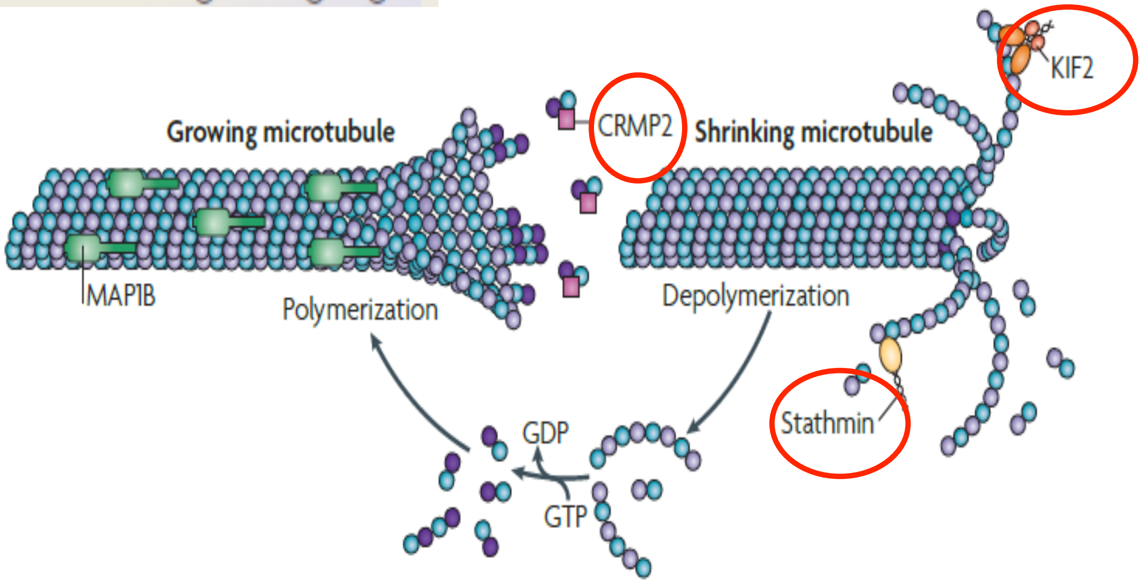
THE BALANCE OF **ANTEROGRADE POLUMERIZATION** AND **RETROGRADE RETRACTION** DETERMINES THE ADVANCE OF THIS ACTIN-RICH STRUCTURES IN THE PERIPHERAL DOMAIN

ACTIN-MICROTUBULES DYNAMIC



- MONOMER BINDING PROTEINS
- NUCLEATION FACTORS
- CAPPING PROTEINS

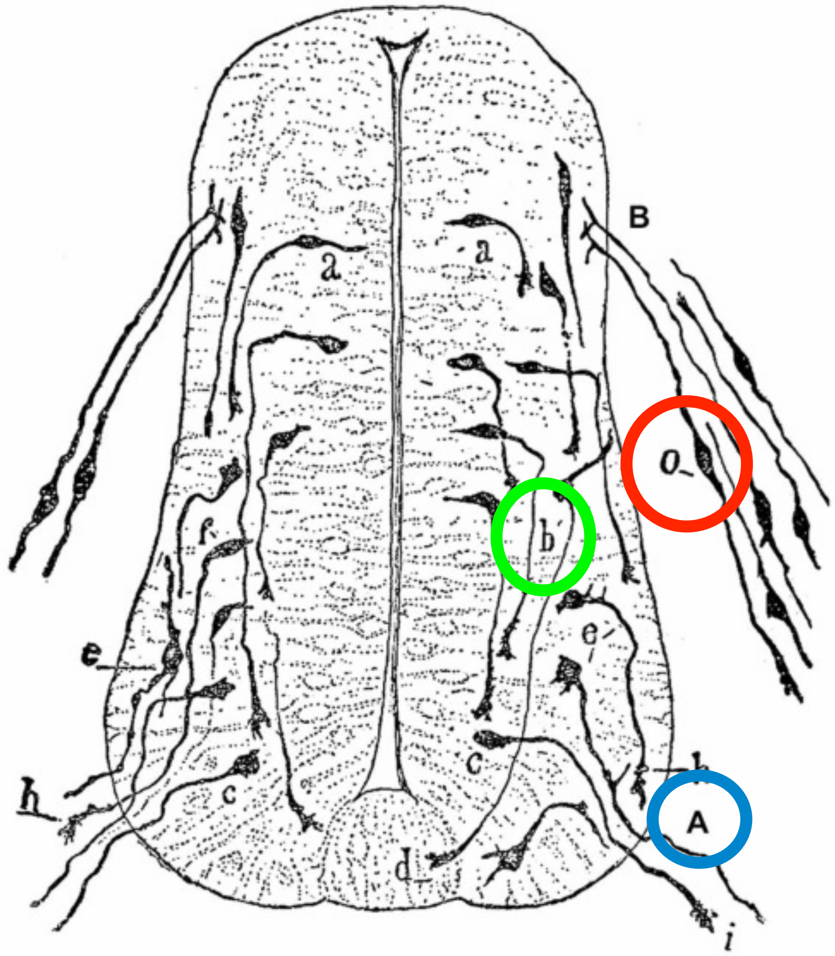
- MAP1B
- DYNEIN AND KINESIN



THE MOVEMENT

DO ALL THE AXONS GROWTH IN SAME WAY?

behaviour of growing nerve fibers was reproducible but depended on their origin



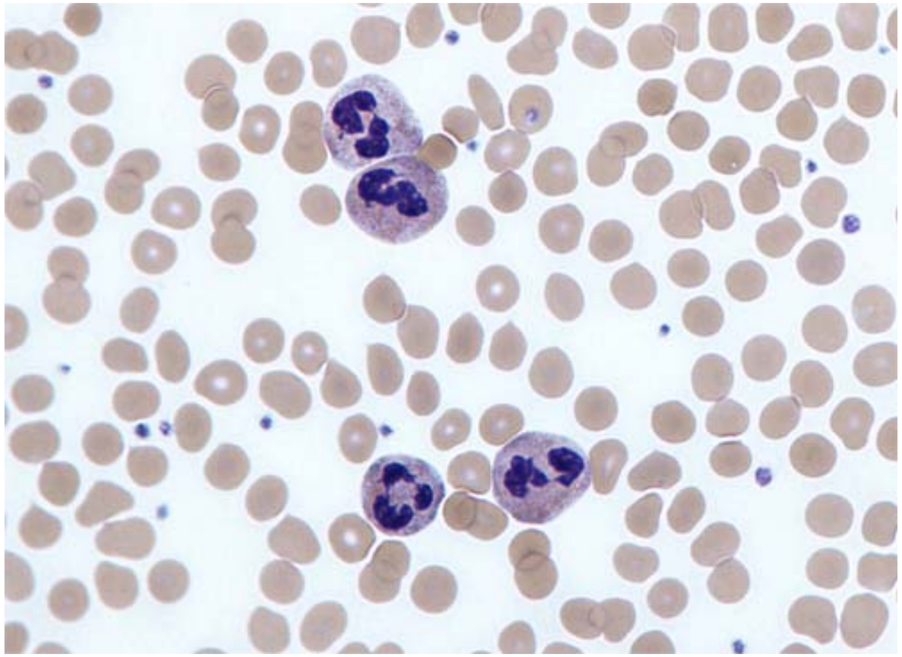
''ADOPT PREDETERMINED DIRECTIONS AND ESTABLISH CONNECTIONS WITH DEFINED NEURAL OR EXTRANEURAL ELEMENTS….WITHOUT DEVIATIONS OR ERRORS, AS IF GUIDED BY AN INTELLIGENCE FORCE ''

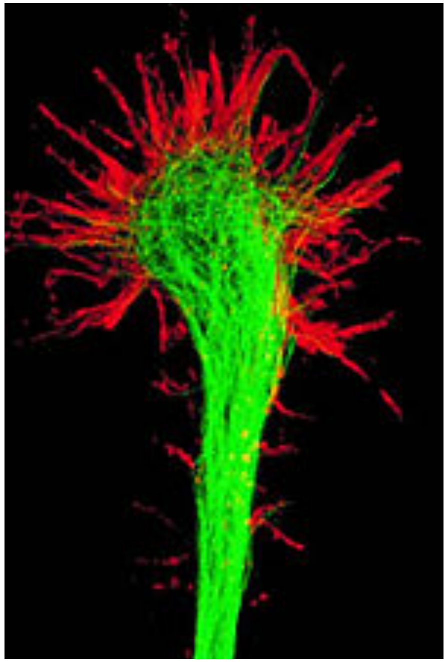
b **COMMISSURAL AXONS**

o **DORSAL ROOT GANGLIA CELLS**

A **VENTRAL NERVE ROOT**

THEY SHARE THE SAME PRINCIPLE!



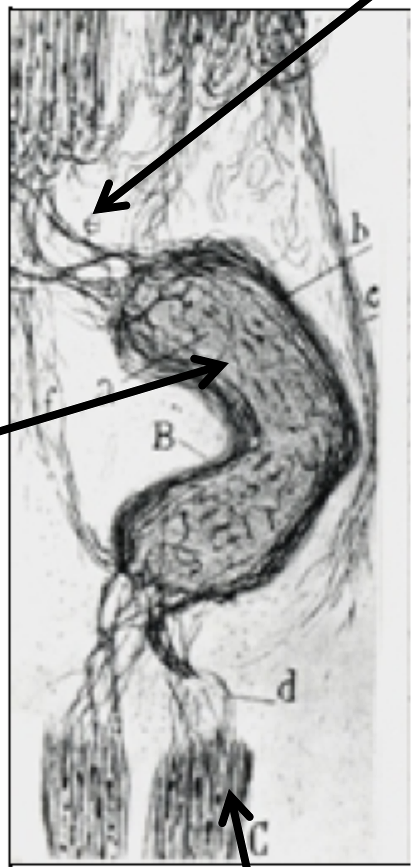


THE CHEMOTACTIC OR NEUROTROPIC HYPOTHESIS

THE NATURE OF THE SUBSTRATE DETERMINE THE ROUTE AXONS TAKE AS THEY EXTENDED

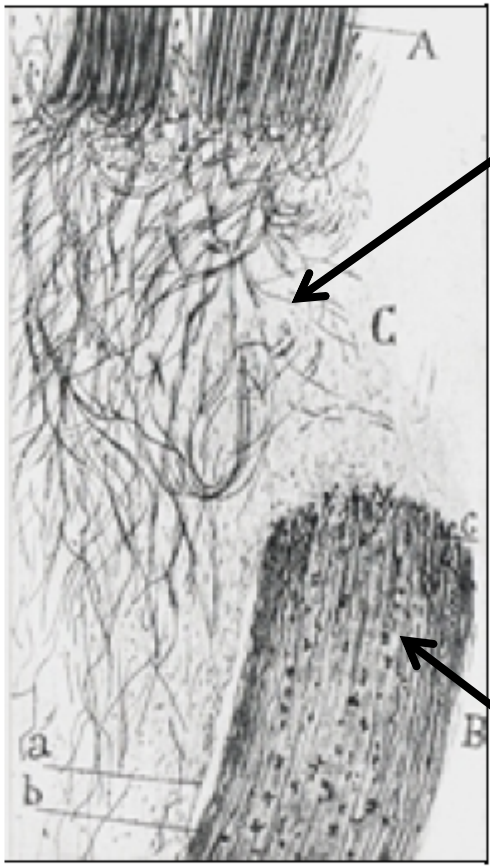
THE EVIDENCE

Fibers from the proximal stump cross the graft.....



Portion of peripheral nerve grafted in the wound of the sciatic nerve of an adult rabbit

Enter the distal stump



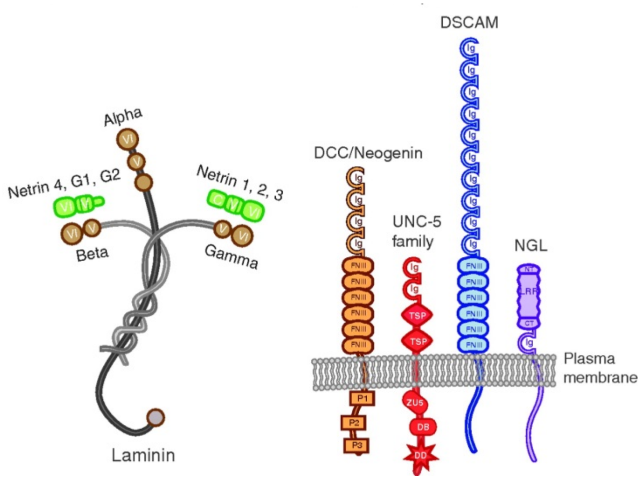
Regenerating fibers leaving the proximal stump do not enter the death graft

Peripheral nerve killed by chloroform

- THEY MOVE AS **IF THEY WERE STRONGLY ATTRACTED**
- **THE SOURCE OF NEUROTROPHIC SUBSTANCES** RESIDES IN SCHWANN CELLS

BOTH COMPONENTS OF CAJAL POSTULATE, THE RESPONSE TO GRADED,
DIFFUSIBLE CHEMICAL SIGNALS AND THE
INTERACTION WITH THE SUBSTRATE,
HAVE BEEN PROVED

WHO ARE THESE COMPONENTS?

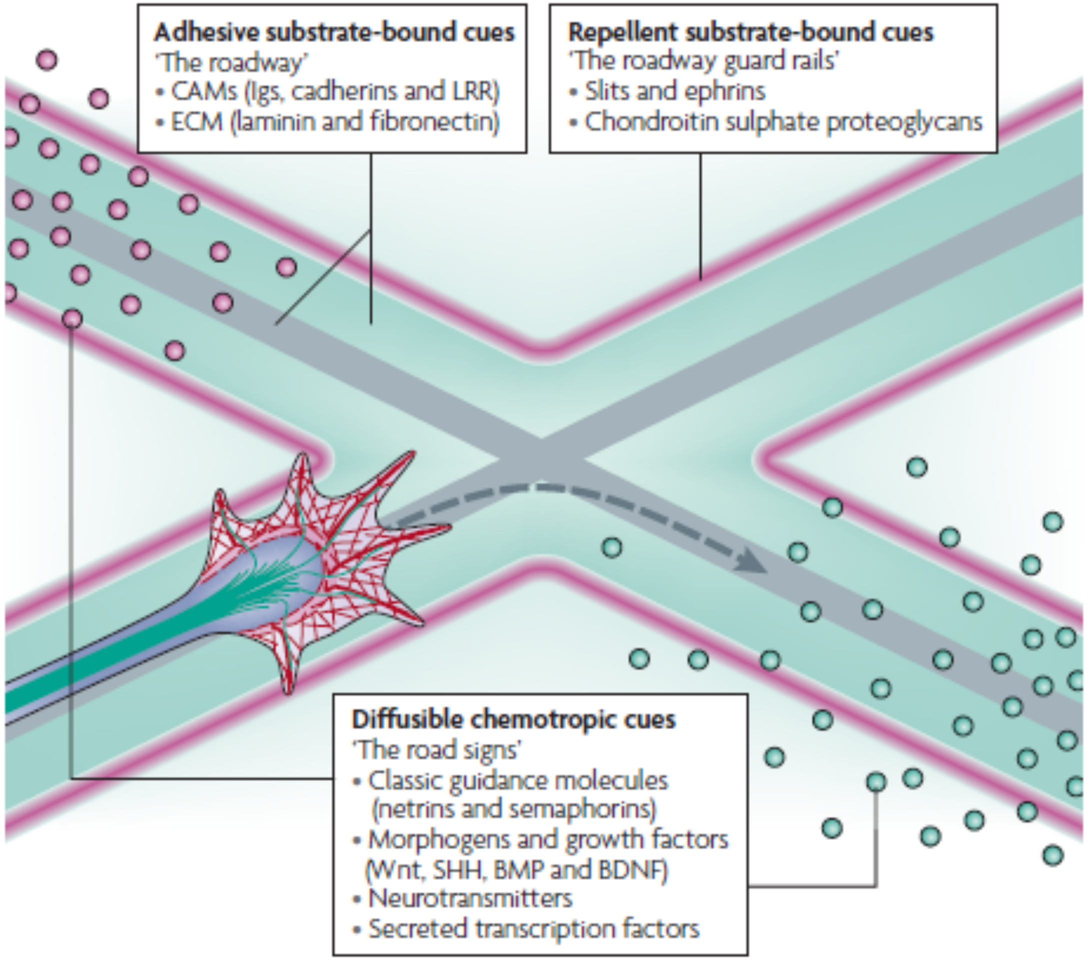


NETRIN-1

TESSIER-LAVIGNE, 1988
KENNEDY ET AL., 1994

AXON GUIDANCE MOLECULES

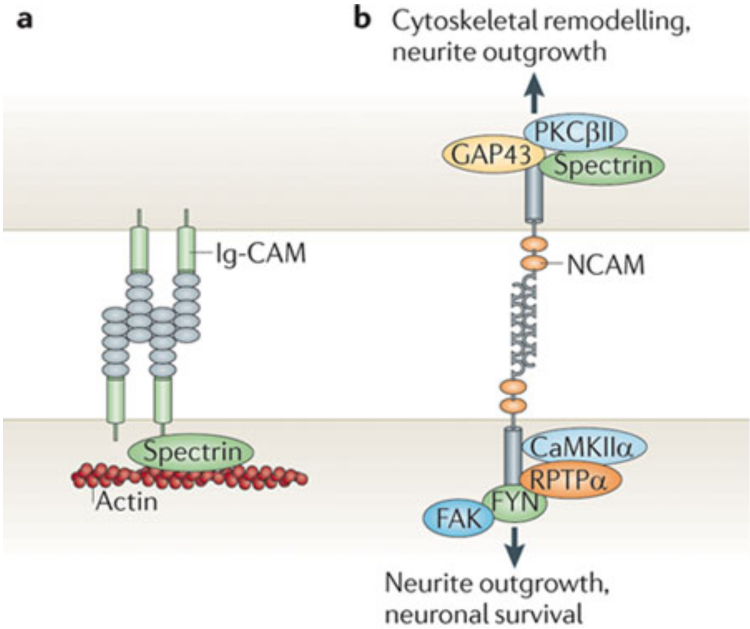
ATTRACTION OR REPULSION DEPENDS ON THE SPECIFIC GROWTH CONE RECEPETORS



4 TYPES OF MOLECULAR CUES:

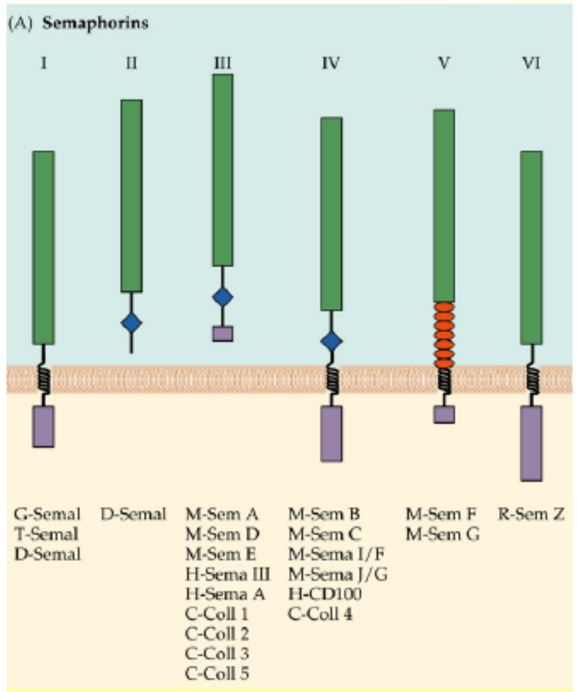
1-CONTACT ADHESION
2-CONTACT REPULSION
3-LONG RANGE ATTRACTION
4-LONG RANGE REPULSION

1-CONTACT ADHESION



EACH AXON TRACT EXPRESS DIFFERENT CAMs SO THAT NEWLY FORMED AXONS FOLLOW PATH DEPENDING ON WHICH CAMs THEY HAVE ON THEIR SURFACE

