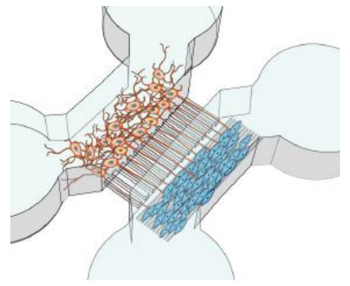

INSTITUT DE GÉNÉTIQUE MOLÉCULAIRE DE MONTPELLIER

Florence Rage

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Team : NMJ (IGMM)

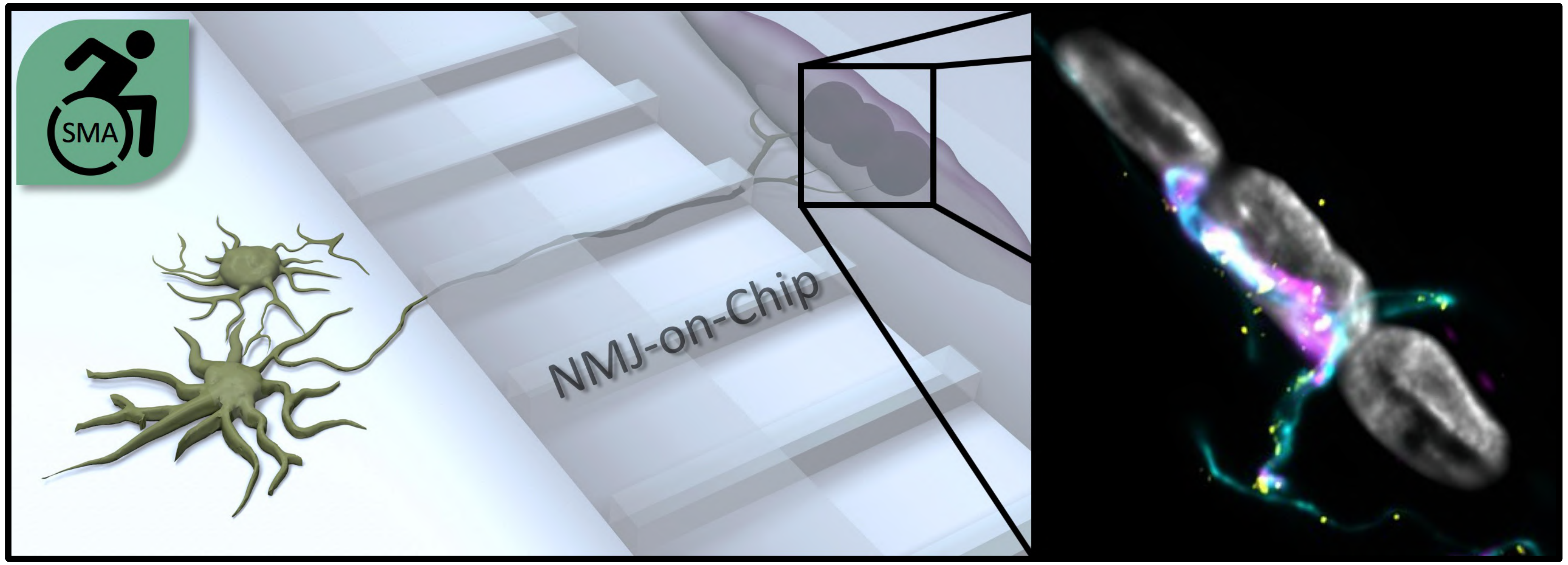
Florence Rage
Johann Soret
Pauline Duc
Audrey Moisan

Team : NMJ (IES)

Benoit Charlot
Audrey Sebban

Team : NMJ (Phymedexp)

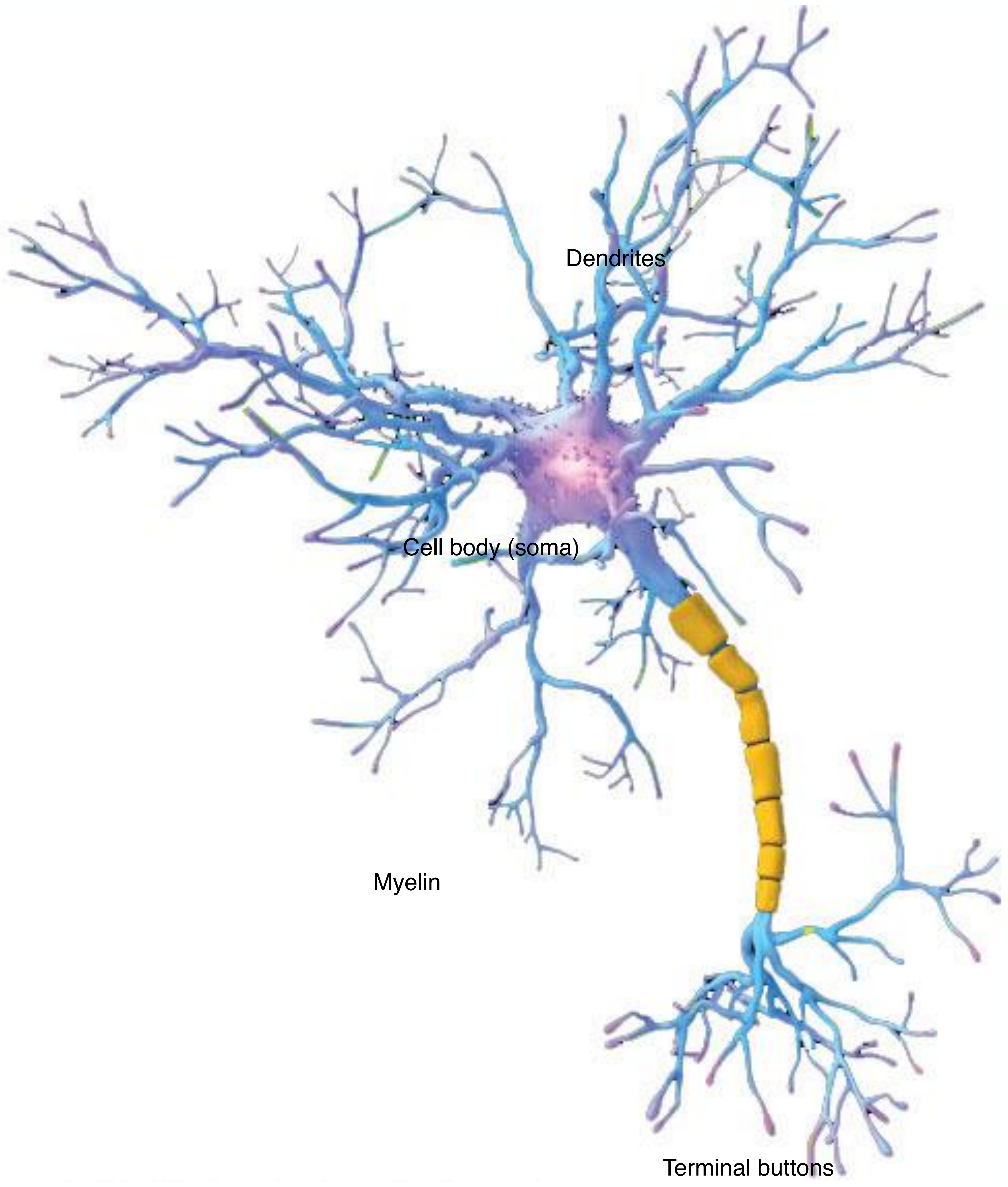
Gilles Carnac
Gerald Hugon



Build a human NMJ in 2D to decipher the molecular mechanisms of mRNA transport and local translation during its formation.
Healthy Individuals vs. SMA Patients