2-CONTACT REPULSION



SEMAPHORINS ARE A FAMILY OF MEMBRANE-BOUND OR SOLUBLE PROTEINS CONTAINING THE SEMA GROUP

THEY BIND THE NEUROPILIN RECEPTORS NP1 AND NP2 AND CAN INTERACT WITH EXTRACELLULAR MATRIX MOLECULES

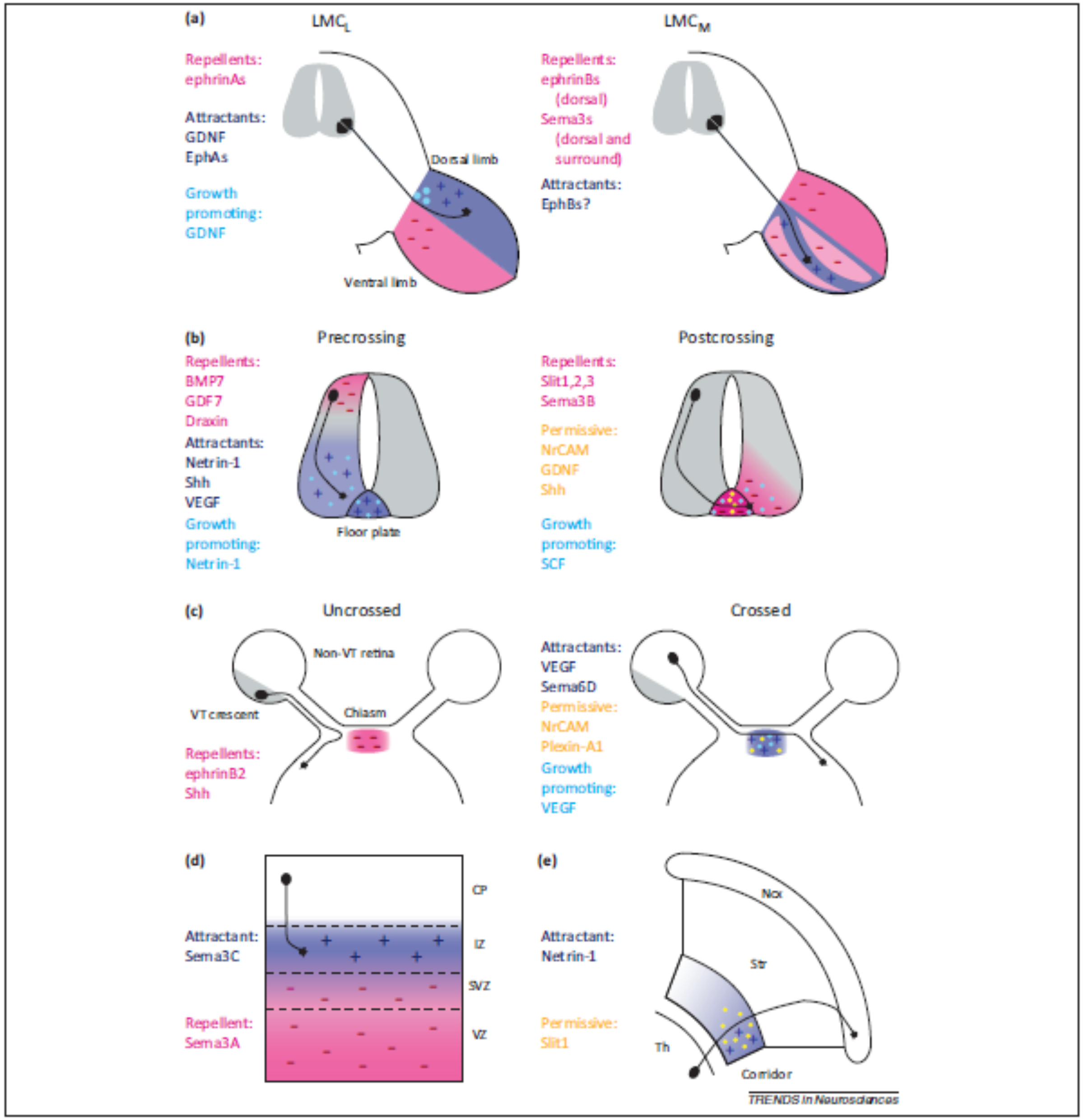
WHICH KIND OF SIGNALS DOES THE GROWTH CONE NEED?

1 AXON GROWTH REQUIRES **SUPPLY OF CYTOPLASM AND MEMEBRANE**

2 CONSTANT AXONAL PROTEIN SYNTHESIS IS REQUIRED TO RESENSITIZE THE GROWTH CONE TO GUIDANCE CUES

3 TROPHIC FACTOR MAY ACT SOLELY TO THE AXON BUT **TO INDUCE AXON ELEONGATION** THEY MUST ACTIVATE PROCESS AT THE CELL BODY (MEMBRANE SUPPLY AND INSERTION, mRNA TRANSLATION)

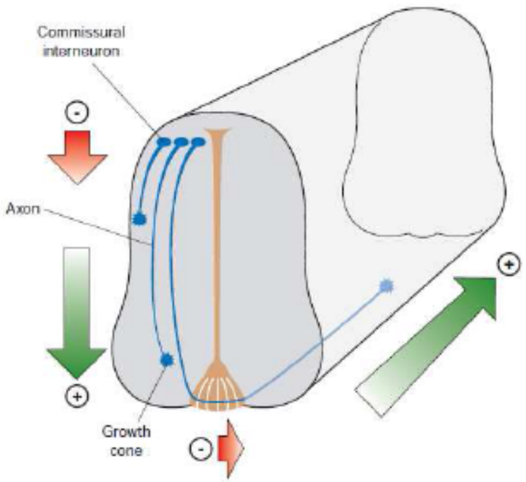
INTEGRATION OF GUIDANCE CUES

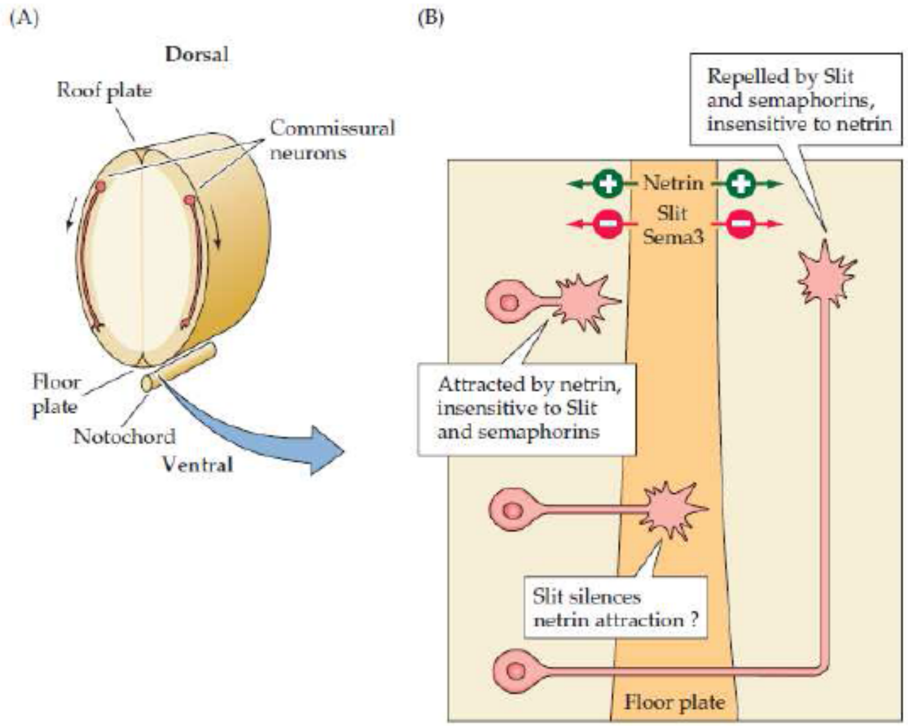


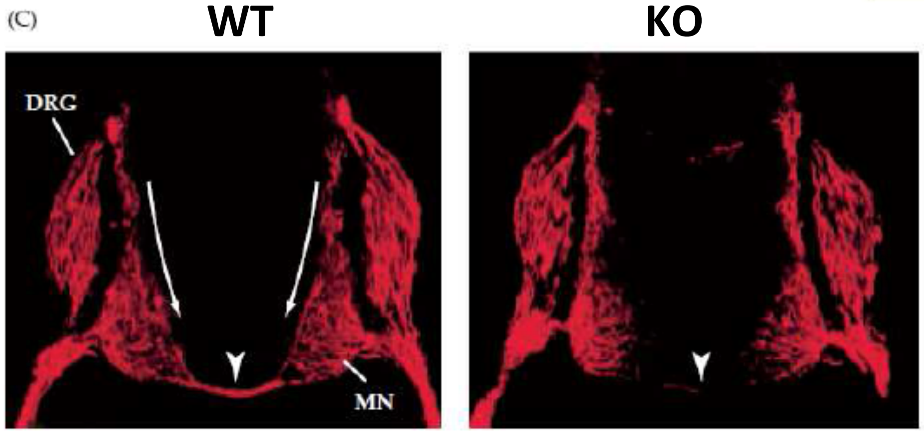
AXONS CAN USE **COMBINATIONS** OF CUES TO GUIDE THEM TO THEIR CORRECT LOCATION.

THESE CUES ARE INTERPRETED BY THE GROWTH CONE AS THE PERCEIVED CUES ACT TO **REGULATE THE ACTIN CYTOSKELETON** AND DETERMINE THE DIRECTION OF THE GROWING AXON

COMMISSURAL NEURONS

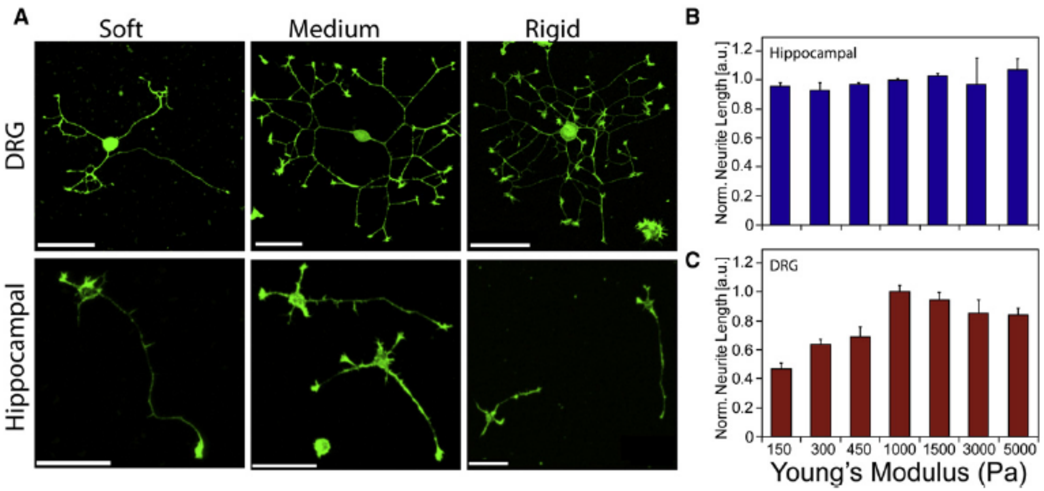


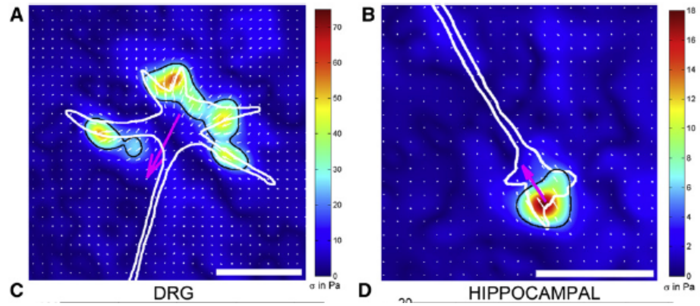


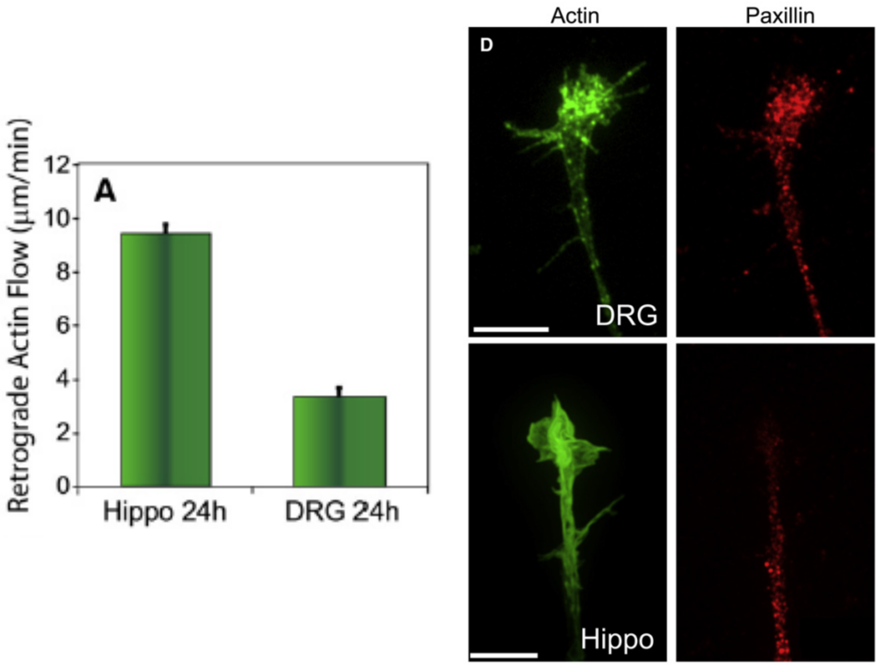


RELAYING PAIN AND TEMPERATURE

AXONS CAN RESPOND DIFFERENTLY





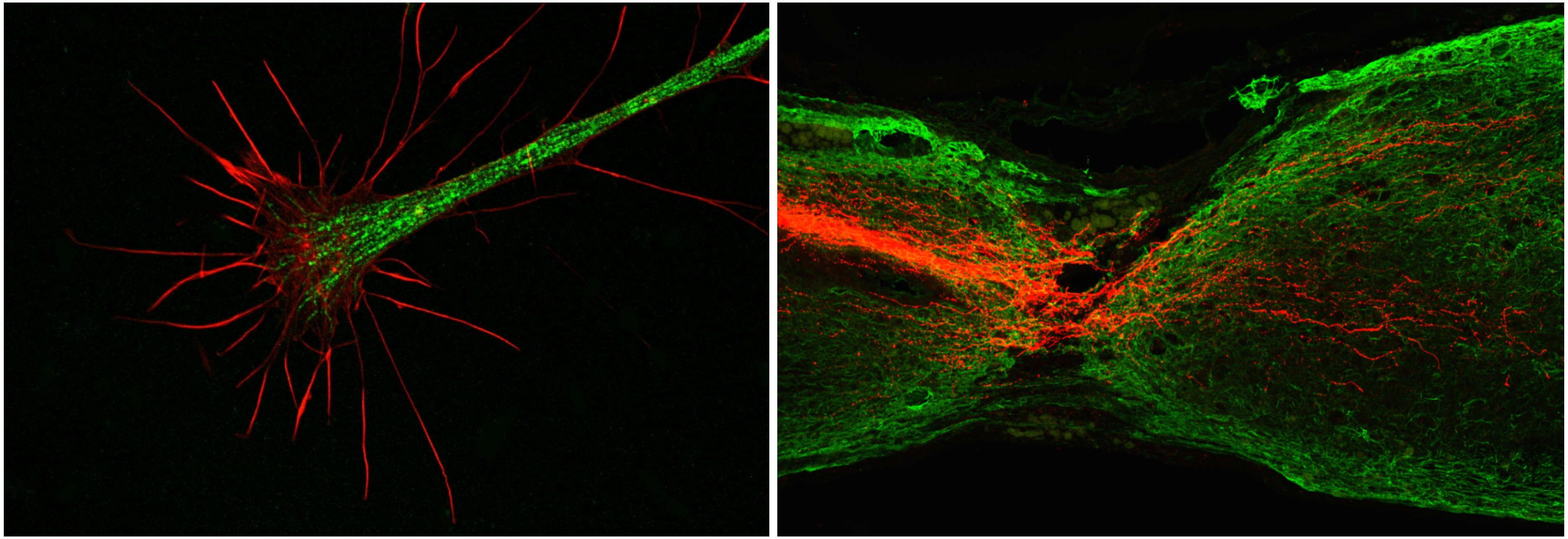


DIFFERENT TRACTION FORCES

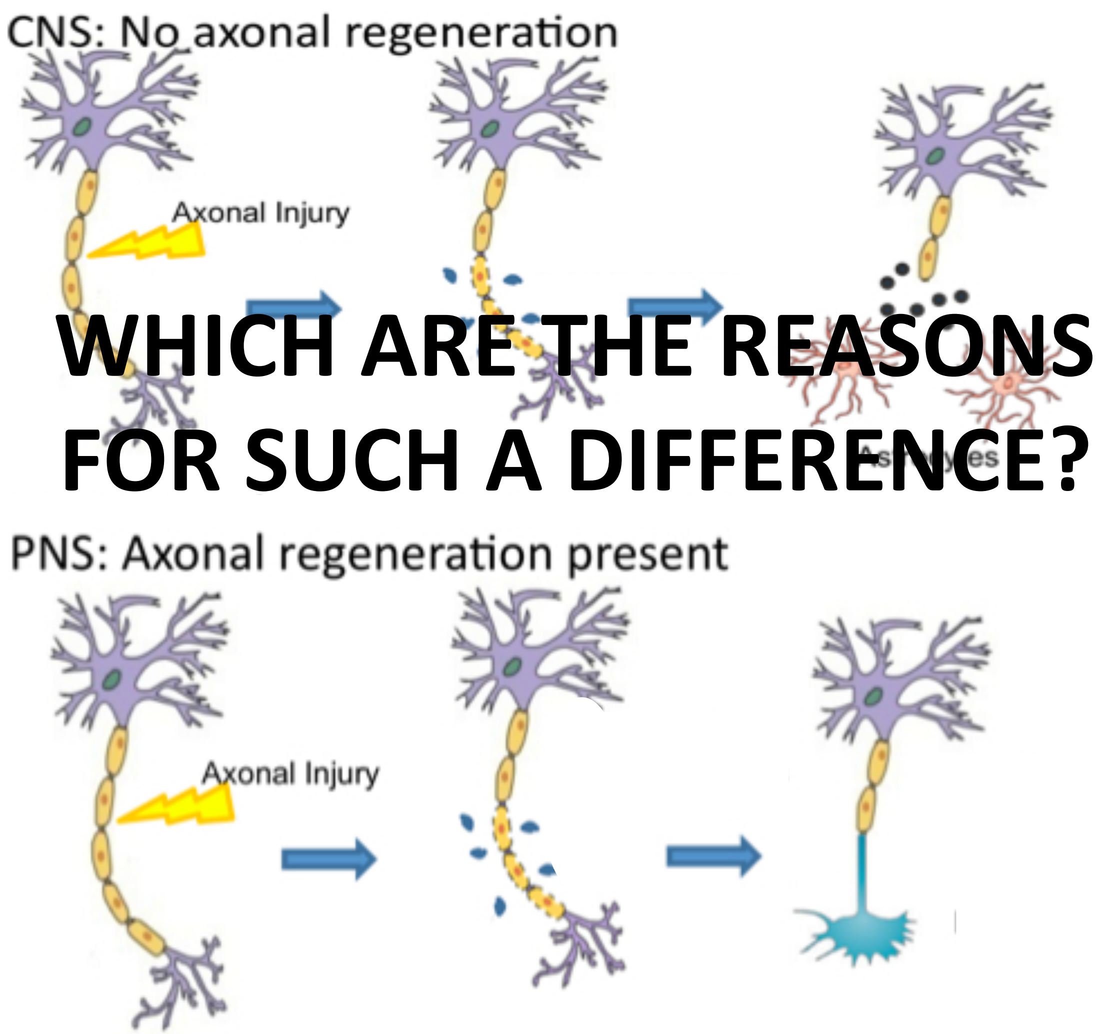
DIFFERENT OUTGROWTH

Daniel Koch et al., Biophysical Journal, 2012

AXON REGENERATION



A BIG DIFFERENCE!