UE : « Physiologie intégrée »
COURS MASTER BIOLOGIE SANTE

The neuron a highly specialized cell

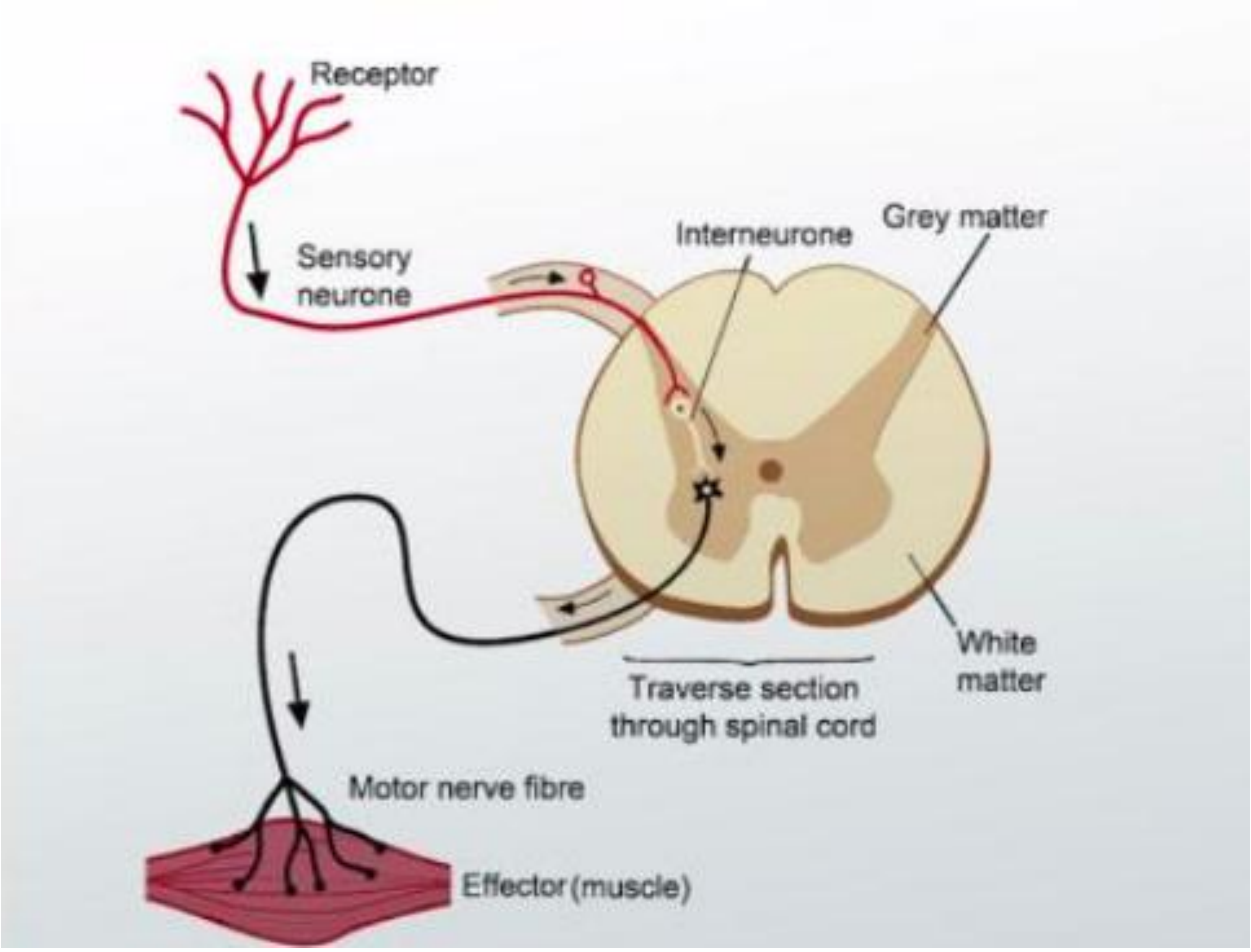


Types of neurons:

Afferent (Sensitive periphery)
nerves conduct signals from sensory neurons
to the central nervous system,
<u>for example the mechanoreceptors in skin.</u>

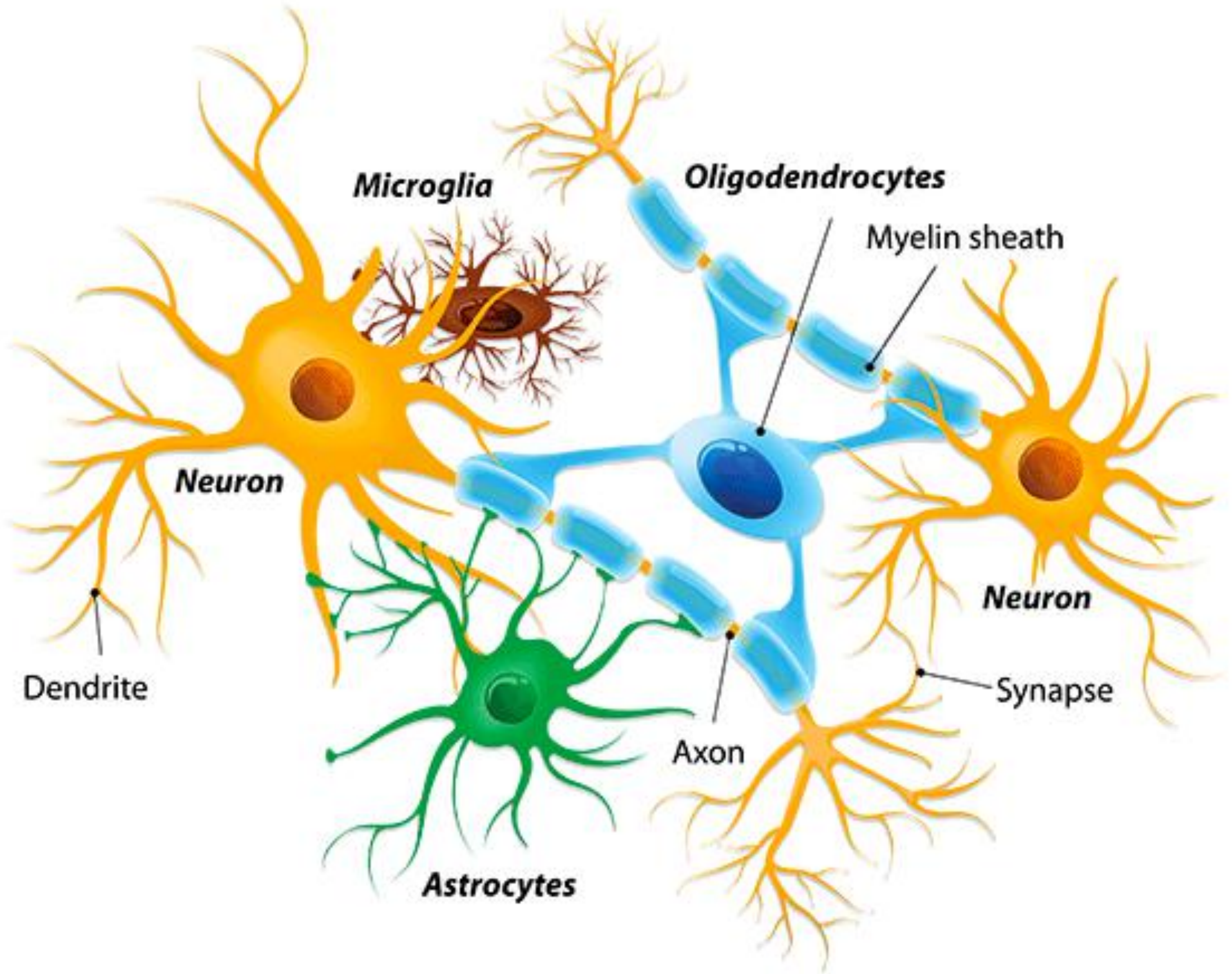
Efferents (muscles and glands)
nerves conduct signals from the central nervous system
along neurons to their target neurons muscles and glands.

Interneurons (Short and long)



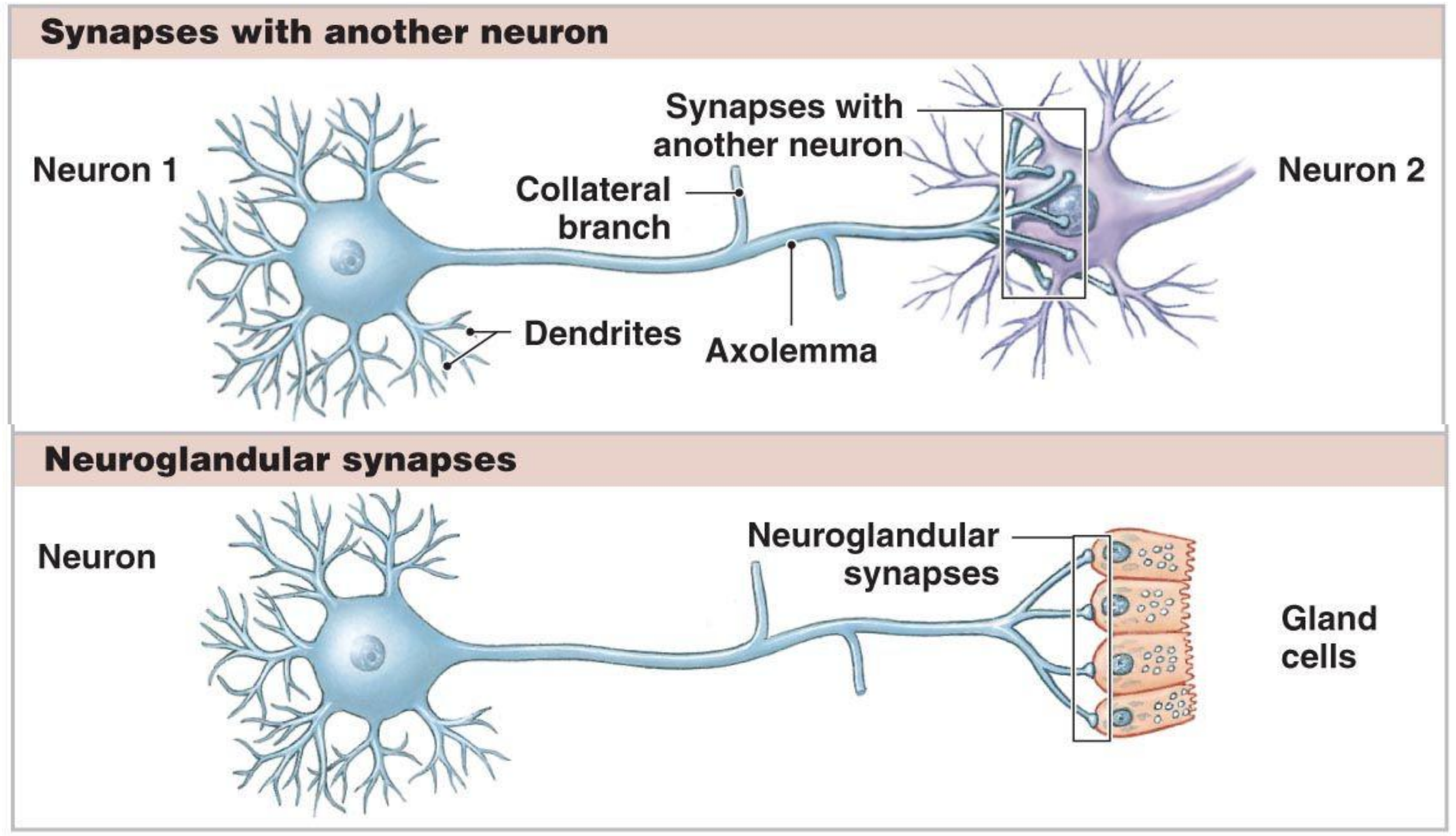
Glial cells = non neuronal:

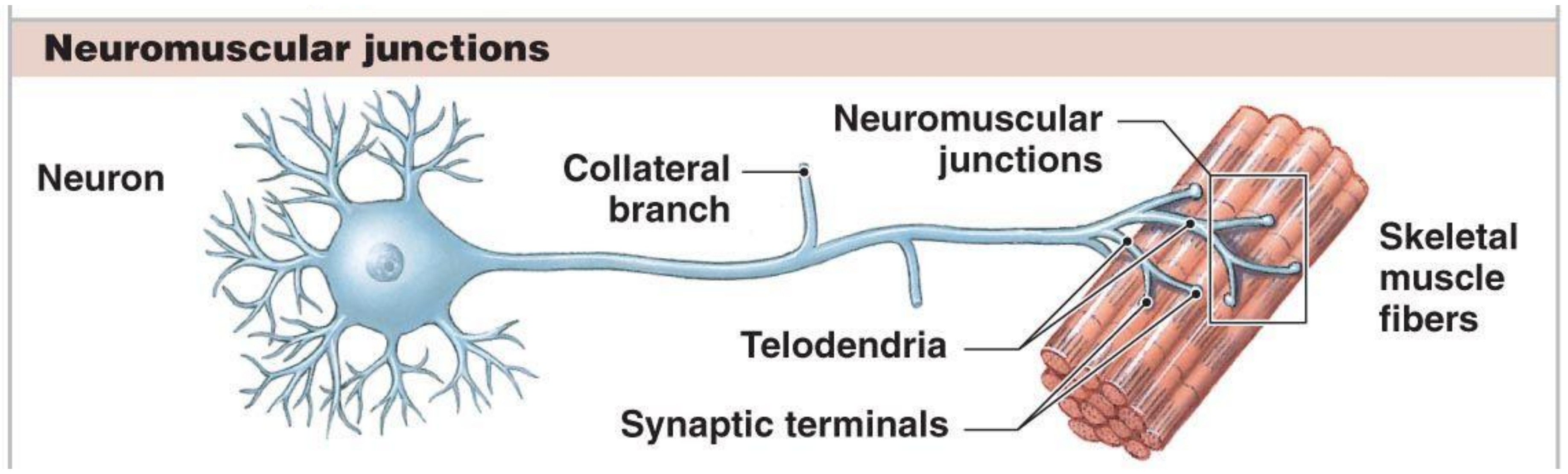
Astrocytes, oligodendrocytes Myelin, Microglia
Provide physical and metabolic support to neurons.