WHICH ARE THE REASONS FOR SUCH A DIFFERENCE?

PNS REGENERATION

- **THEY ARE VULNERABLE TO CUTS AND TRAUMA**
- **THEY CAN REGENERATE**
- **THE REGENERATION PROCESS DEPENDS ON THREE FACTORS:**

  -AMOUNT OF DAMAGE

  -NEUROLEMMOCYTES SECRETION OF NGFs

  -DISTANCE BETWEEN THE DAMAGE SITE AND THE EFFECTOR ORGAN

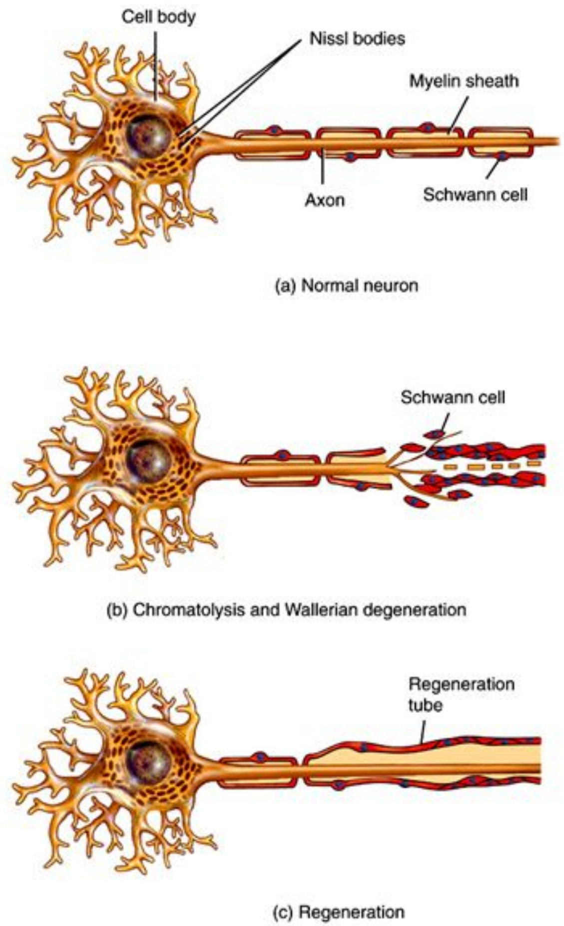
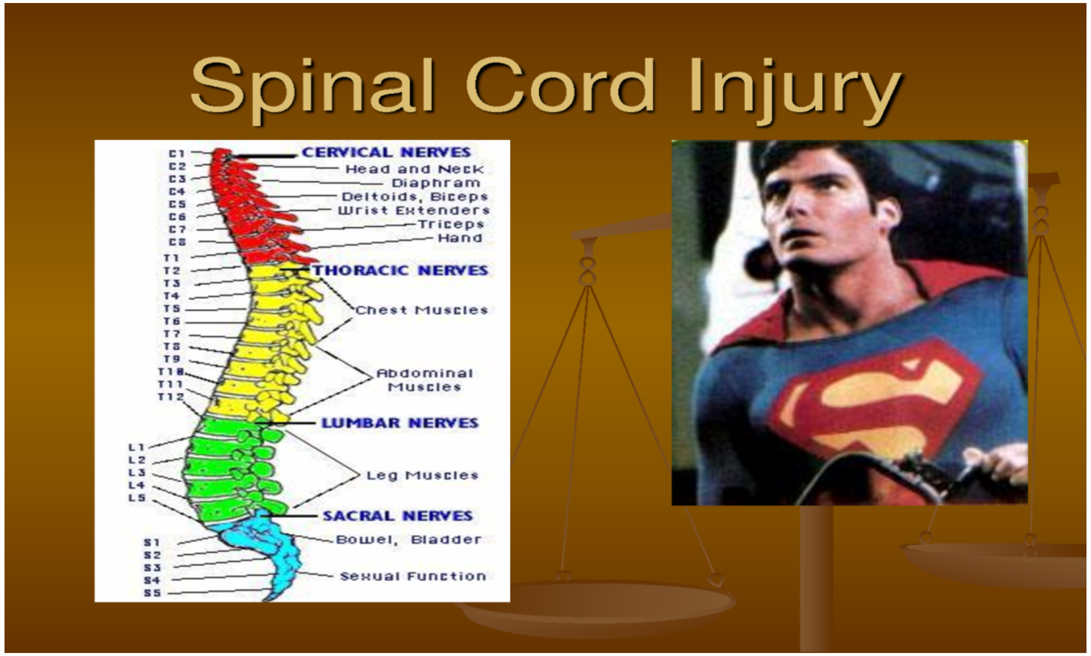


Figure 12.29 Tortora - PAP 12/e

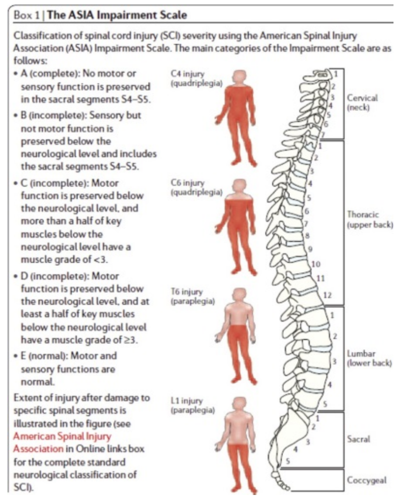
CNS REGENERATION?!



CHRISTOPHER & DANA REEVE FOUNDATION
TODAY'S CARE. TOMORROW'S CURE.

SPINAL CORD INJURY

- THE PROGNOSIS DEPENDS ON THE **DAMAGE EXTENT**
  - PROPMTNESS OF INTERVENTION
  -TETRAPLEGIC, PARAPLEGIC
  -MOTOR, SENSORY, AUTONOMIC AND REFLEX FUNCTIONS

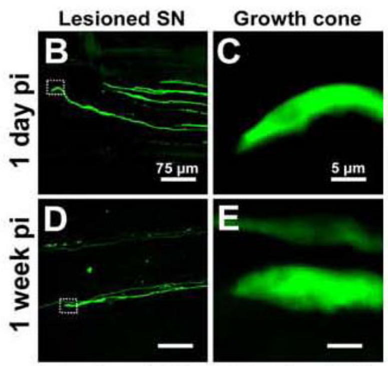

Box 1 | **The ASIA Impairment Scale**

Classification of spinal cord injury (SCI) severity using the American Spinal Injury Association (ASIA) Impairment Scale. The main categories of the Impairment Scale are as follows:

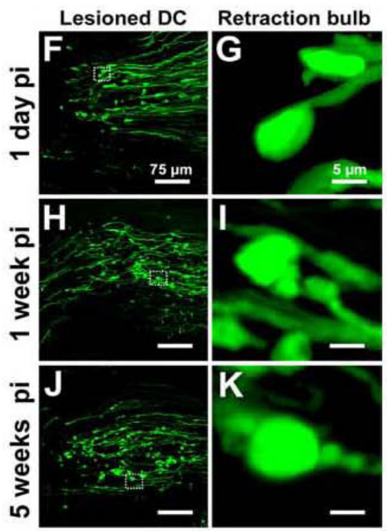
- A (complete): No motor or sensory function is preserved in the sacral segments S4–S5.
- B (incomplete): Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4–S5.
- C (incomplete): Motor function is preserved below the neurological level, and more than a half of key muscles below the neurological level have a muscle grade of <3.
- D (incomplete): Motor function is preserved below the neurological level, and at least a half of key muscles below the neurological level have a muscle grade of ≥3.
- E (normal): Motor and sensory functions are normal.

Extent of injury after damage to specific spinal segments is illustrated in the figure (see American Spinal Injury Association in Online links box for the complete standard neurological classification of SCI).

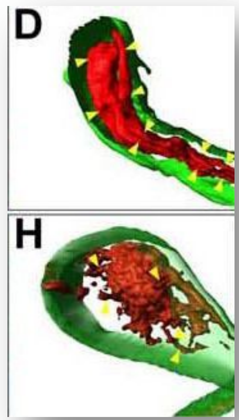
- THEY CAN NOT **REGENERATE**



GROWTH CONES (PNS)

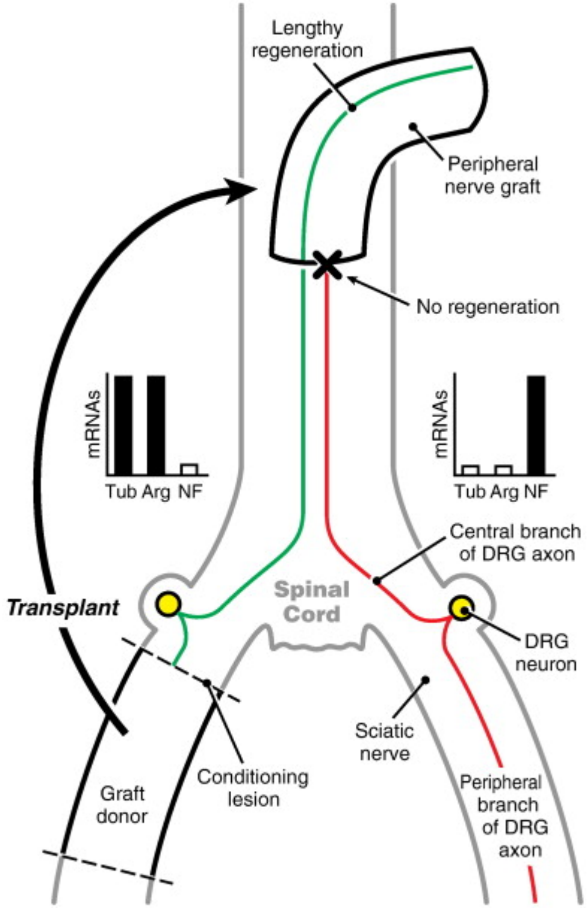


RETRACTION BULBS (CNS)

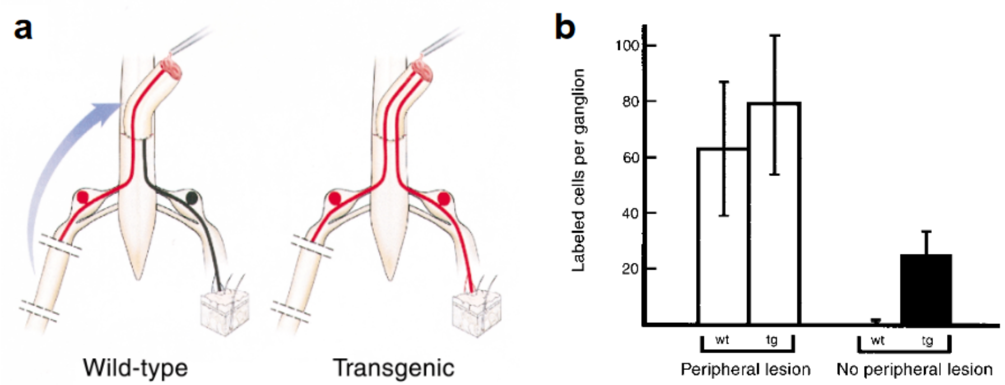


Erturk A et al., J Neurosci, 2007

THEY CAN NOT REGENERATE……?!



Hoffman PN, Exp Neurol, 2010

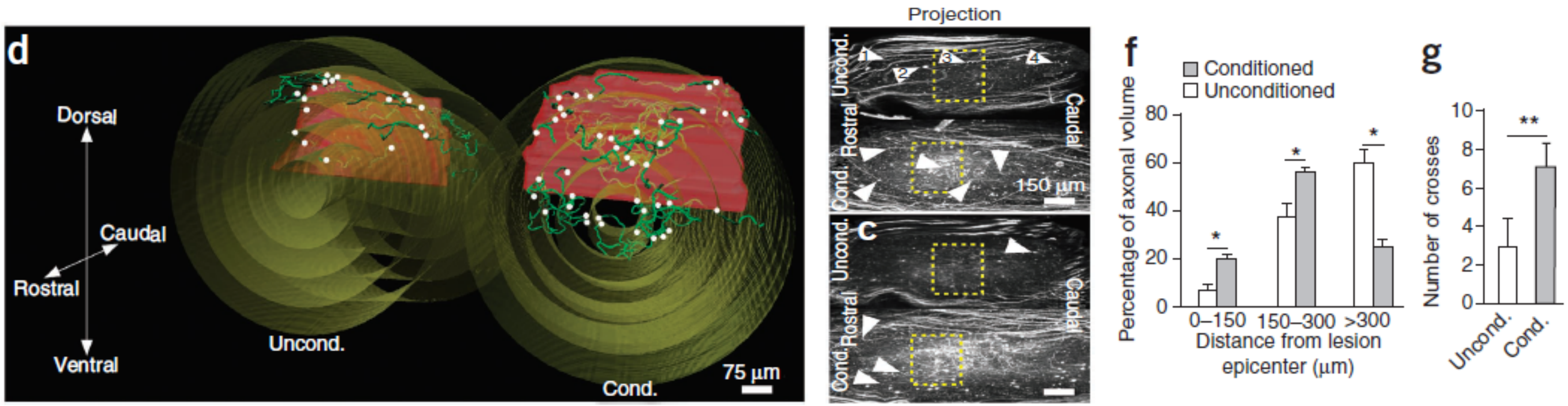


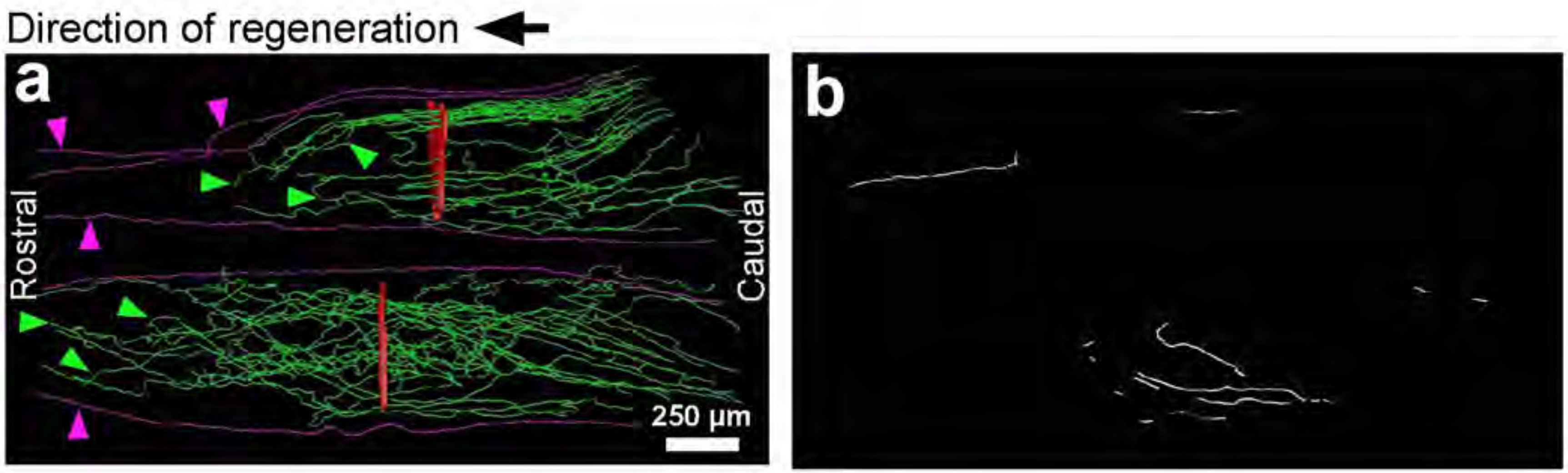
Bomze HM et al., Nat Neurosci, 2001

- THE SPECIFIC CHANGES NECESSARY FOR THE REGENERATION OF CNS AXONS IN A PERIPHERAL NERVE GRAFT HAVE NOT BEEN IDENTIFIED

- THE CHANGES IN GENE EXPRESSION INDUCED BY A CL OF PERIPHERAL BRANCHES SUPPORT THE LENGTHY REGENERATION OF CENTRAL BRANCHES IN A PERIPHERAL NERVE GRAFT

TECHNICAL BIAS





Erturk A et al., Nature Medicine, 2011

CNS "REGENERATION"

SPINAL CORD INJURY

IN VIVO MODEL FOR SPINAL CORD INJURY

COMPRESSION

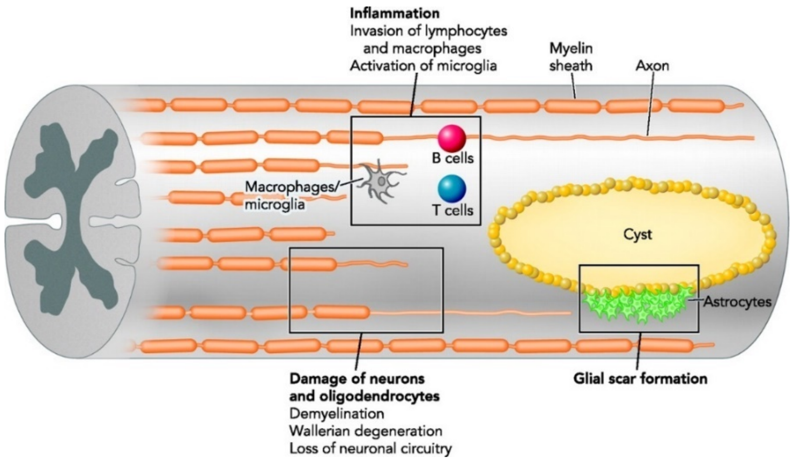
- DROPPING WEIGHTS, INFLATING BALOONS, ANEURYSM CLIP
- NEUROPATHOLOGICAL AND HISTOLOGICAL FEATURES RESEMBLING THE HUMAN ONES
- VARIABLE DAMAGE
- PARTIAL SPARING OF MANY AREAS
- NEURAL REGROWTH STUDY

TRANSECTION

- CUTTING WITH SHARP INSTRUMENT
- TOTAL OR PARTIAL
- COMPLETE DISCONNECTION WITH THE TARGET
- NEURAL REGROWTH AND FUNCTIONAL RECOVERY

SPINAL CORD INJURY

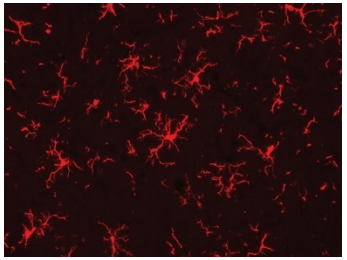
PHISIOPATHOLOGICAL MECHANISM

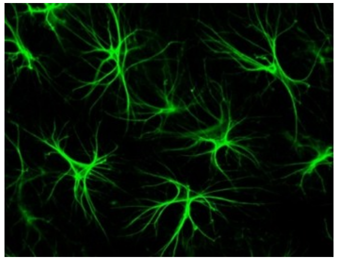


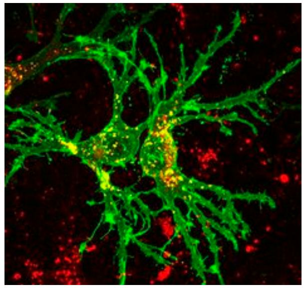
CONSECUTIVE PHASES

- IMMEDIATE
- ACUTE
- INTERMEDIATE
- CHRONIC

CELLS INVOLVED IN SCI

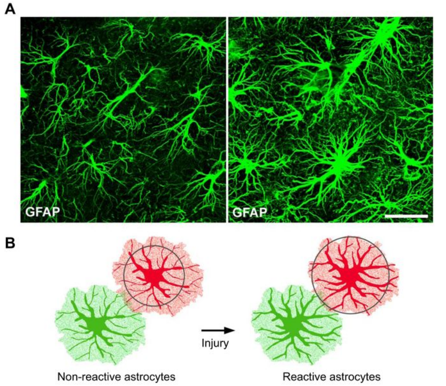






NEURONS
OLIGODENDROCYTES
ASTROCYTES
MICROGLIA AND MACROPHAGES
ENDOTHELIAL CELLS

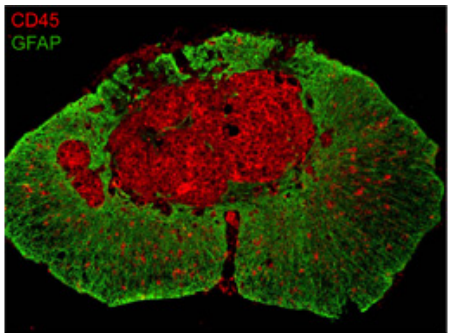
INFLAMMATION GOOD OR BAD?