Different type of synapses

The types of synapses

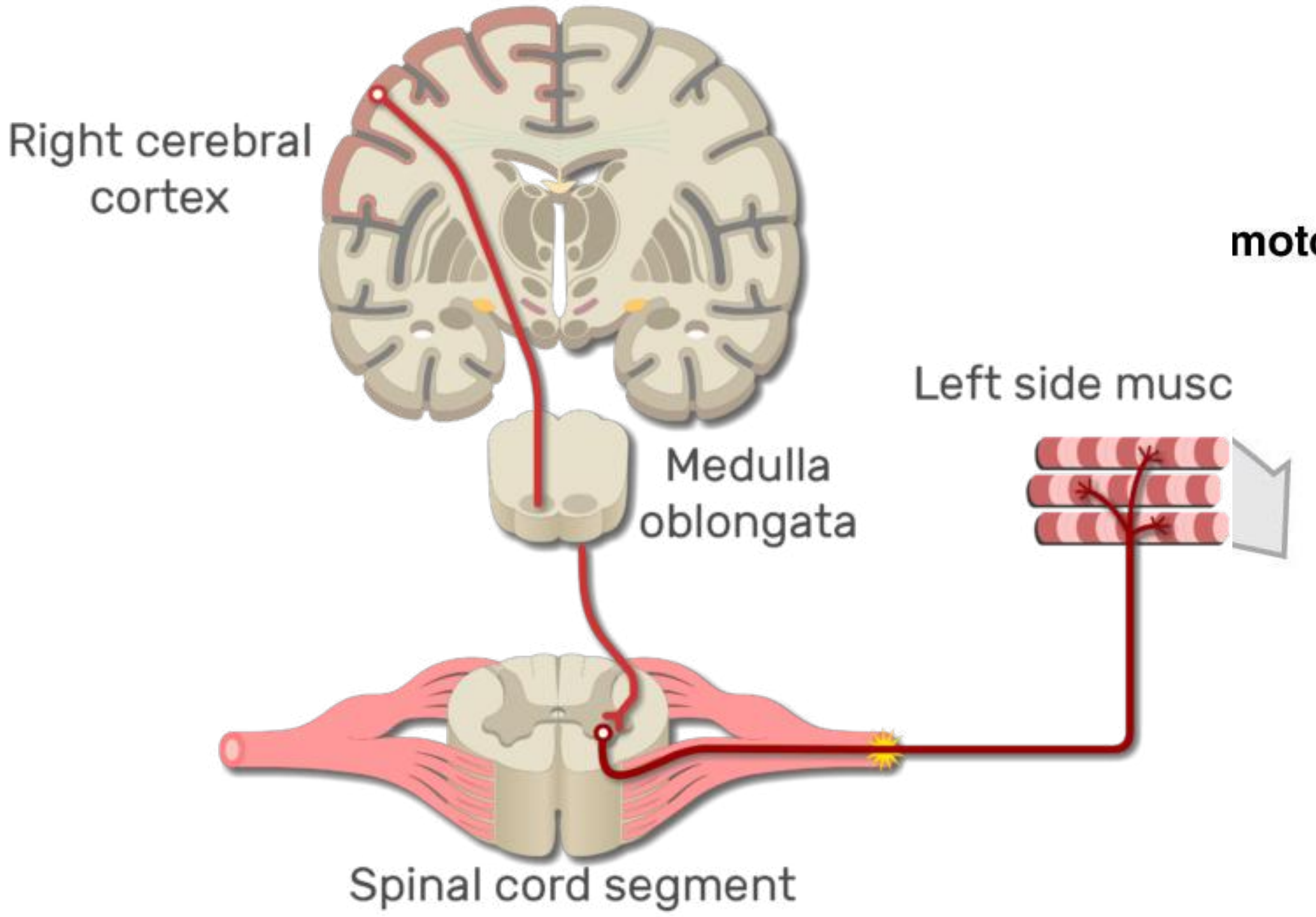


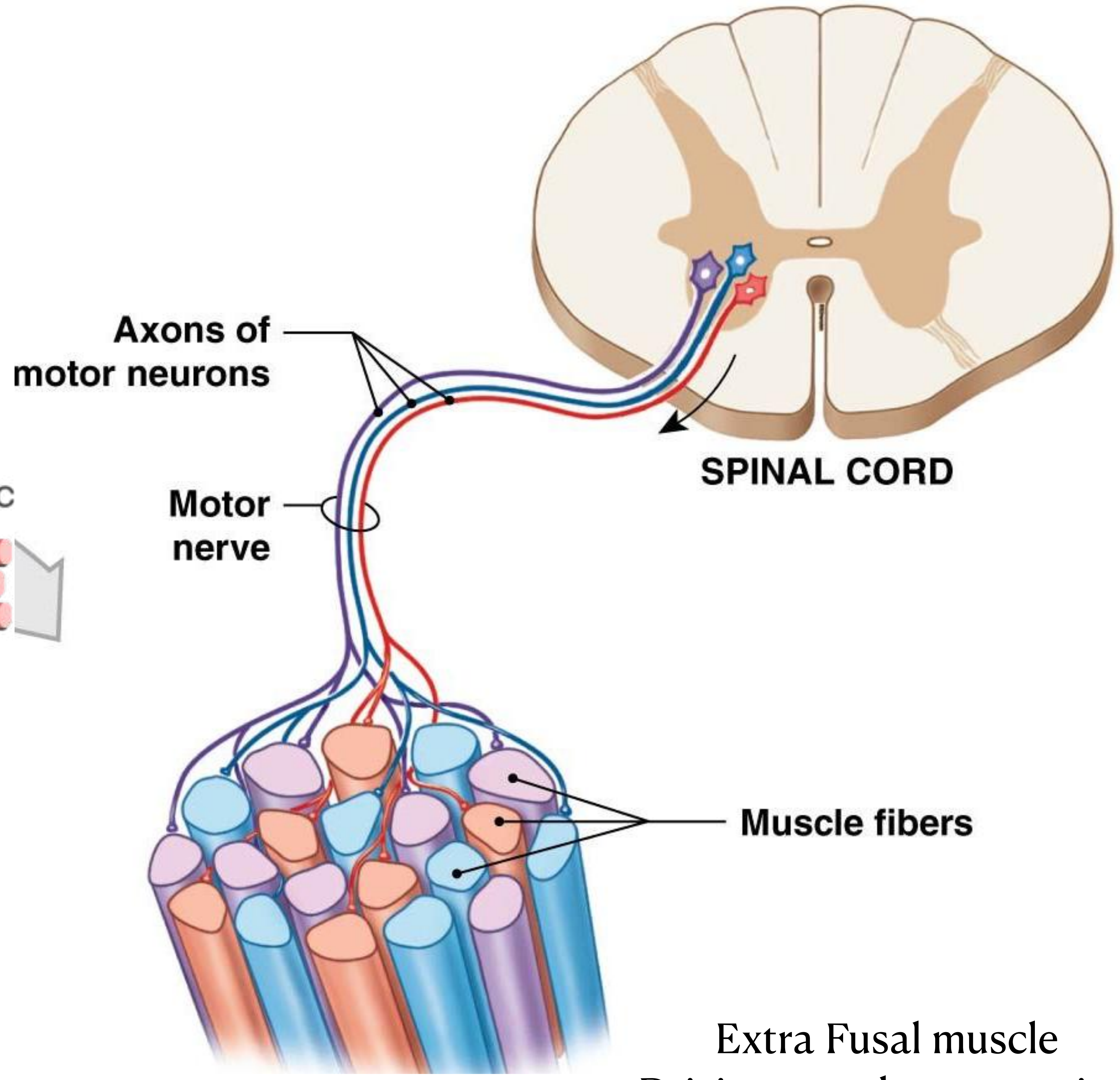


Specialized Synapse

The NMJ : origin of the motor neurons.

CNS



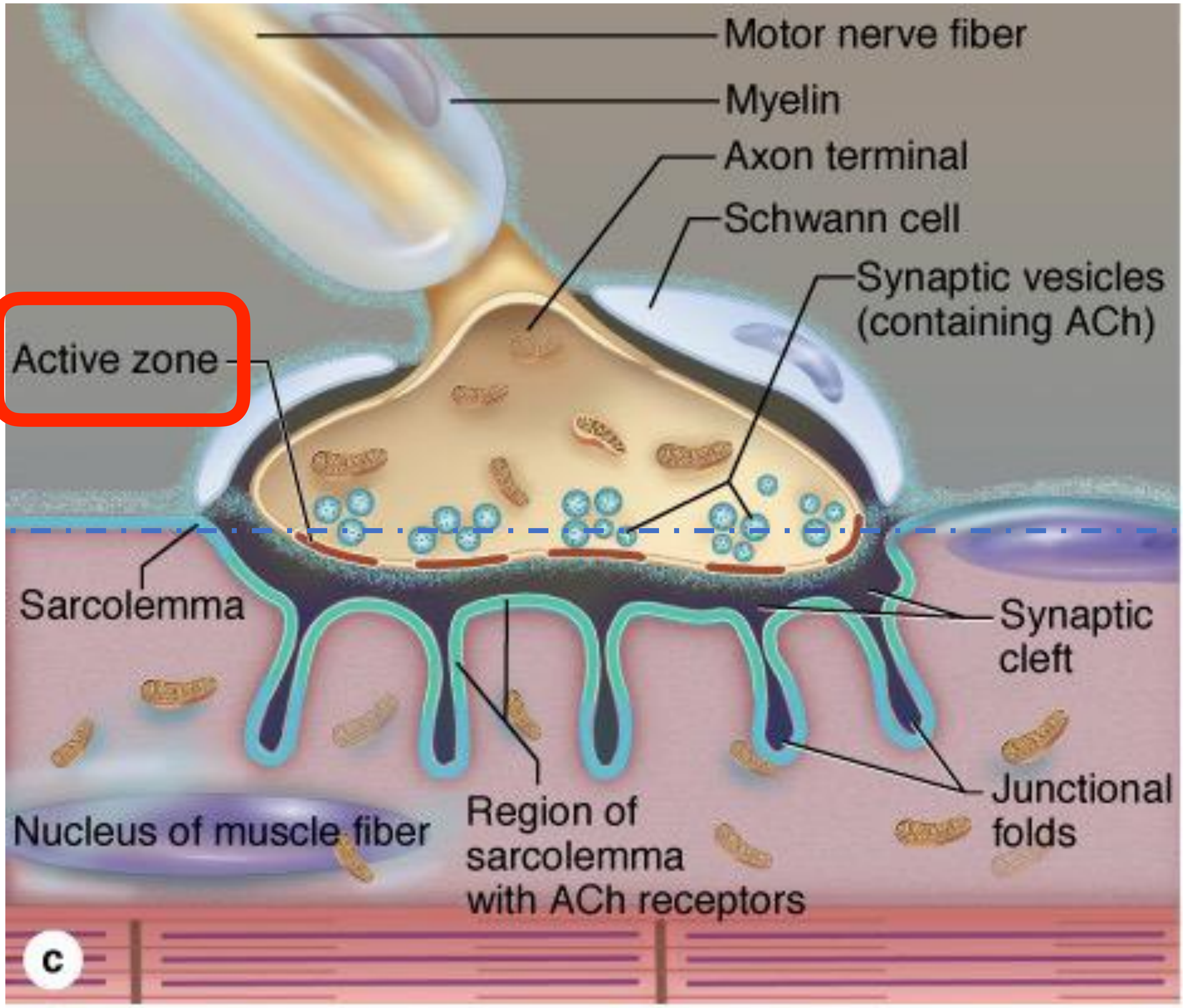


Extra Fusal muscle
Driving muscle contraction

The NMJ : Cellular constituents.