C

Some functions of astrocytes in the healthy and diseased CNS

Astrocytes in the healthy CNS	Reactive astrocytes in the diseased/injured CNS	
	Acute phase	**Chronic phase**
Maintenance of homeostasis	Restoration of homeostasis	Restoration of homeostasis
Regulation of blood flow	Regulation of blood flow	Regulation of blood flow
Regulation of blood-brain barrier	Restoration of blood-brain barrier	Regulation of blood-brain barrier
Recycling of neurotransmitters	Recycling of neurotransmitters	Recycling of neurotransmitters
Activity-dependent regulation of synapse number and function	Synapse protection Tonic inhibition of neurons	Inhibition of synapse formation? Tonic inhibition of neurons
Regulation of neurogenesis	?	Inhibition of the integration of newly formed neurons
	Neuroprotection through the secretion of neurotropic factors	Neuroprotection through the secretion of neurotropic factors?
	Lesion demarcation	Glial scar formation
		Inhibition of axonal growth
		Inhibition of regeneration and functional recovery



- REGULATION OF HOMEOSTASIS
- REGULATION OF BLOOD FLOW
- REGULATION OF BLOOD BRAIN BARRIER
- REGULATION OF NEUROTRANSMITTERS
- NEUROPROTECTION
- GLIAL SCAR FORMATION
- INHIBITION OF AXON GROWTH
- INHIBITION OF FUCNTIONAL RECOVERY

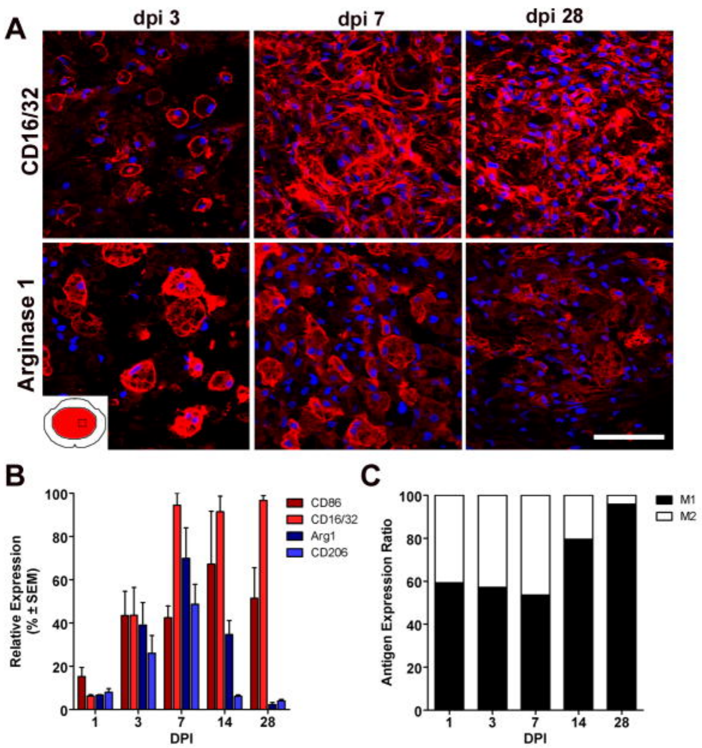
J Neurosci. 2009 Oct 28;29(43):13435-44. doi: 10.1523/JNEUROSCI.3257-09.2009.

Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord.

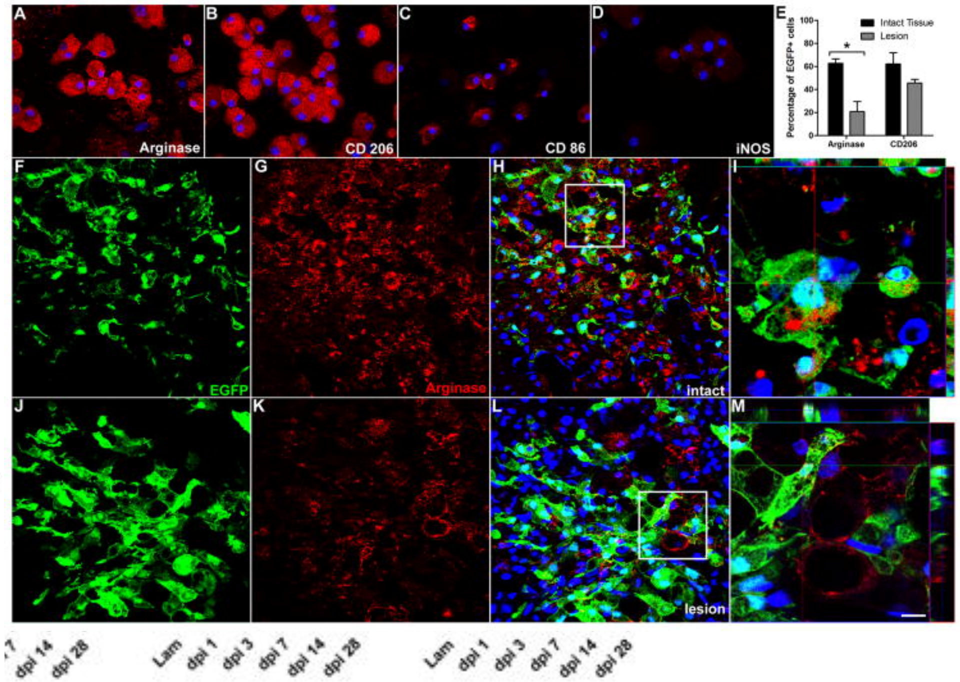
Kigerl KA[1], Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG.

M1

M2



M2



- M1 AND M2 GENES WERE RAPIDLY INDUCED.
- M1-M2 COEXISTENCE DURING THE FIRST WEEKS
- M2 GENE EXPRESSION WAS TRANSIENT AND IN MOST CASES RETURNED TO PRE-INJURY LEVELS BY 7 DPI.
- M1 PREVALENCE
- M1 GENE EXPRESSION WAS MAINTAINED FOR UP TO ONE MONTH POST-INJURY
- M2 MACROPHAGES PHENOTYPE IS DOWREGULATED IN THE INJURED SPIANL CORD

J Neurosci. 2009 Oct 28;29(43):13435-44. doi: 10.1523/JNEUROSCI.3257-09.2009.

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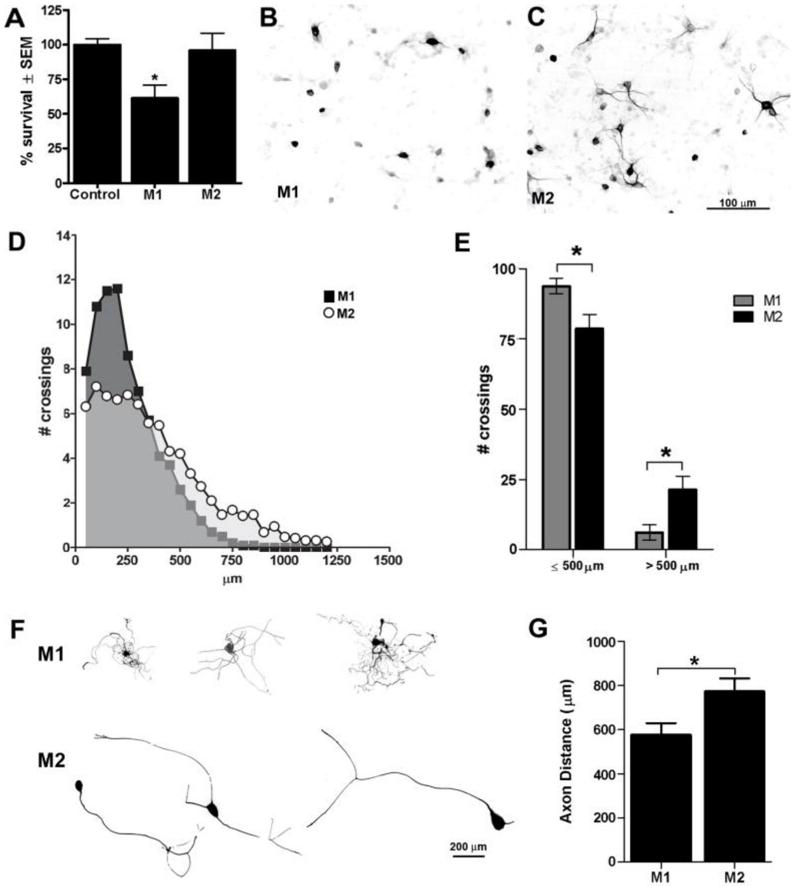
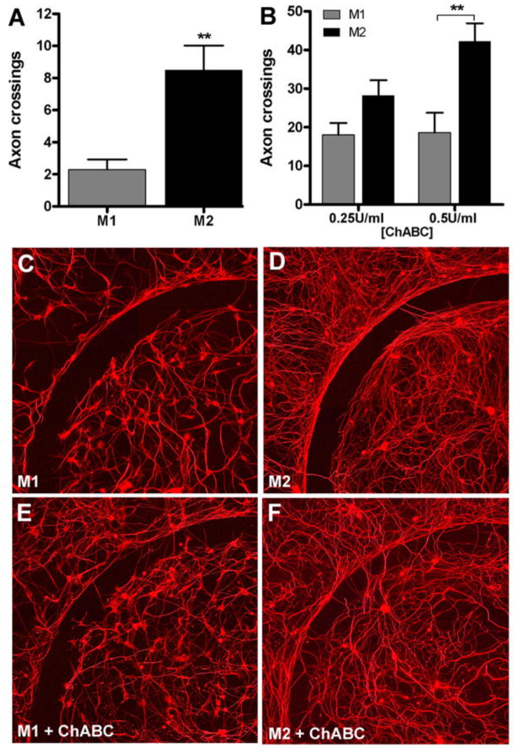
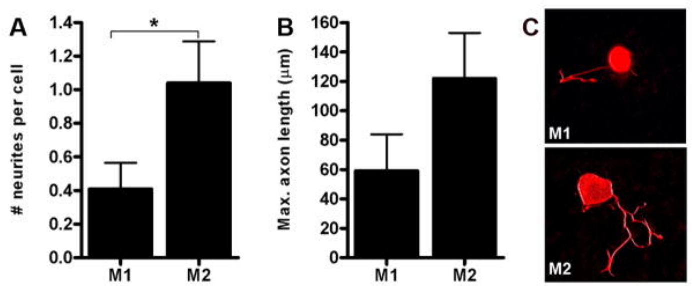
Kigerl KA[1], Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG.

M1 AND M2 EXERT DISPARATE EFFECTS ON NEURON SURVIVAL AND AXON GROWTH







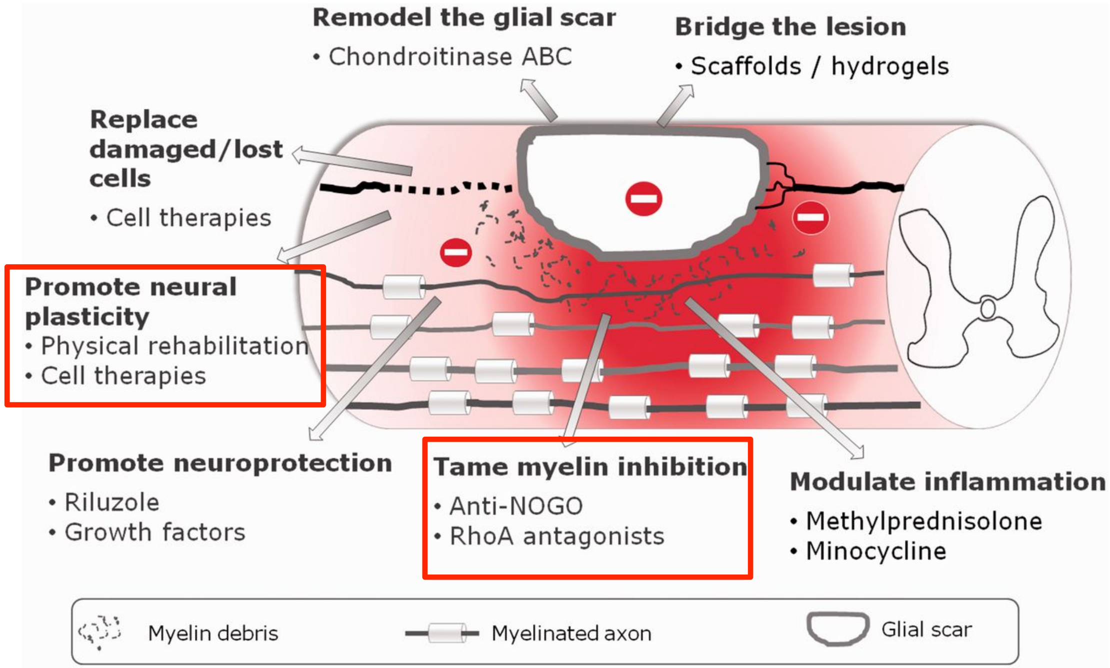
J Neurosci. 2009 Oct 28;29(43):13435-44. doi: 10.1523/JNEUROSCI.3257-09.2009.

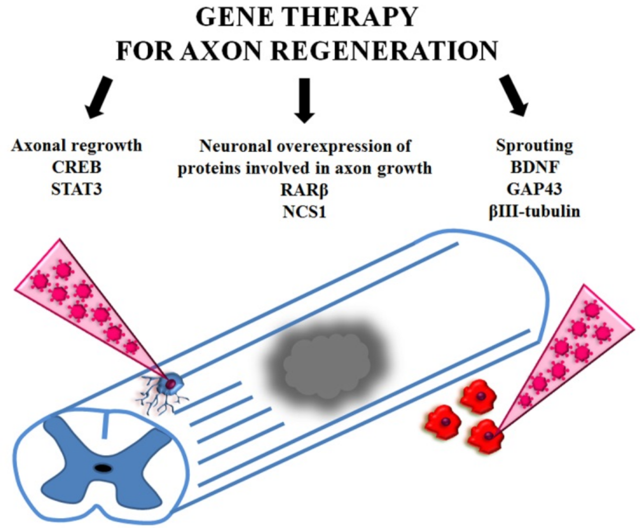
Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord.

Kigerl KA[1], Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG.

- A robust and protracted macrophage response accompanies all forms of CNS trauma.
- After SCI depletion or inhibition of CNS macrophages consistently confers neuroprotection and promotes functional recovery.
- Paradoxically, the controlled activation or even augmentation of this response can enhance various indices of CNS repair
- These divergent effects may be explained by the induction of a macrophage response that is both phenotypically and functionally heterogeneous.

SCI THERAPEUTIC APPROACHES

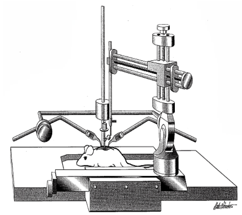




"*Combined treatment by cotransplantation of mesenchymal stem cells and neural progenitors with exercise and enriched environment housing in mouse spinal cord injury*"

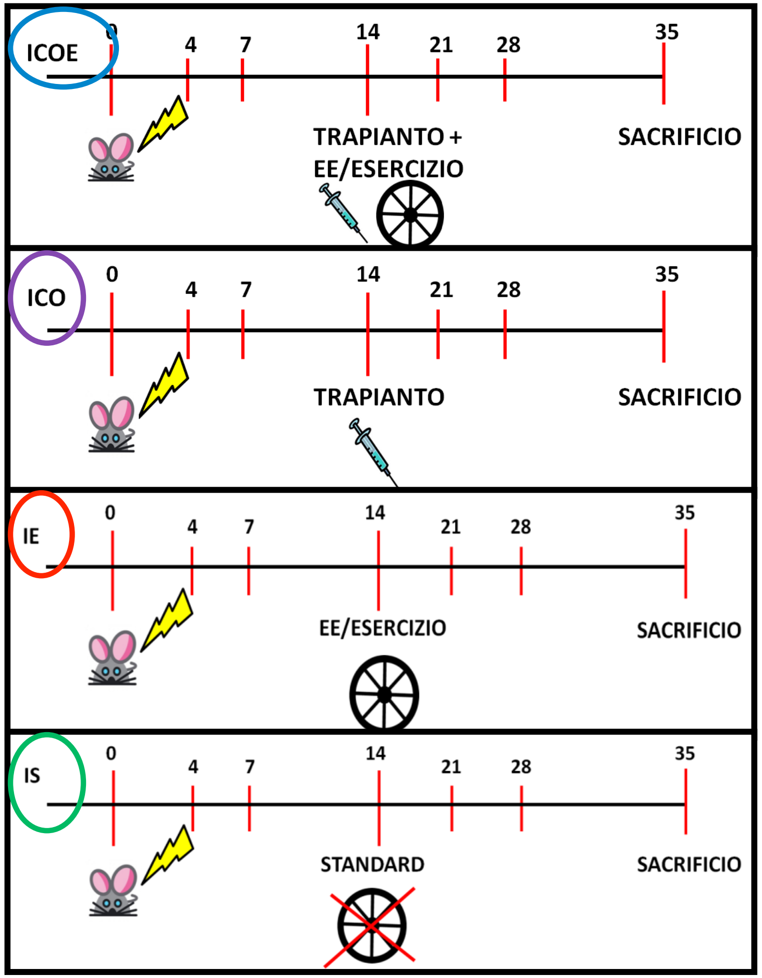
Boido Marina, Niapour Ali, Salehi Hossein, De Amicis Elena, Ghibaudi Matilde and Vercelli Alessandro, Advances in stem cells, 2014

SCI COMPRESSION MODEL T13



CELLULAR GRAFT
MSC-NPS
SEPARATELY/TOGETHER

EXERCISE
ENRICHED ENVIRONMENT



"*Combined treatment by cotransplantation of mesenchymal stem cells and neural progenitors with exercise and enriched environment housing in mouse spinal cord injury*"

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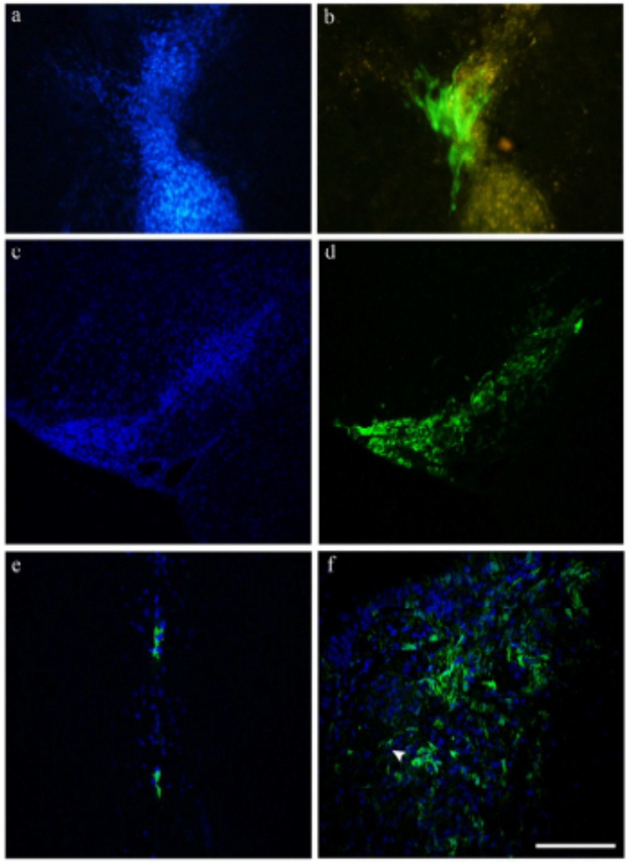


Figure 2- Survival and Distribution of NPs and MSCs

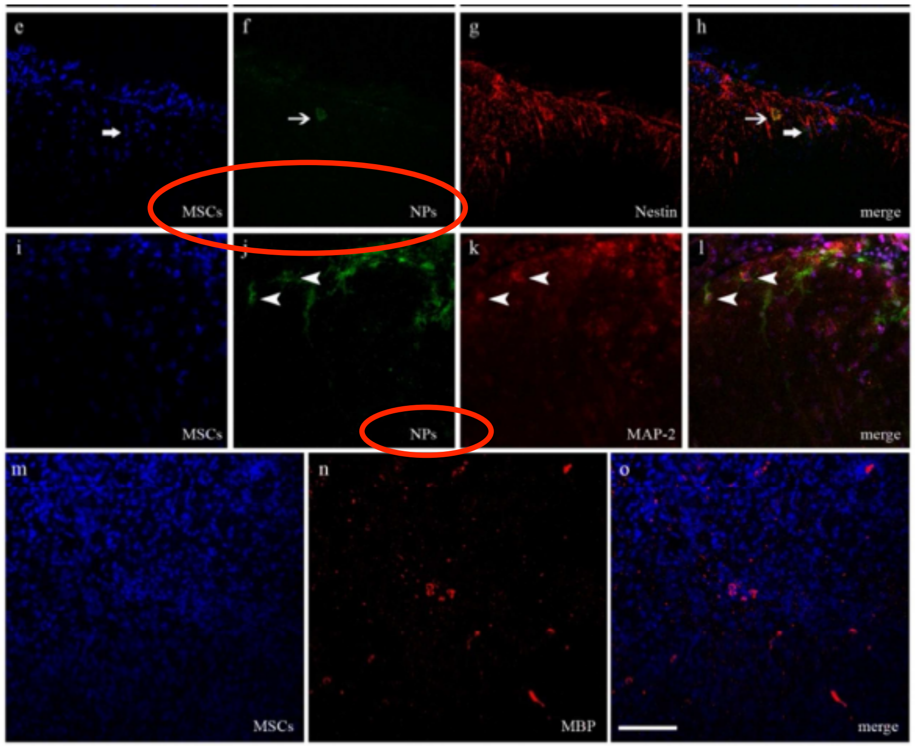
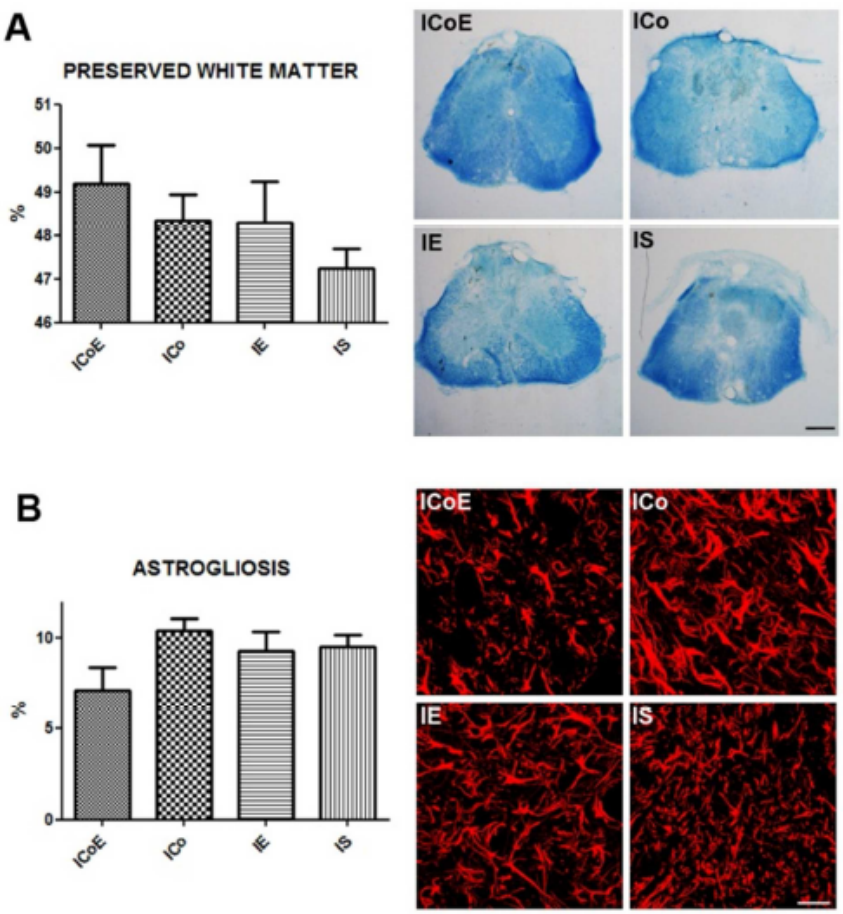
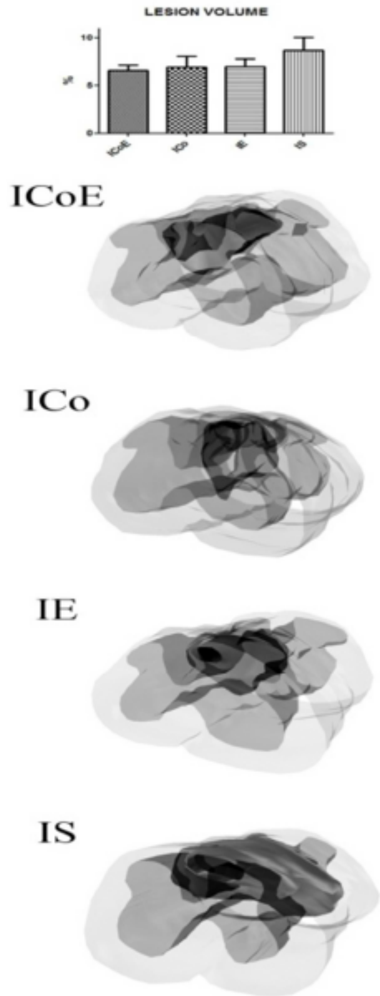


Figure 3 - Differentiation of NPs and MSCs

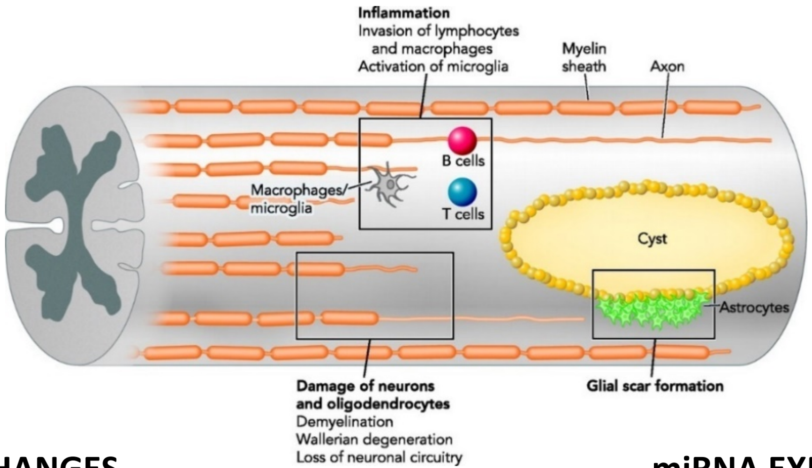
"Combined treatment by cotransplantation of mesenchymal stem cells and neural progenitors with exercise and enriched environment housing in mouse spinal cord injury"

Boido Marina, Niapour Ali, Salehi Hossein, De Amicis Elena, Ghibaudi Matilde and Vercelli Alessandro, Advances in stem cells, 2014

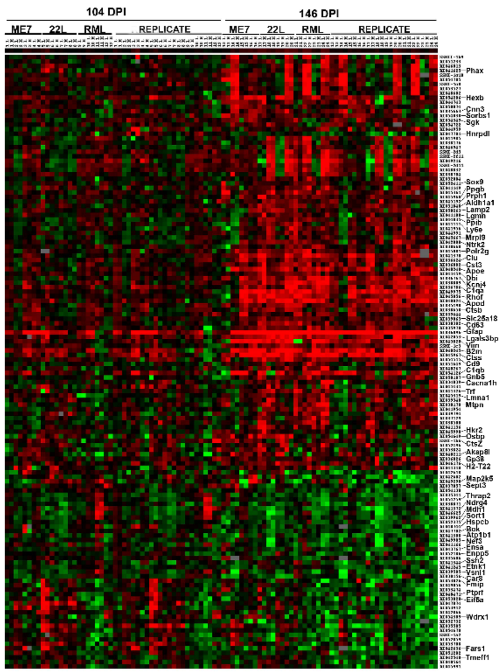




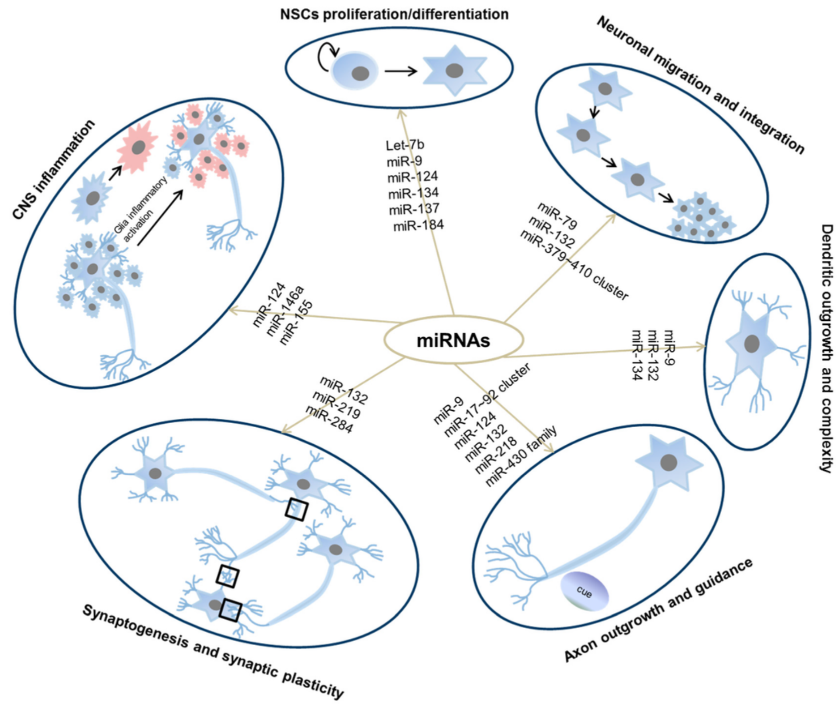
HOW THESE EVENTS ARE CONTROLLED?



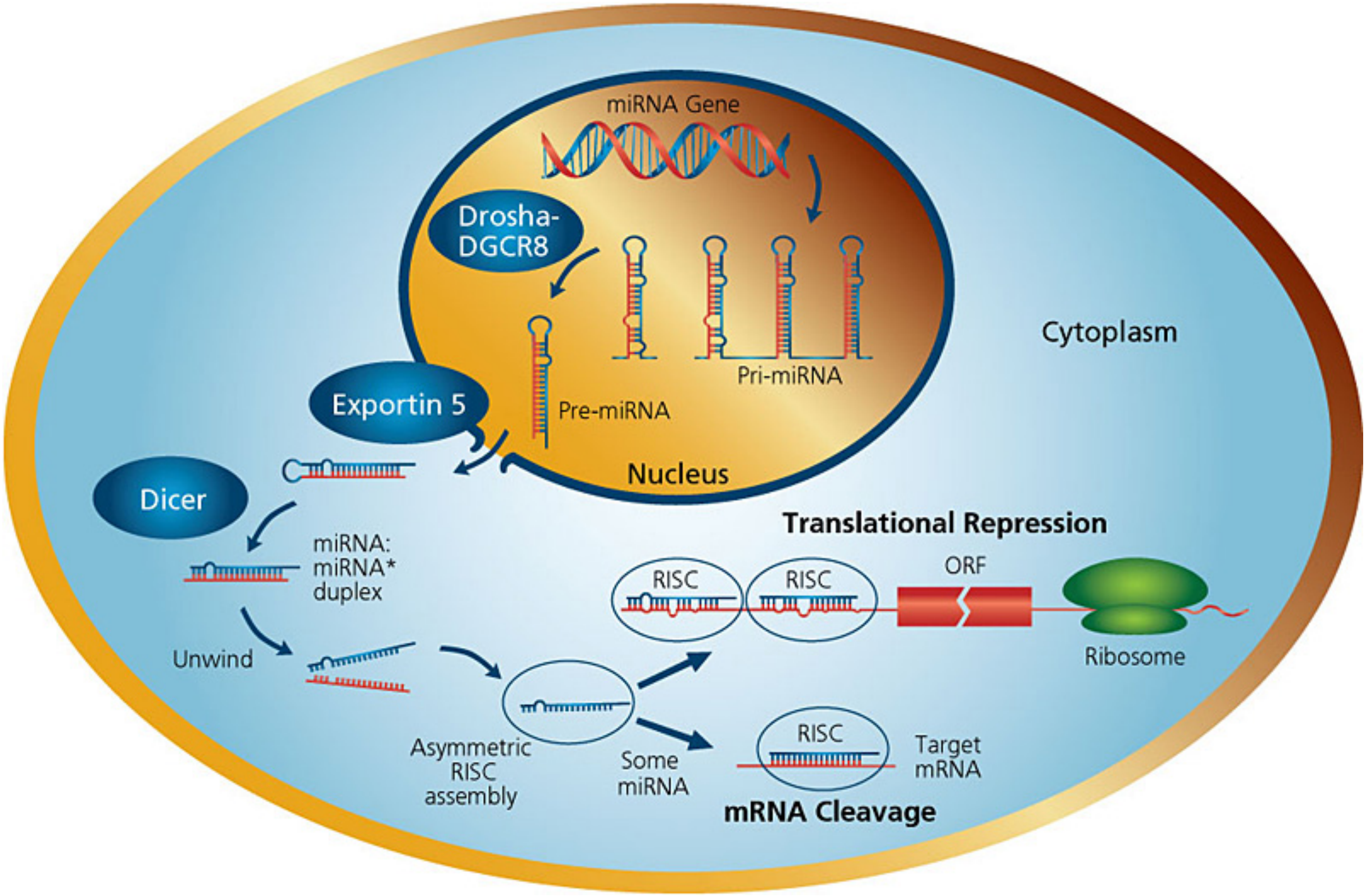
GENE EXPRESSION CHANGES



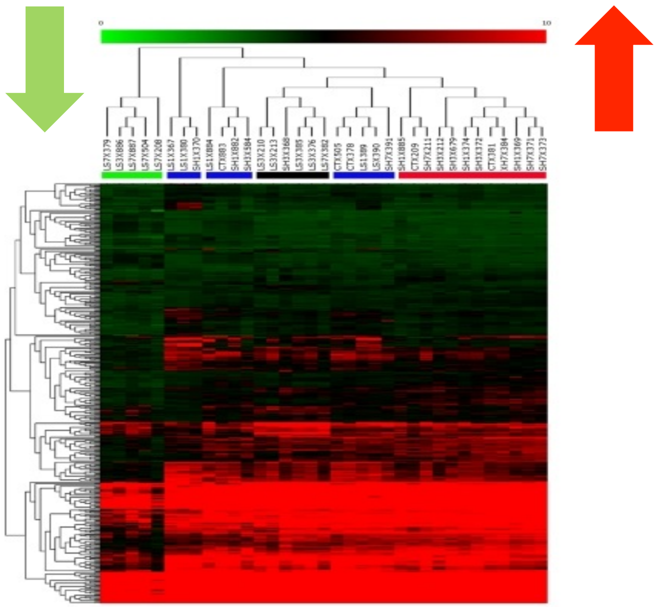
miRNA EXPRESSION CHANGES



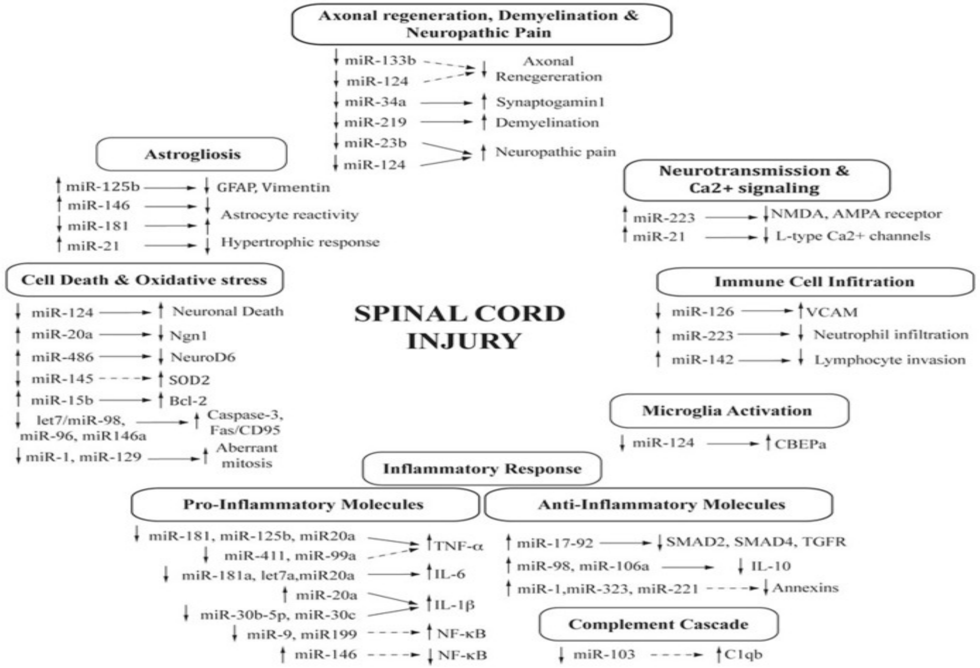
ACTIVATION-INACTIVATION OF SPECIFIC GENE PROGRAMS



WHAT WE KNOW FROM LITERATURE



Yunta M et al., 2012



Nieto Diaz M et al., 2014

Altered microRNA expression following traumatic spinal cord injury
Liu NK et al., Exp Neurol,2009

Microrna dysregulation following spinal cord contusion: implications for neural plasticity and repair
Strickland ER et al., Neuroscience,2011

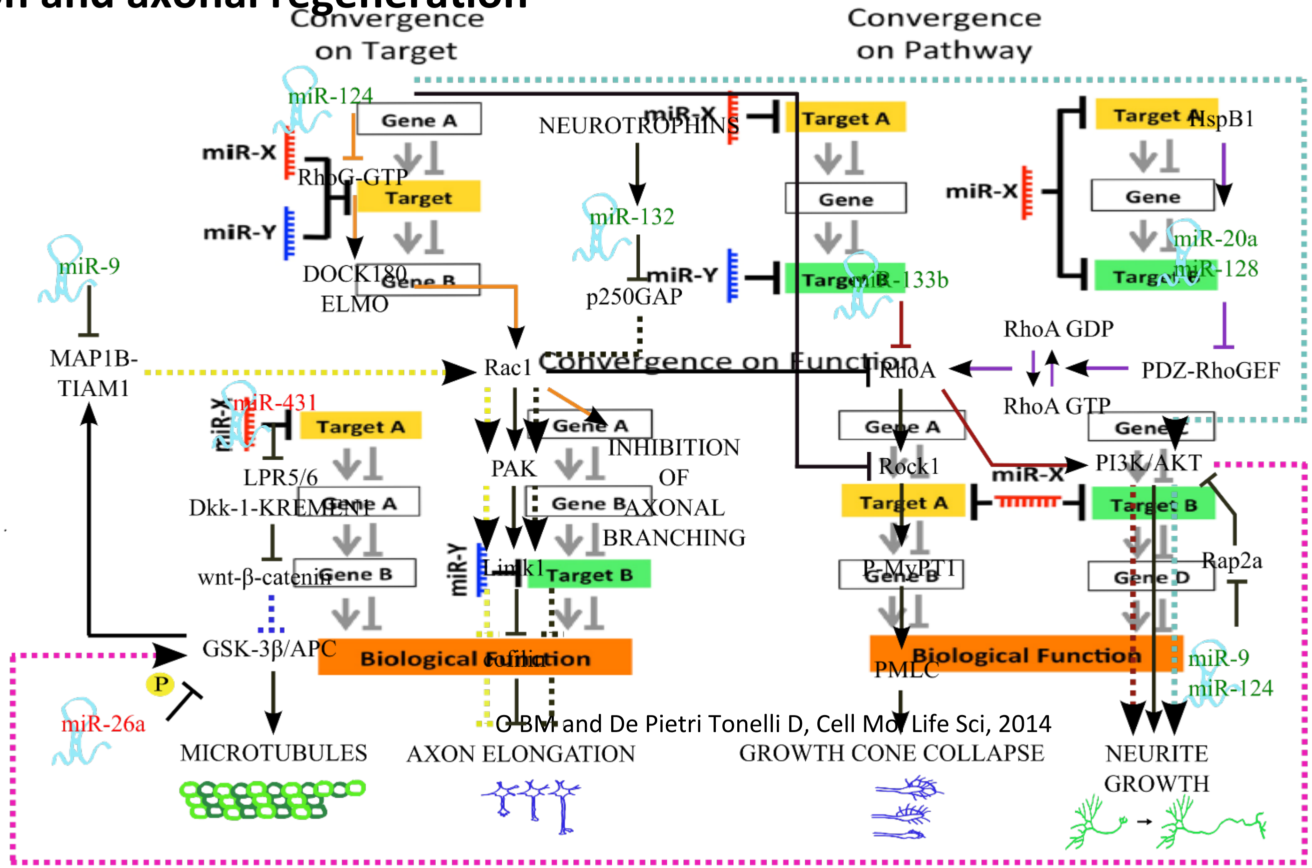
MicroRNA-9 regulates axon extension and branching by targeting Map1b in mouse cortical neurons
Dajas-Bailador F et al., Nat Neurosci,2012

MicroRNA 486 is a potentially novel target for the treatment of spinal cord injury
Jee MK et al., Brain,2012

HOW DO THEY WORK?