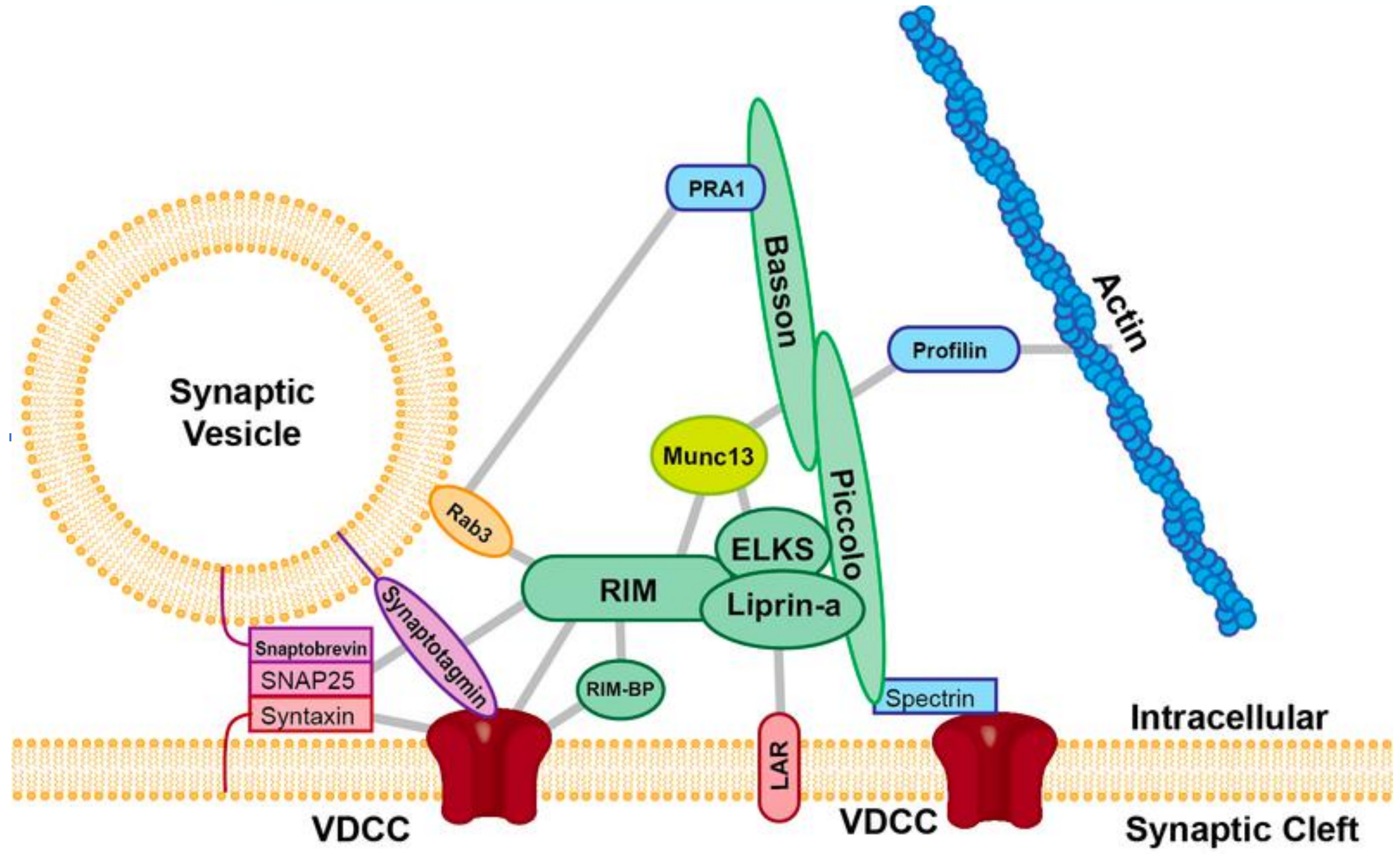
PRESYNAPTIC

POSTSYNAPTIC

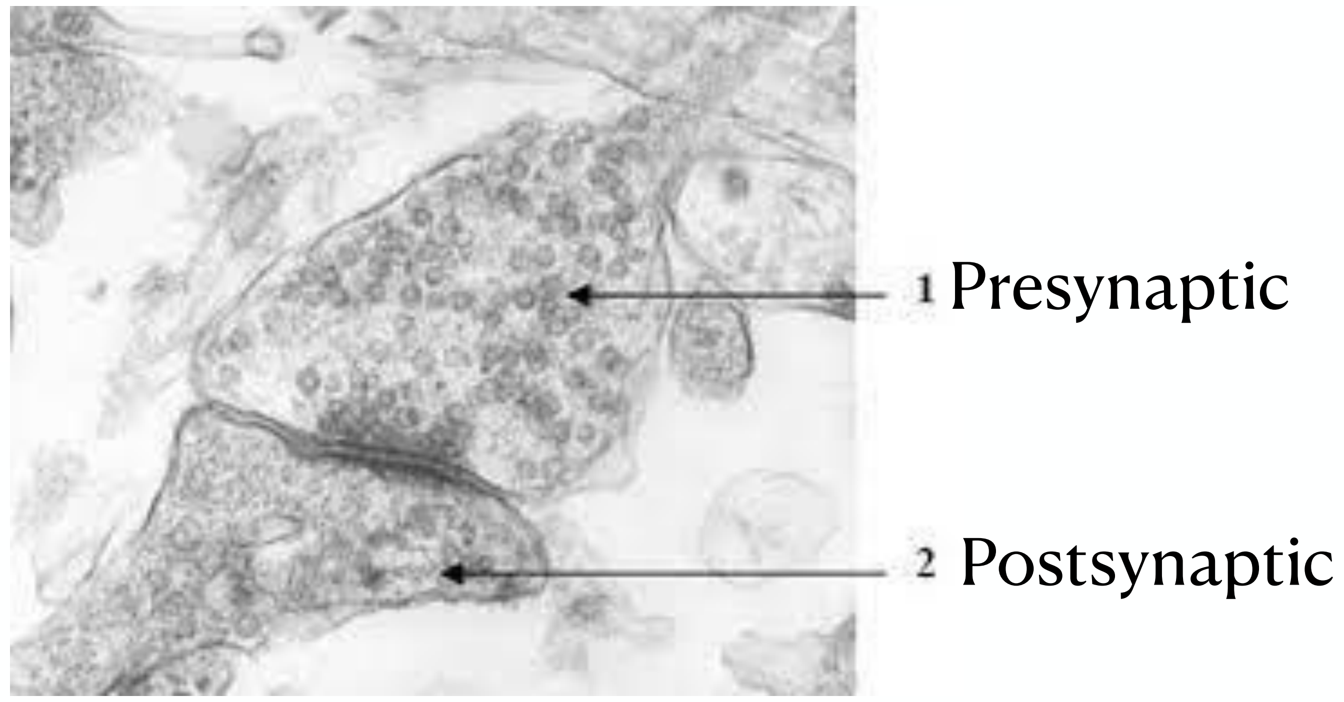


Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition*: http://www.accessmedicine.com