CONVERGENCE miRNA ACTION

Functional integration of complex miRNA networks in central and peripheral lesion and axonal regeneration

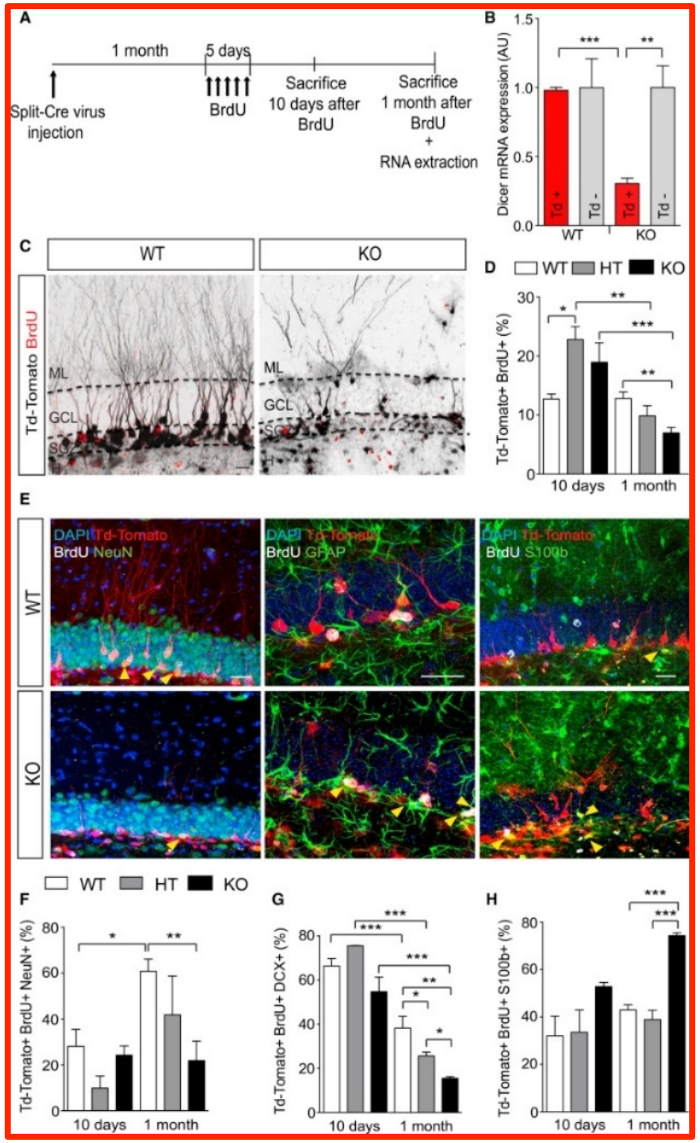


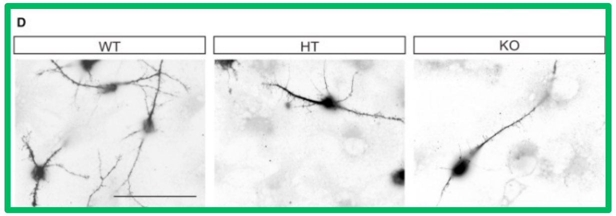
Ghibaudi M, Boido M and Vercelli A, submetted to Progress in Neurobiology

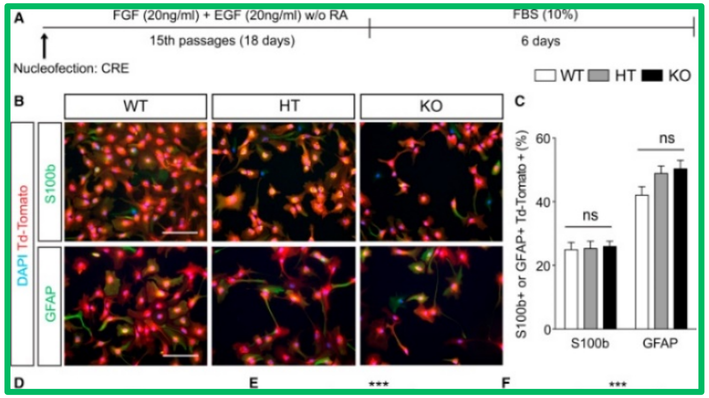
Stem Cell Reports. 2017 Apr 11;8(4):1046-1061. doi: 10.1016/j.stemcr.2017.02.012. Epub 2017 Mar 16.

Synergic Functions of miRNAs Determine Neuronal Fate of Adult Neural Stem Cells.

Pons-Espinal M[1], de Luca E[1], Marzi MJ[2], Beckervordersandforth R[3], Armirotti A[4], Nicassio F[2], Fabel K[5], Kempermann G[5], De Pietri Tonelli D[6].







IN VIVO-DICER ABLATION IMPAIRS NEURAL DIFFERENTIATION AND SURVIVAL BUT NOT ASTROGLIOGENESIS

IN VITRO DICER ABLATION IMPAIRS NEUROGENESIS AND NEURAL MATURATION WITHOUTH AFFECTTING ASTROGLIOGENESIS

Stem Cell Reports. 2017 Apr 11;8(4):1046-1061. doi: 10.1016/j.stemcr.2017.02.012. Epub 2017 Mar 16.

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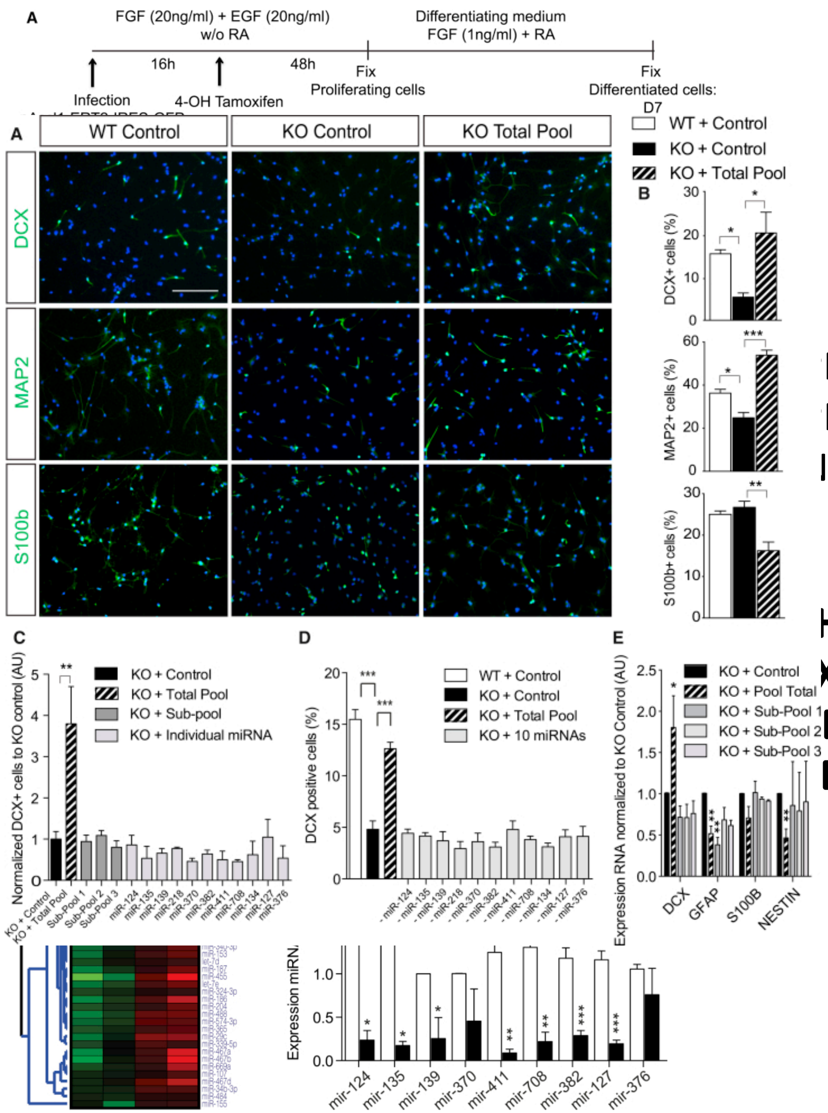


- **A POOL OF 11 MIRNAS SYNERGISTICALLY RESCUES *DICER* CKO IMPAIRMENT OF ADULT NEUROGENESIS IN VITRO**

ROFILING OF MIRNA EXPRESSION UNDER ROLIFERATING CONDITIONS AND DURING IEURONAL DIFFERENTIATION

- ALL OF THE 11 MIRNAS ARE SUFFICIENT TO RESCUE NEURONAL COMMITMENT

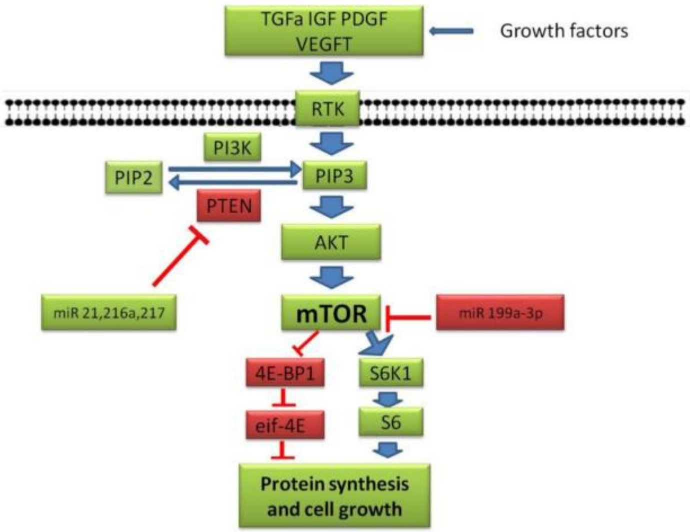
- A SUBSTANTIAL FRACTION OF THE PREDICTED TARGETS (37%, I.E., 1,817 OUT OF 4,890) WERE SHARED BY AT LEAST TWO OF THE 11 MIRNAS AND HAD PREVIOUSLY BEEN SHOWN TO BE EXPRESSED IN DEVELOPING ASTROCYTES OR NEURONS

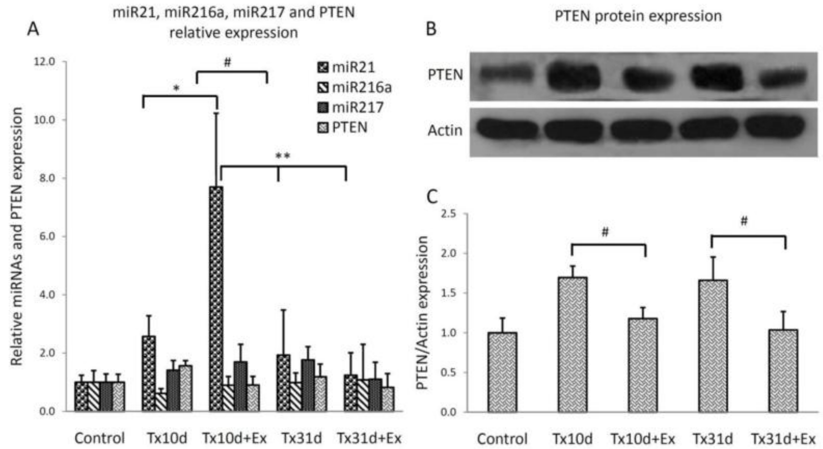
HEY HYPOTHESIZED THAT MIRNAS, WHOSE XPRESSION IS ASSOCIATED WITH EARLY STEPS OF EURONAL FATE CHOICE, MIGHT RESCUE A DICER-EPENDENT IMPAIRMENT ON NEUROGENESIS.

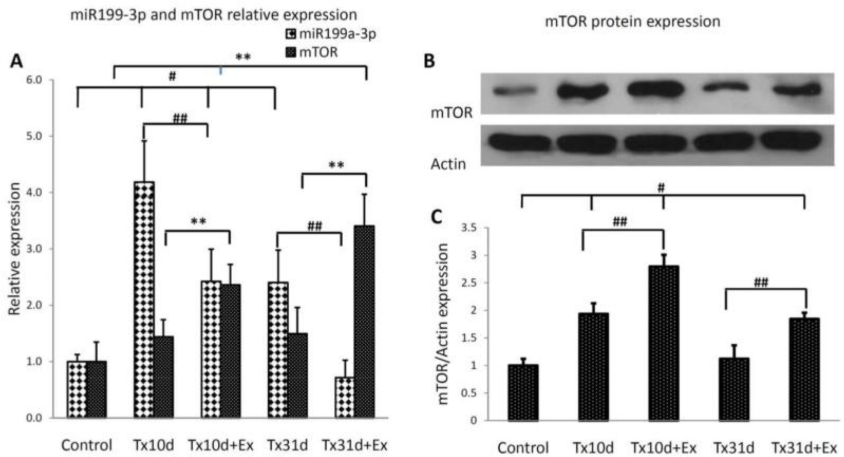
Exp Neurol. 2012 Jan;233(1):447-56. doi: 10.1016/j.expneurol.2011.11.018. Epub 2011 Nov 19.

Exercise modulates microRNAs that affect the PTEN/mTOR pathway in rats after spinal cord injury.

Liu G[1], Detloff MR, Miller KN, Santi L, Houlé JD.







EXERCISE SIGNIFICANTLY INCREASED MIR21 EXPRESSION

SCI CAUSES A 2 FOLD INCREASE IN MIR199A-3P
EX SIGNIFICANTLY REDUCED THE LEVEL OF MIR199A-3P

Exp Neurol. 2012 Jan;233(1):447-56. doi: 10.1016/j.expneurol.2011.11.018. Epub 2011 Nov 19.

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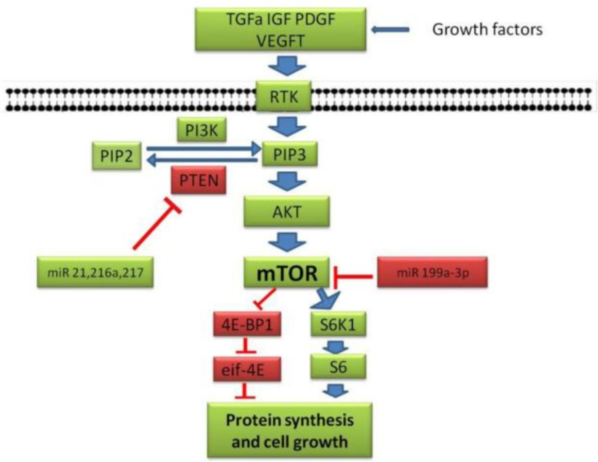
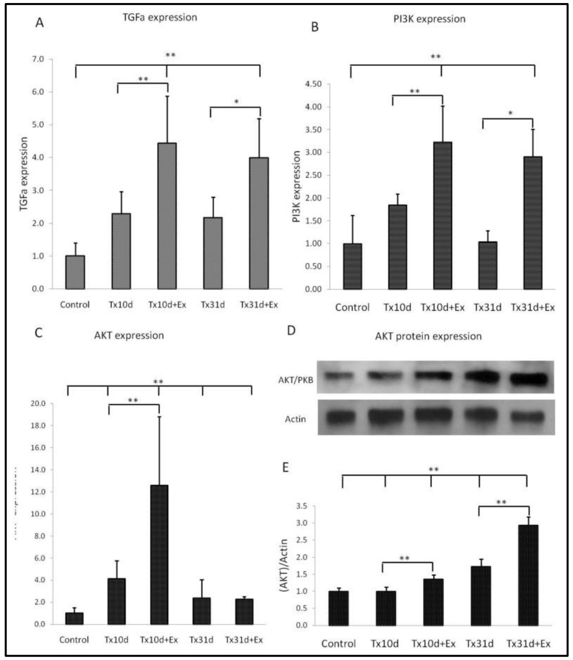
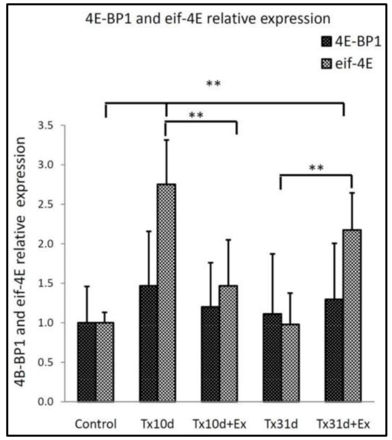
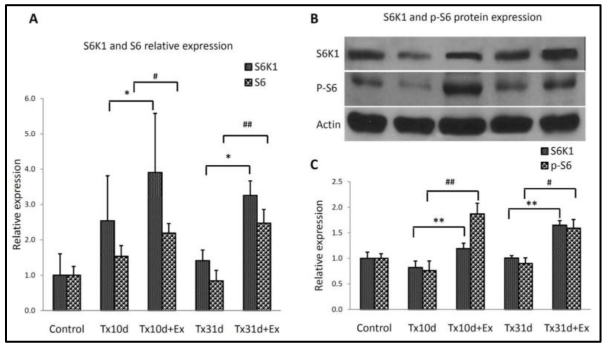


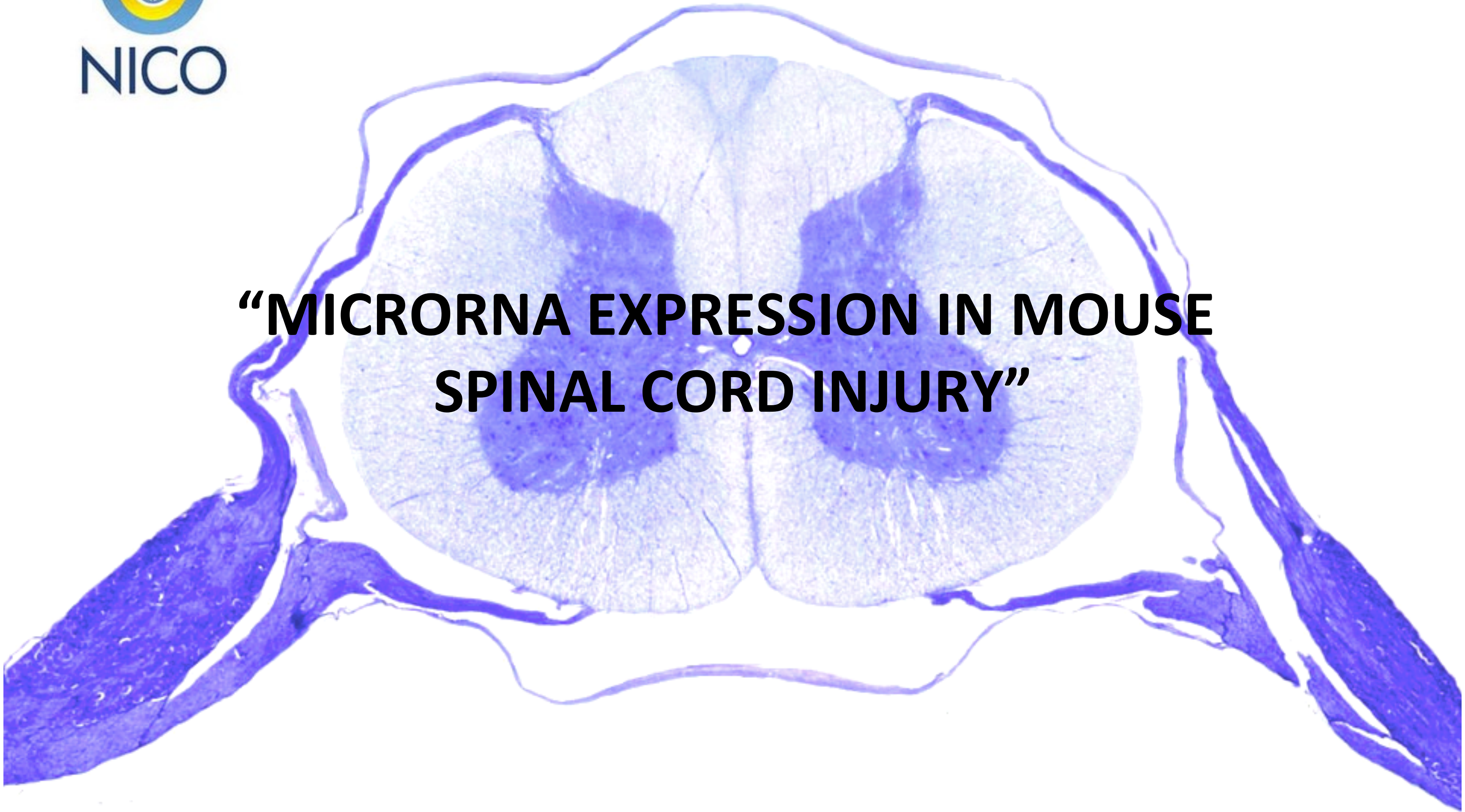




TGFα, PI3K AND AKT INCREASING
Eif-4 E REDUCTION
S6K1 AND S6 INCREASING



EX AFTER SPINAL CORD INJURY MAY PROVIDE A REHABILITATIVE STRATEGY TO IMPROVE THE REGENERATIVE CAPACITY OF DAMAGED AXONS AND HELP REGULATE SPINAL CORD PLASTICITY AFTER INJURY



"MICRORNA EXPRESSION IN MOUSE SPINAL CORD INJURY"

Matilde Ghibaudi
PhD student

MICRORNA SENSORIMOTOR CORTEX PROFILE

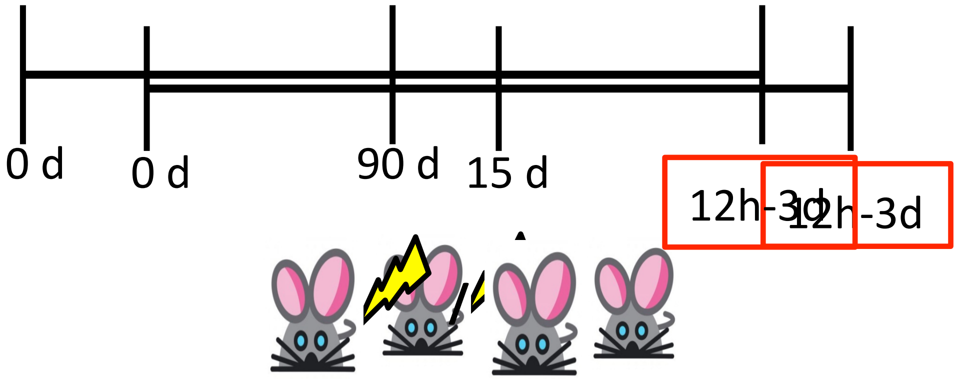
EXPERIMENTALMODEL
EXPERIMENTAL MODEL

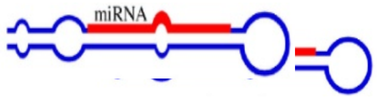
C57BL/6J
P15
C57BL/6J
P90

COMPLETE TRANSECTION INJURY MODEL (C7-T1)
COMPLETE TRANSECTION INJURY MODEL (C7-T1)

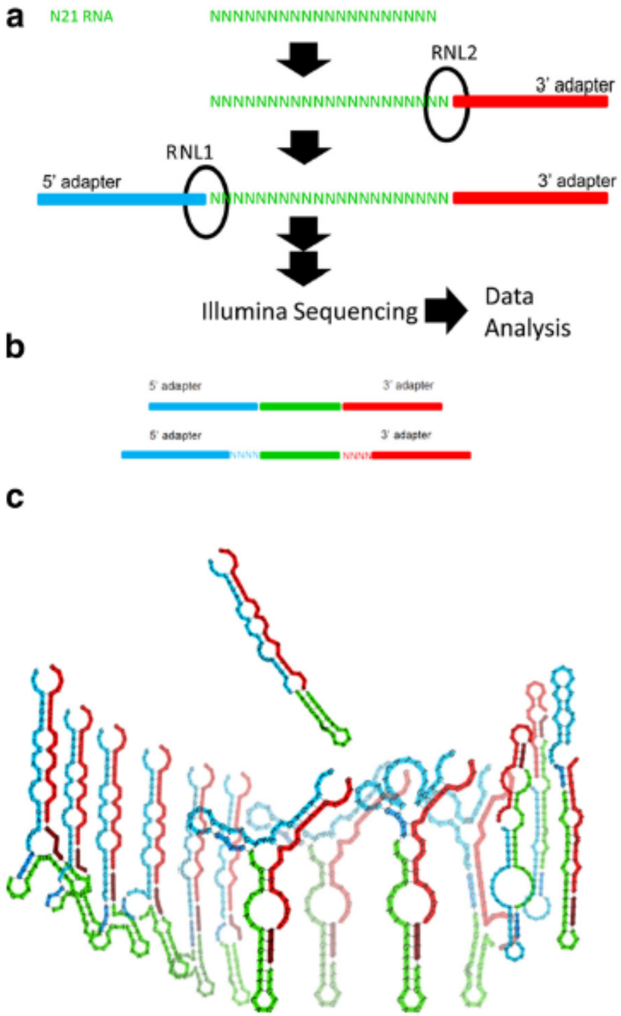
EXPERIMENTAL DESIGN
EXPERIMENTAL DESIGN

SCI/SHAM
SACRIFICE AND CORTEX DISSECTION
SCI/SHAM
SACRIFICE AND CORTEX DISSECTION
\+
RNA EXTRACTION ENRICHED FOR MicroRNAs
\+
RNA EXTRACTION ENRICHED FOR MicroRNAs





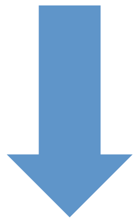
MICRORNA LIBRARY



University of East Anglia

Norwich, UK

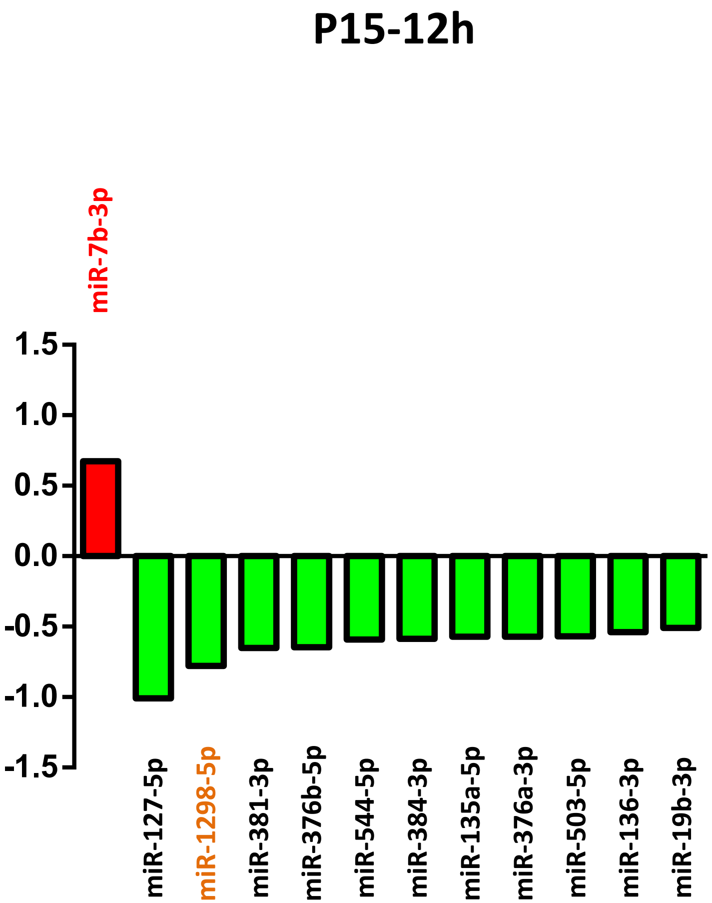
PROFESSOR T. DALMAY
School of Biological Sciences

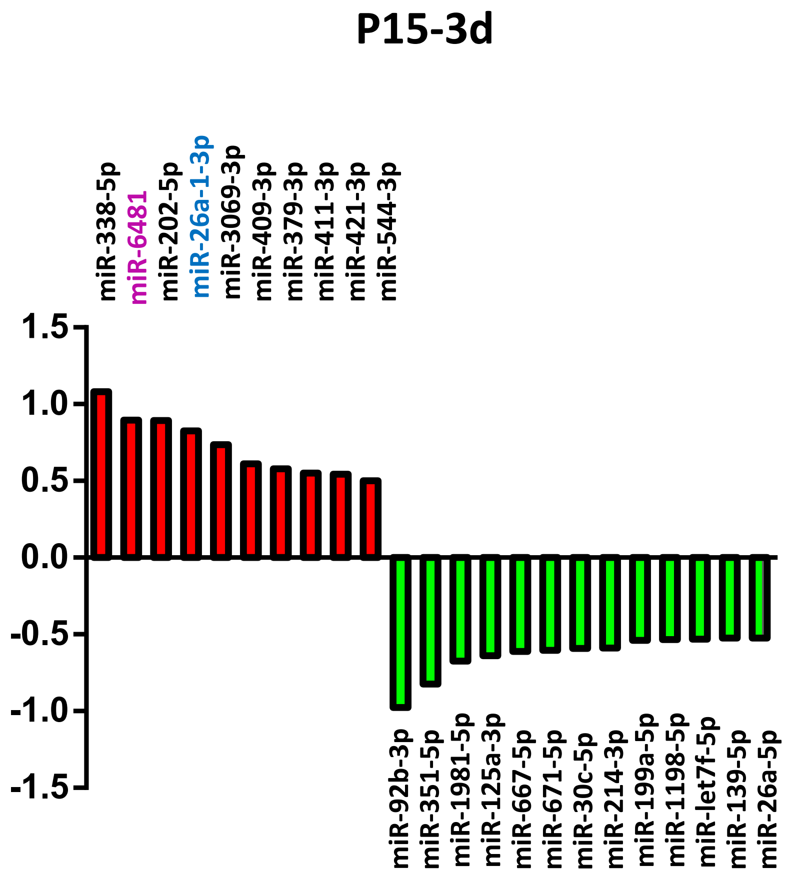


NEXT GENERATION SEQUENCING

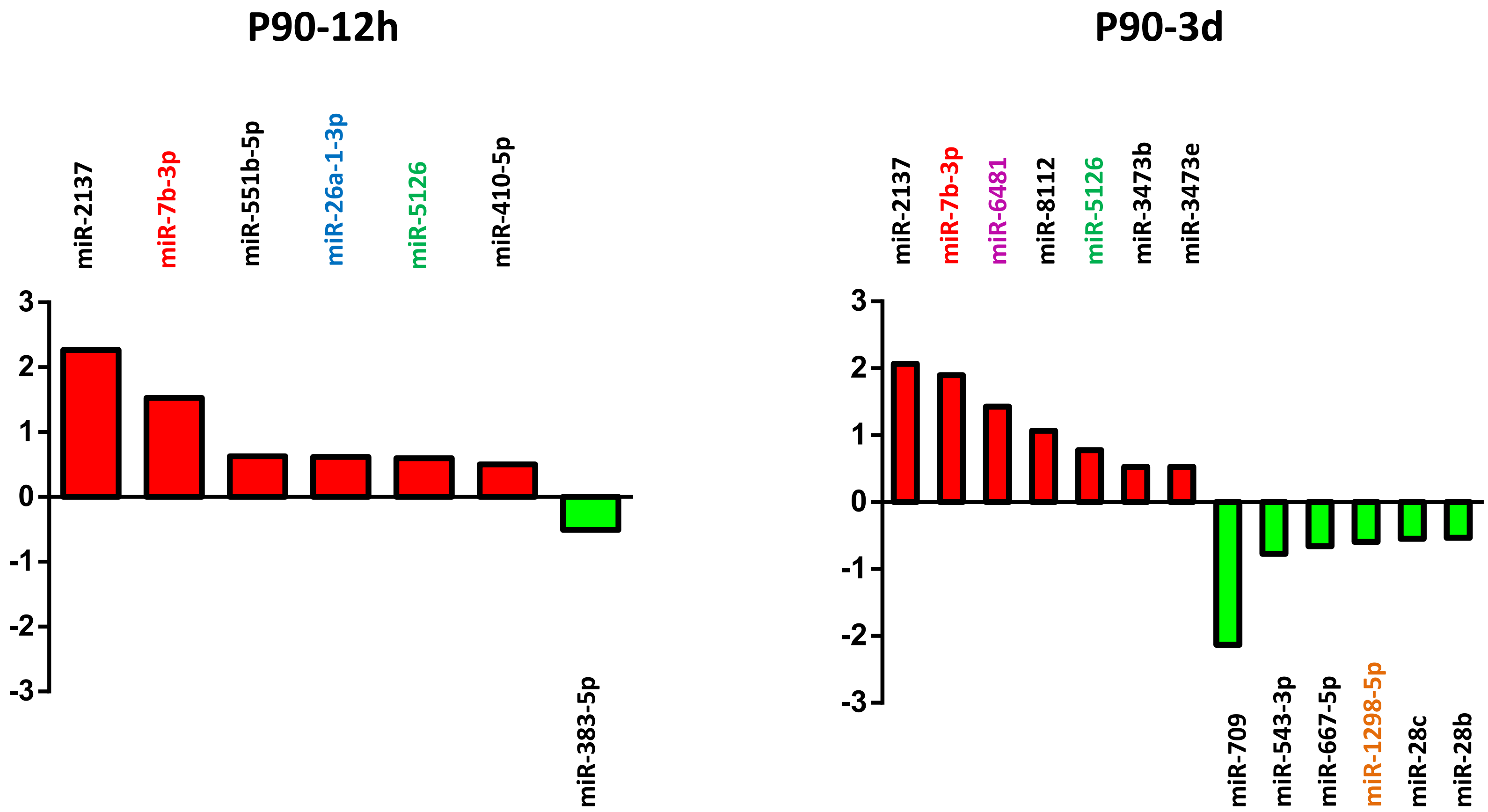
TGAC
The Genome Analysis Centre™
BBSRC
Greater Norwich Development Partnership

MICRORNA PROFILE



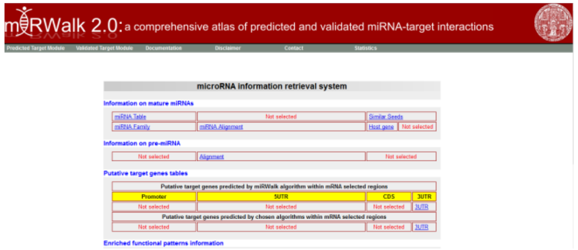


MICRORNA PROFILE



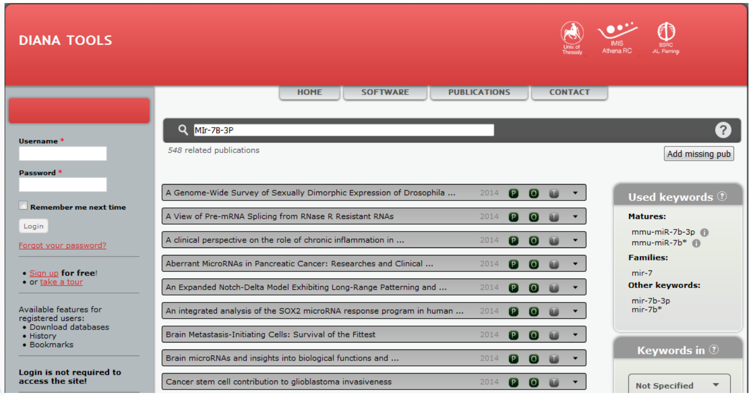
MICRORNA SELECTION

MIRWALK



DAVID

MIRPUB

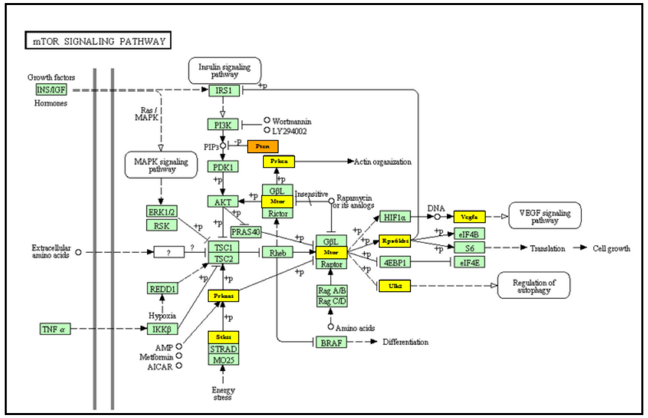


VALIDATED AND PUTATIVE TARGET **FUNCTIONAL ANALYSIS** **LITERATURE**

- miR-19b-3p
- miR-381-3p
- miR-127-5p
- miR-214-3p
- miR-7b-3p
- miR-338-5p

NEEDED A RT-PCR VALIDATION!

miRpath v.3 miRNA analysis



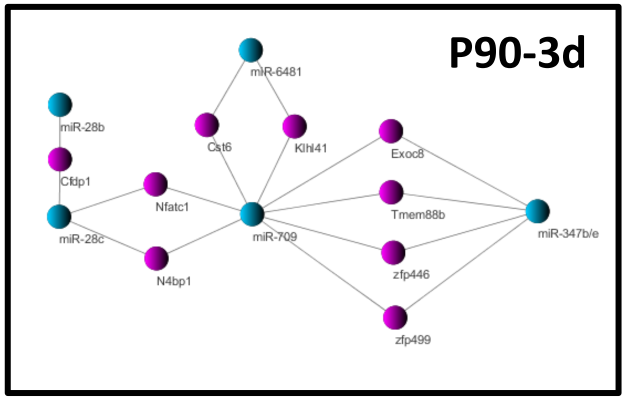
mmu-miR-2137
mmu-miR-7b-3p
mmu-miR-551b-5p
mmu-miR-26a-1-3p
mmu-miR-5126
mmu-miR-410-5p
mmu-miR-383-5p