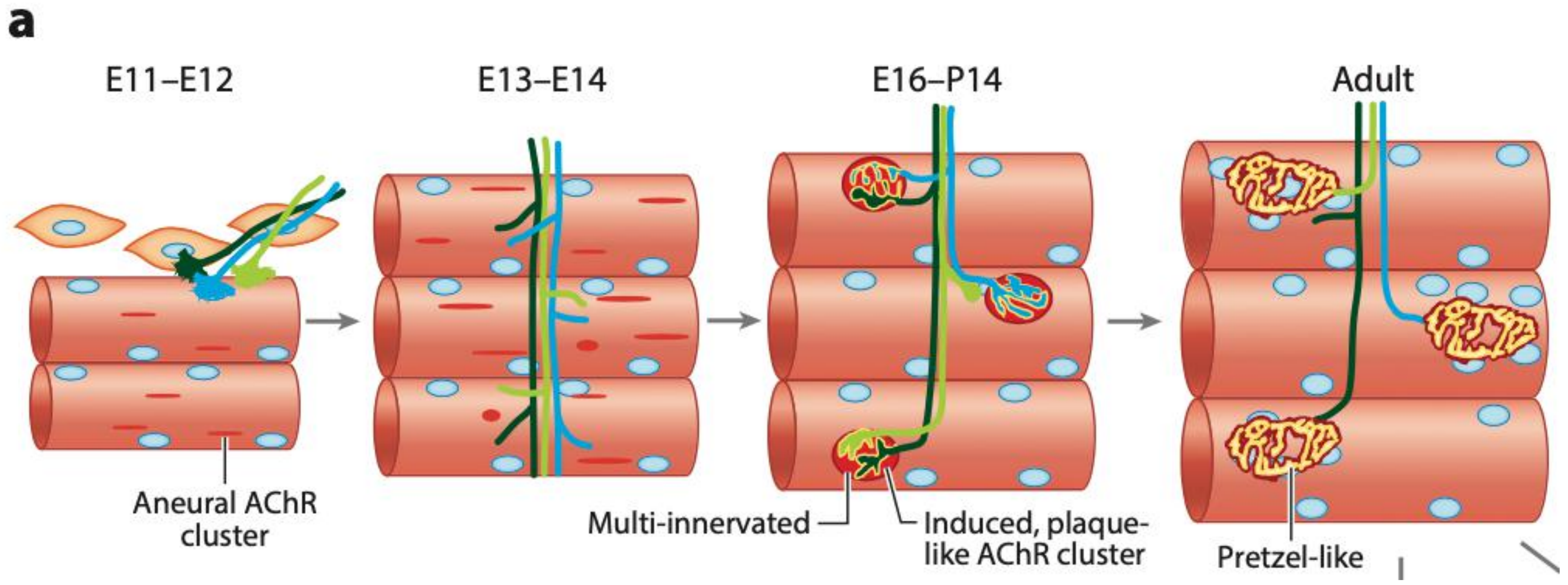
The **active zone** is the region in the presynaptic bouton that mediates neurotransmitter release and is composed of the presynaptic membrane and a dense collection of proteins called the cytomatrix at the **active zone** (CAZ). The CAZ is seen under the electron microscope to be a dark (electron dense) area close to the membrane.



12.14.2014Cellular Intelligence
Complexity of the Glia Neuromuscular Junction

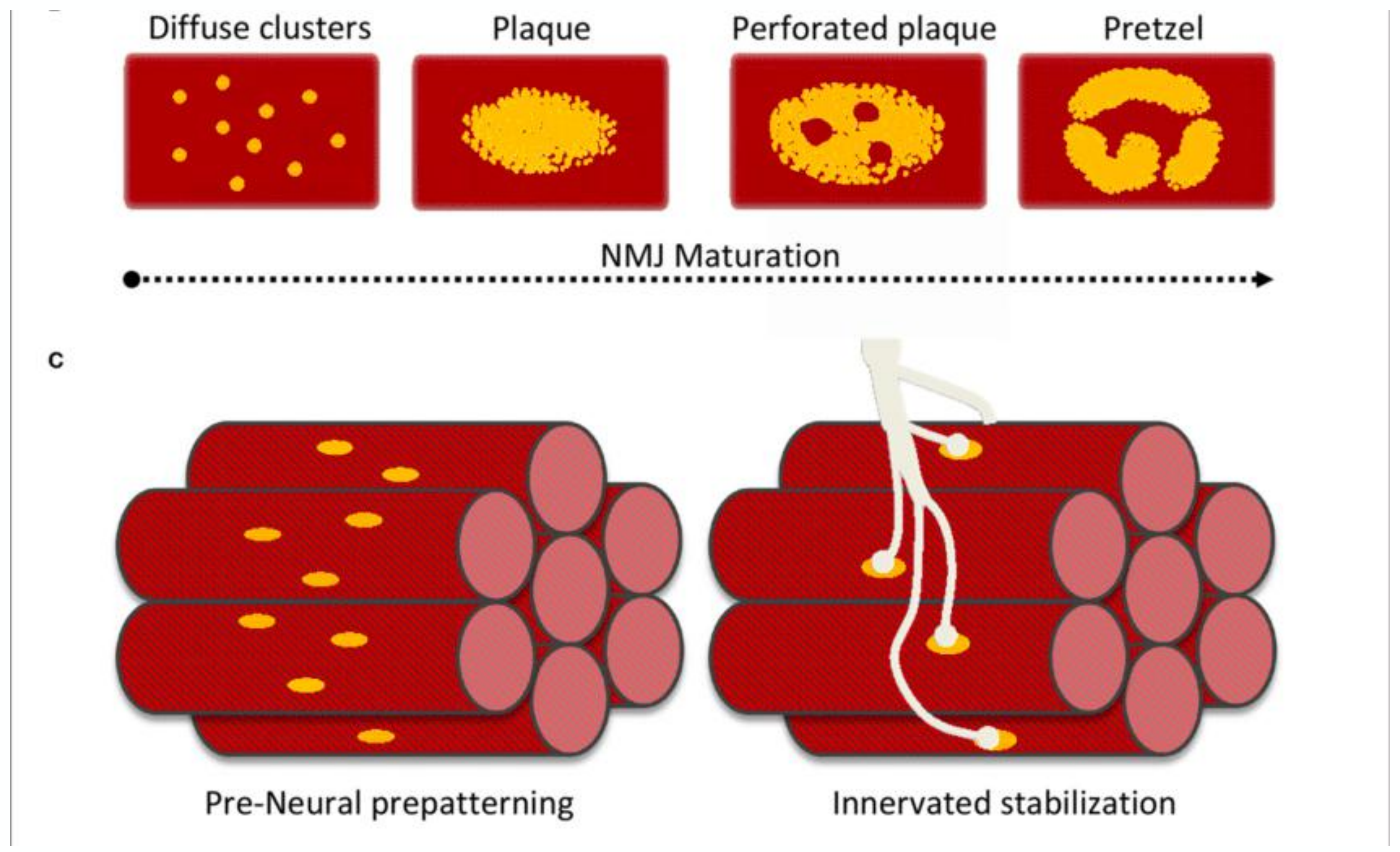


NMJ : The Pre-Patterning



Annu. Rev. Physiol. 2018. 80:159–88

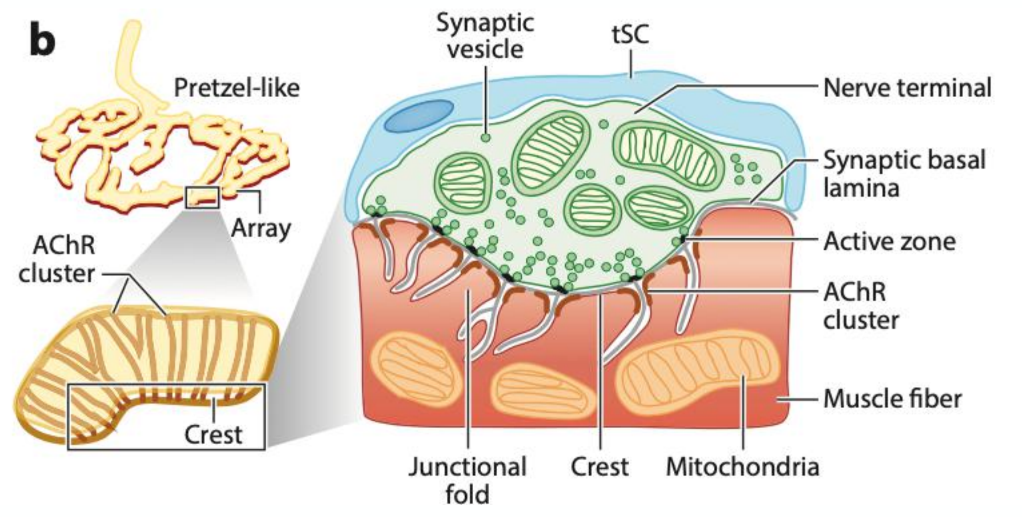
Prior to the arrival of nerve terminals, myotubes form primitive, small, thin AChR clusters that are distributed in a broad middle region (axons E11–E12; E13–E14). Nerve-induced clusters are initially oval plaques, often innervated by multiple axons (E16–P14). As NMJs mature, AChR clusters become perforated and complex, resembling pretzels with arrays or branches that are innervated by one axon per NMJ (adult).



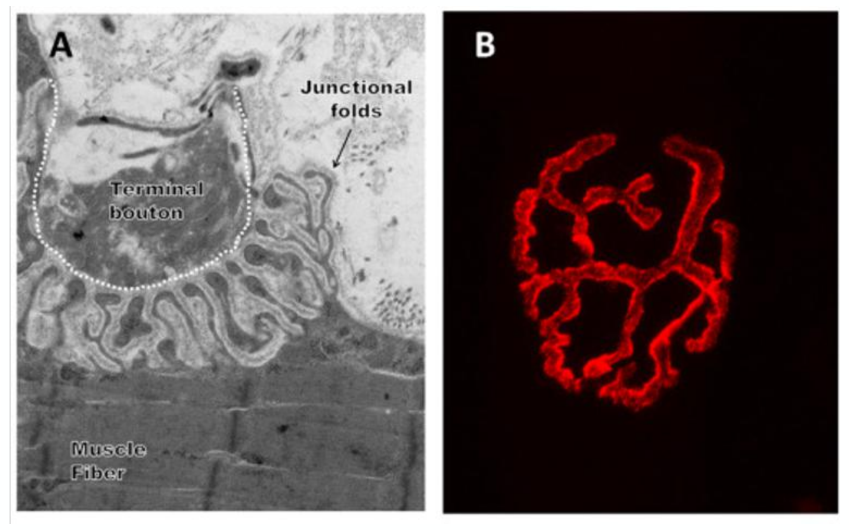
Annu. Rev. Pshysiol. 2018. 80:159–88

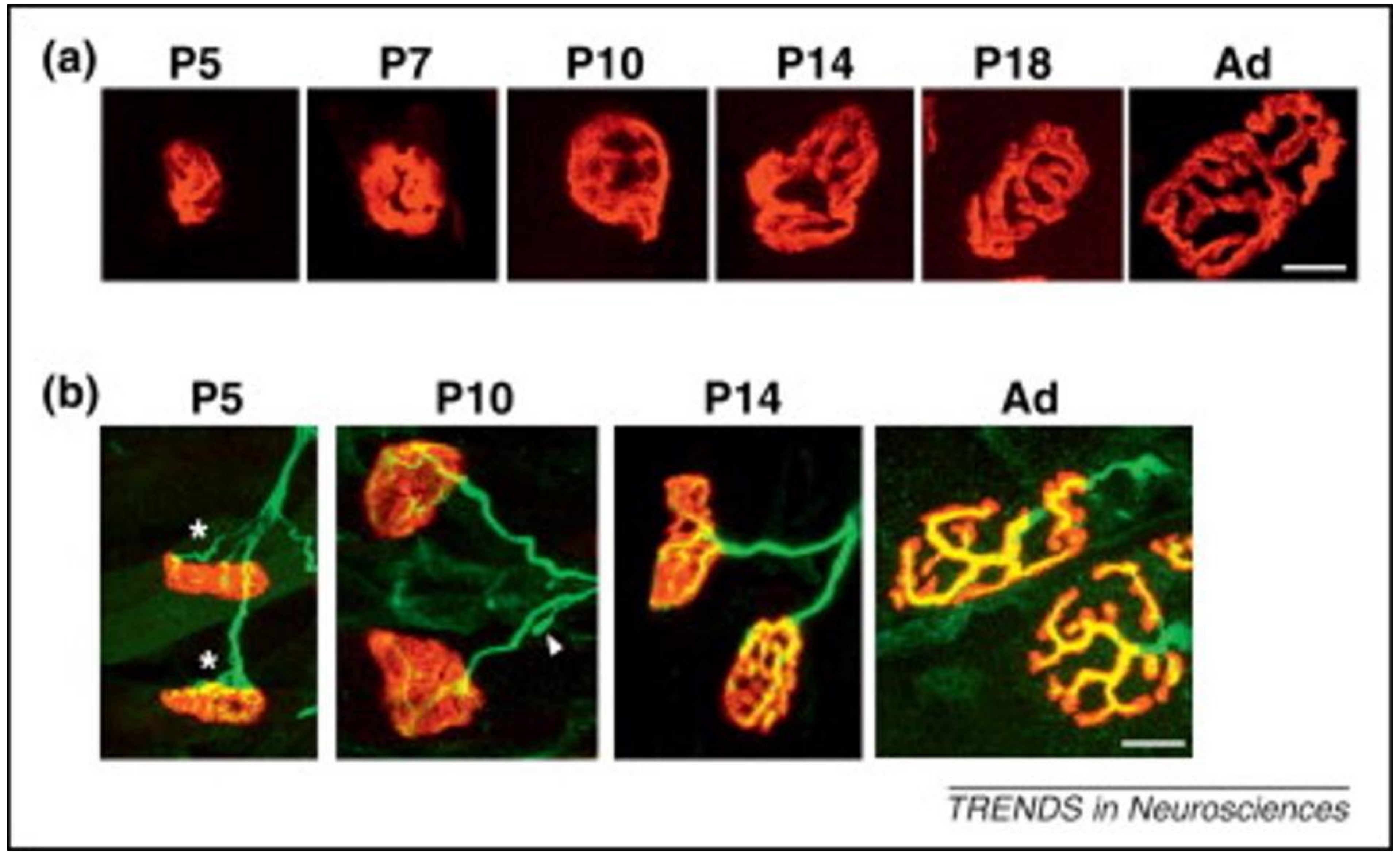
Frontiers in Molecular Bioscience 2020 6:156

Receptors clusterization means pretzel



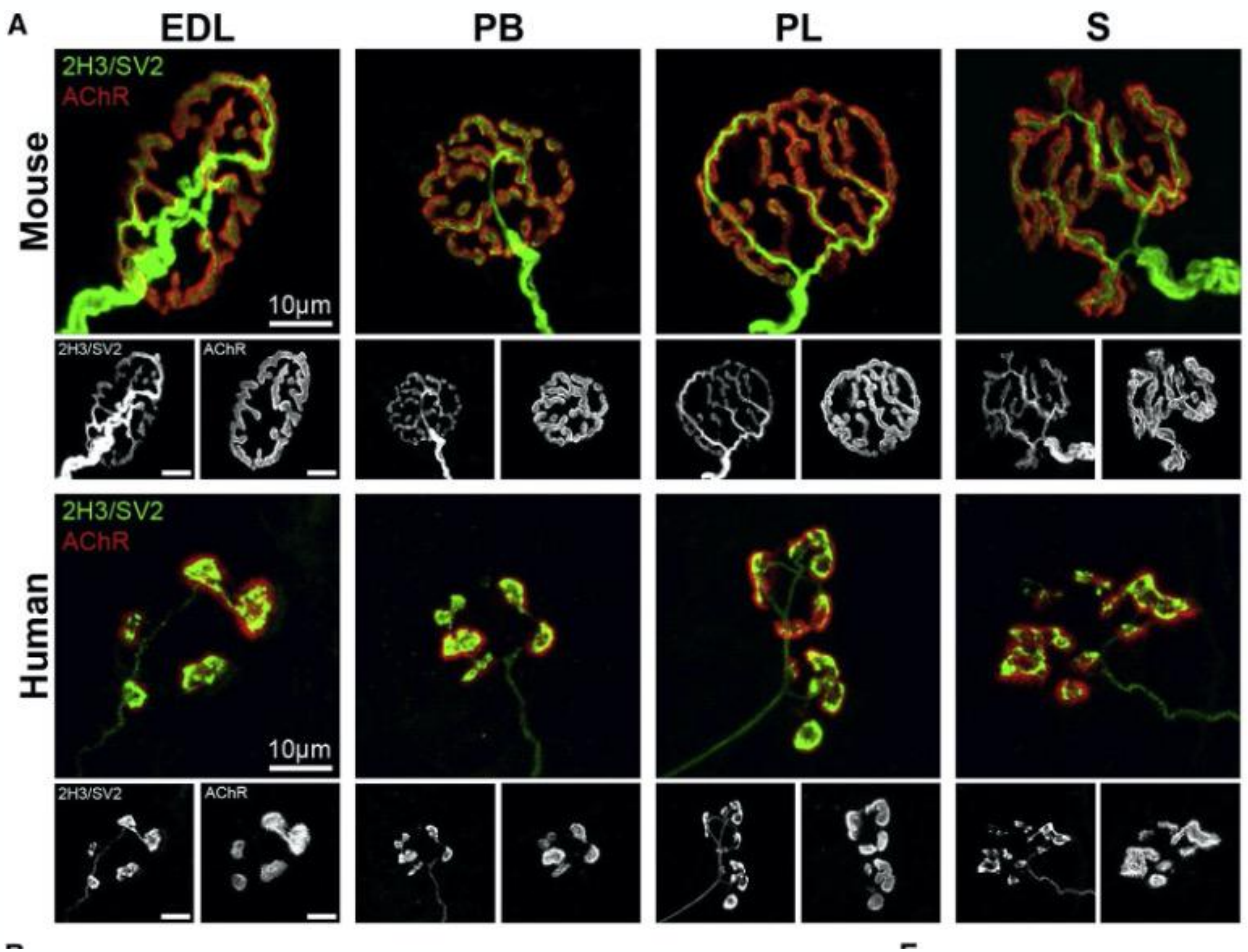
Annu. Rev. Physiol. 2018. 80:159–88





Trends in Neurosciences 2012 35441-453DOI: (10.1016/j.tins.2012.04.005)