KEGG PATHWAY

CELL GROWTH
DIFFERENTIATION

miRna predicted gene target analysis

miRwalk shared genes
cytoscape

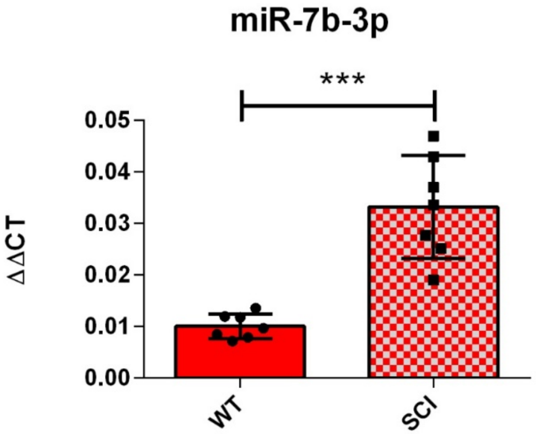


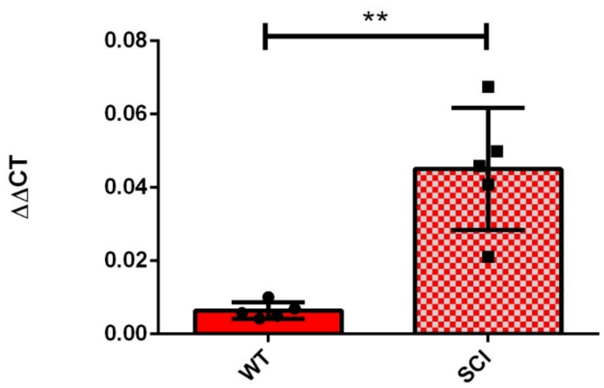
P15-12h
P15-3d
P90-12h

mir-7b-3p upregulation after SCI

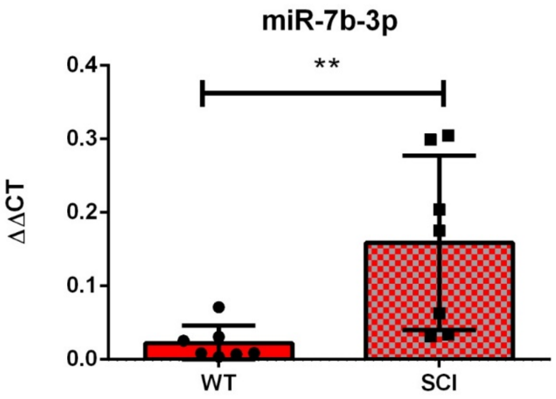
P15 | P90

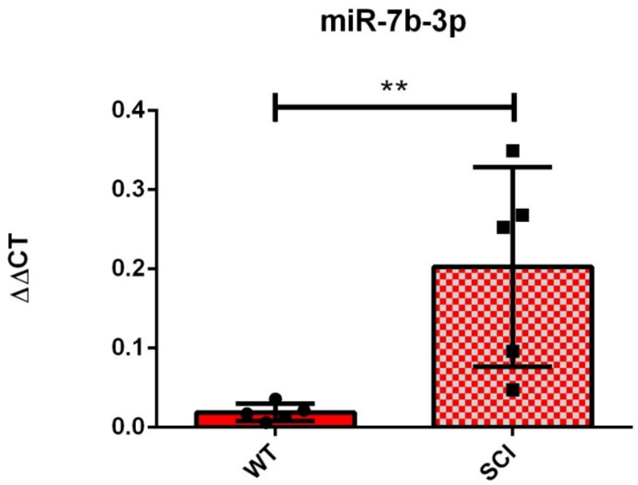
12h





3d





miRna functional analysis

miR-7b-3p PUTATIVE FUNCTION BY miRWalk-NO VALIDATED TARGETS

GOBP
Nervous system development
Axon guidance
Actin cytoskeleton Axonogenesis
Neuron differentiation
Synaptic transmission

KEGG
Axon guidance
Regulation of actin cytoskeleton
Neurotrophin signaling pathway
Long term potentiation
mTOR signaling pathway

IN SCI

- functional neuroprotection
- miR-7a is downregulated
- apoptosis-related

mir-7 function (Chen H et al., 2014)

NEUROBLASTOMA CELL LINE DIFFERENTIATION

- miR-7 PRECURSOR NEURITE OUTGROWTH
- miR-7 INHIBITOR NEURITE OUTGROWTH

PRIMARY CORTICAL NEURONS IN VITRO

- miR-7 INCREASES DURING DIV 12

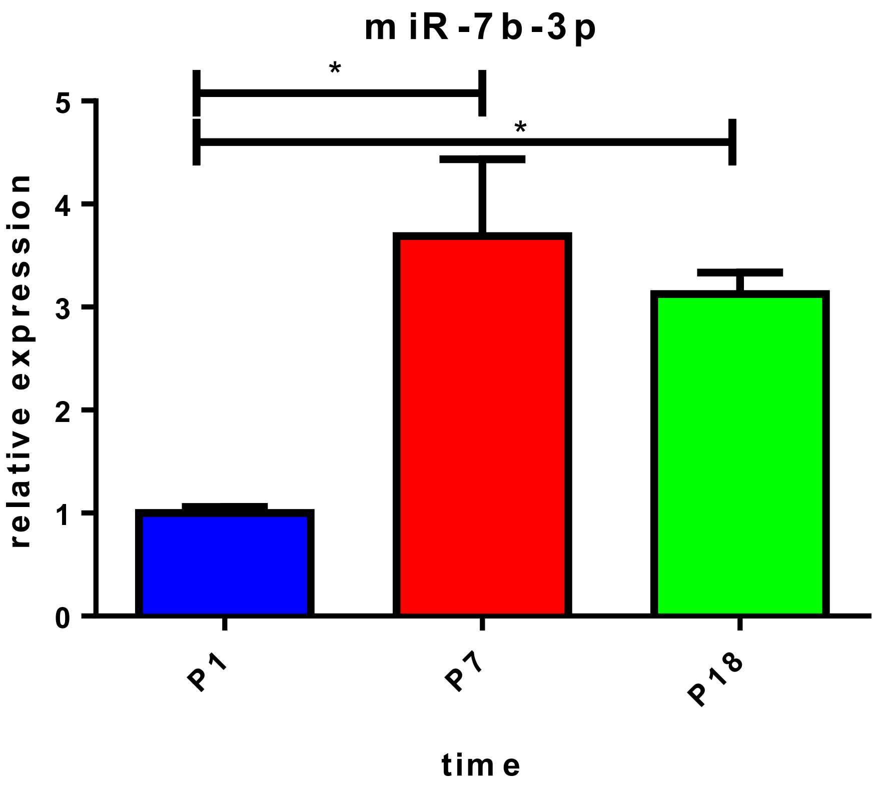
DURING EMBRYONIC AND POSTNATAL DEVELOPMENT

- miR-7 INCREASES ONLY IN POSTNATAL DEVELOPMENT (FROM E13-6W)

DURING DIFFERENTIATION OF ES CELLS

- miR-7 IRREGULARLY DECREASES

mir-7b-3p in primary cortical neuron E 14.5



ANATOMY

SENSORYMOTOR CORTEX

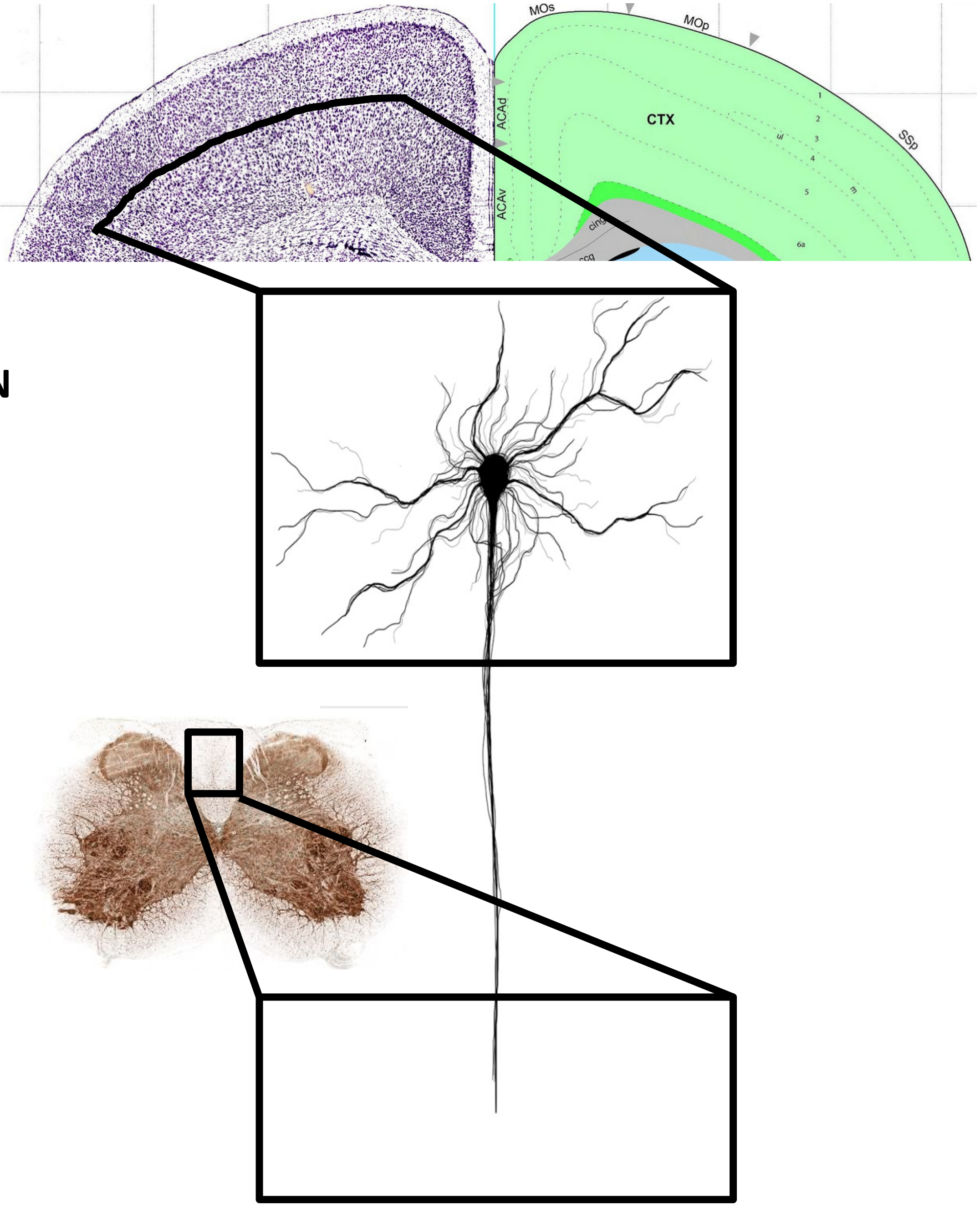
↓

CORTICOSPINAL MOTOR NEURON
CELL BODY

DORSAL
CORTICOSPINAL TRACT

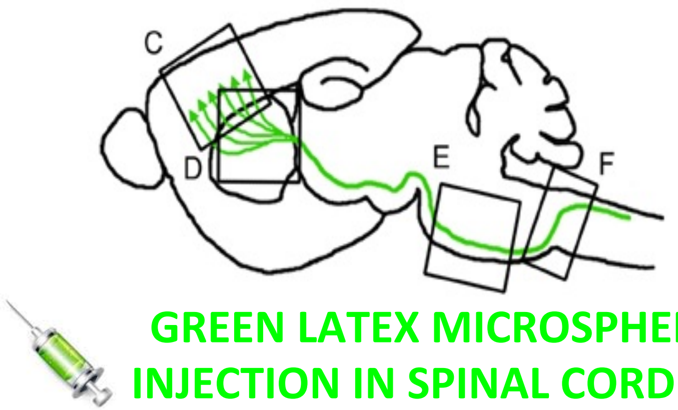
↓

CORTICOSPINAL MOTOR NEURON
AXON



Single cell population labeling

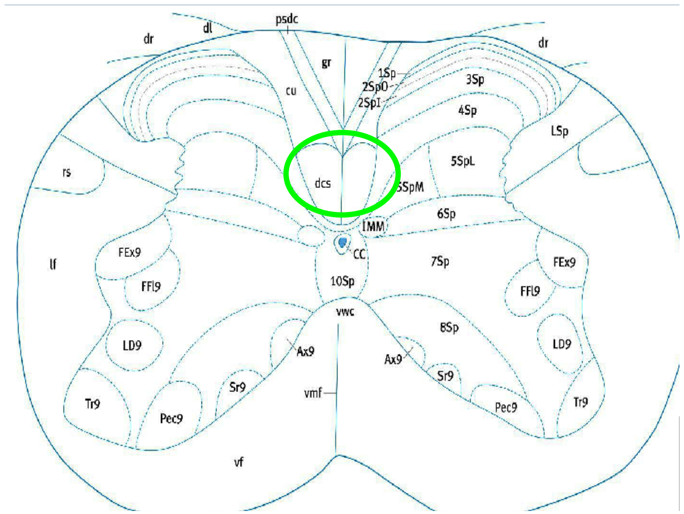
GLM FEATURES
RETROGRADE TRACER
DO NOT DIFFUSE
STABLE LABELING



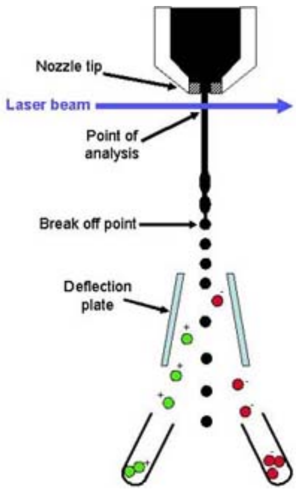
GREEN LATEX MICROSPHERES
INJECTION IN SPINAL CORD (C7)

CSMN CELL BODY
LAYER V

CSMN FIBERS
DORSAL CORTICOSPINAL TRACT

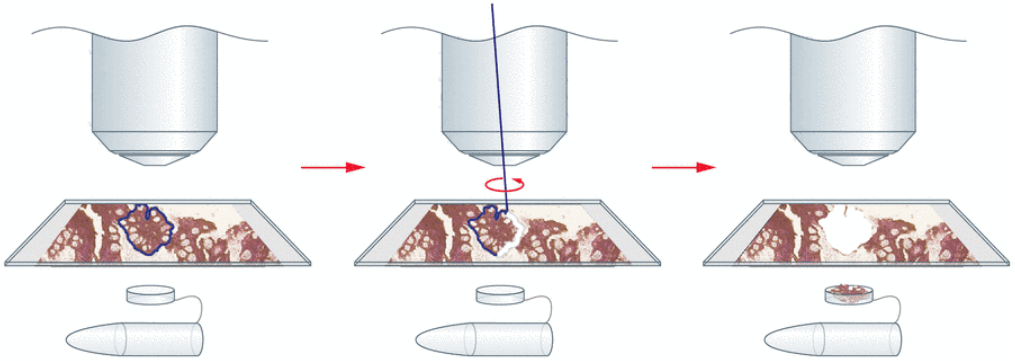


Two different techniques

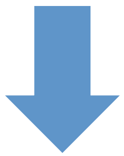


CSMN FACS SORTING

/

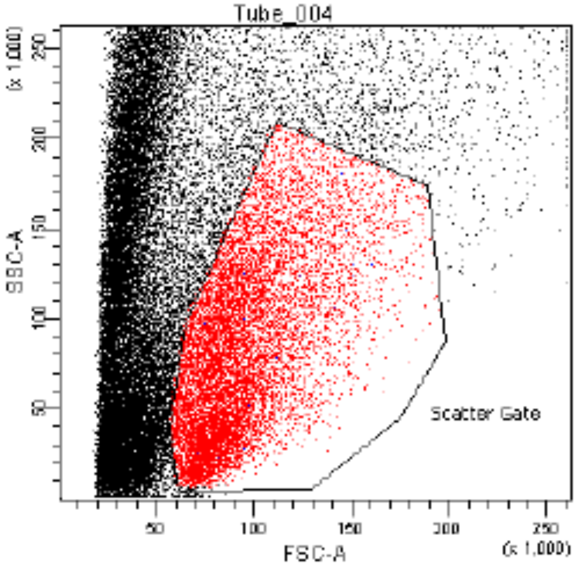


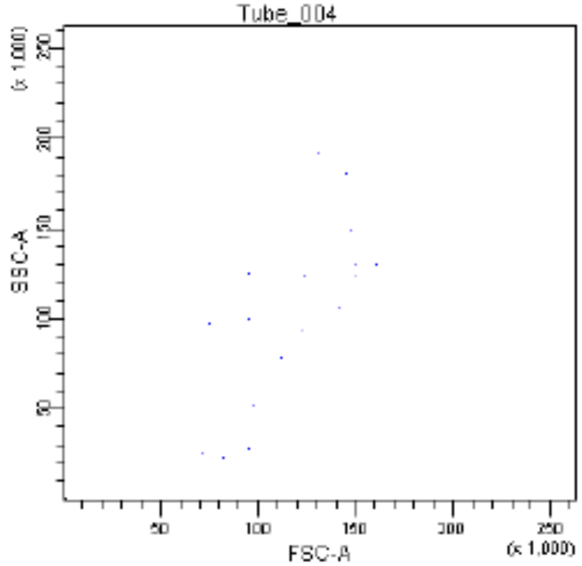
LASER CAPTURE MICRODISSECTOR

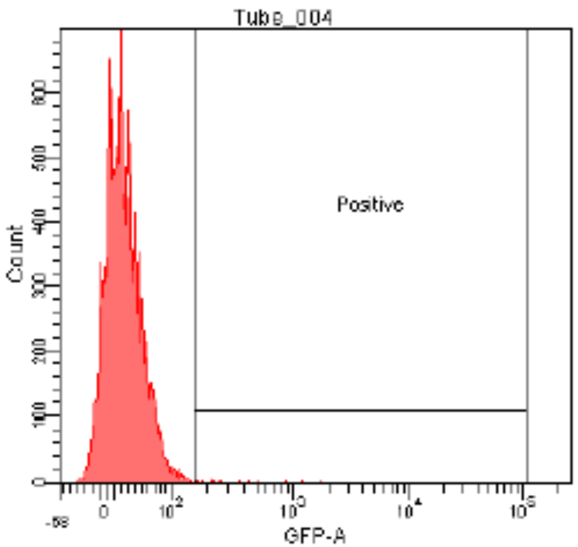


MICRORNA PROFILING
FROM CSMN SINGLE CELL POPULATION!

Results







Tube: Tube_004

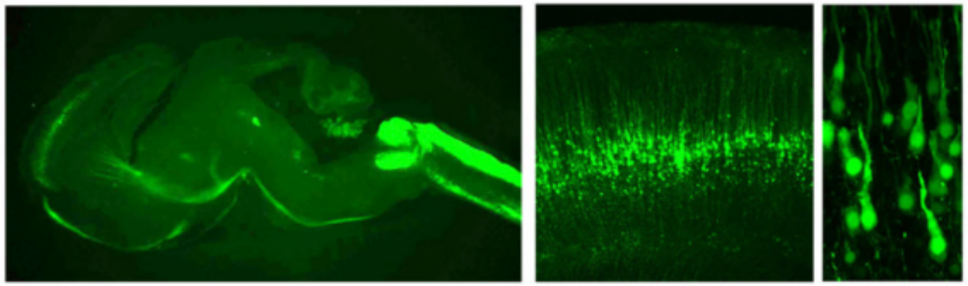
Population	#Events	%Parent	%Total
All Events	50,000	####	100.0
Scatter Gate	11,152	22.3	22.3
Positive	17	0.2	0.0

Experiment Name: 016 jan 16
Specimen Name: Specimen_001
Tube Name: Tube_004
Record Date: Jan 22, 2016 3:38:11 PM

Population	#Events	%Parent	FSC-A Mean	SSC-A Mean	GFP-A Mean
All Events	50,000	####	78,078	147,055	27
Scatter Gate	11,152	22.3	95,099	81,979	24
Positive	17	0.2	117,705	102,377	444

Future perspective

Tg Uchl1-EGFP mice



Yasvoina MV, 2013

UCHL1-eGFP mice express **Enhanced Green Fluorescent Protein** under the control of the ***Uchl1* promoter** and can be used to visualize **corticospinal motor neurons**

Mice that are **hemizygous** for the transgene are **viable and fertile**. The Donating Investigator reports that homozygotes are expected to be viable


THANK YOU
FOR YOUR
ATTENTION!