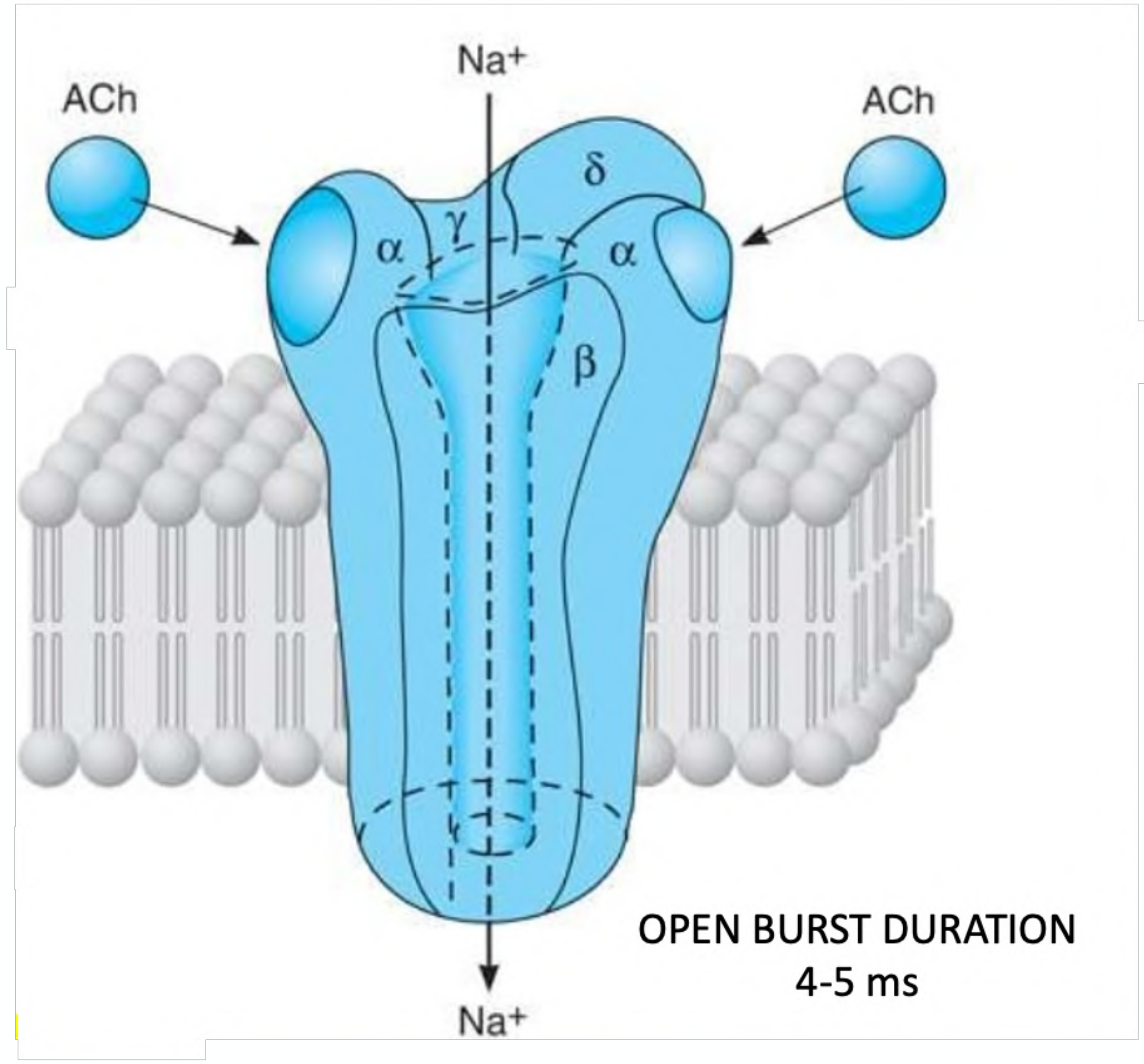
Difference between rodents and Humans



Jones et al., 2017, Cell Reports 21, 2348–2356

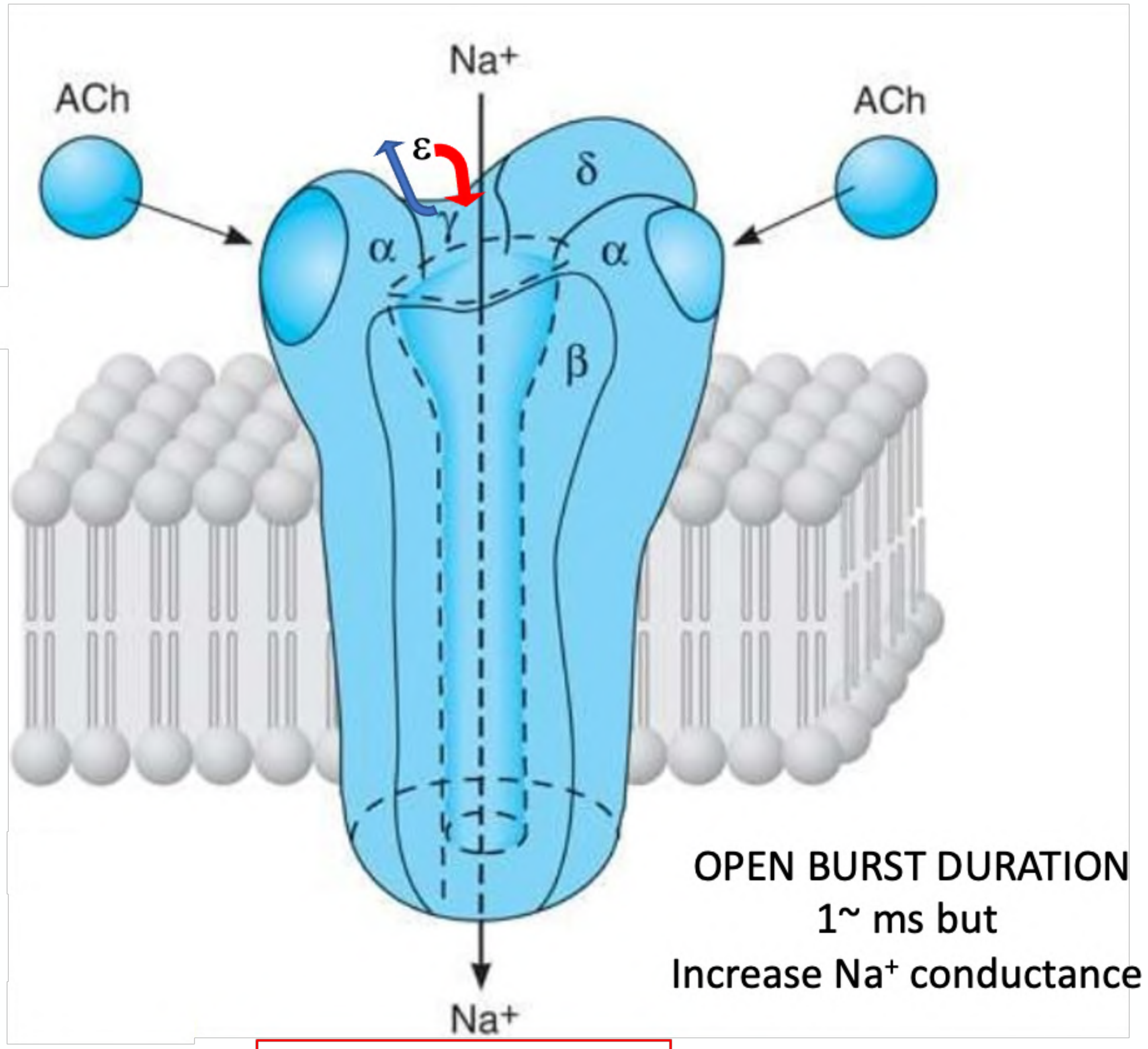
The NMJ : stage of development shift in nicotinic receptor subunits

Fetal



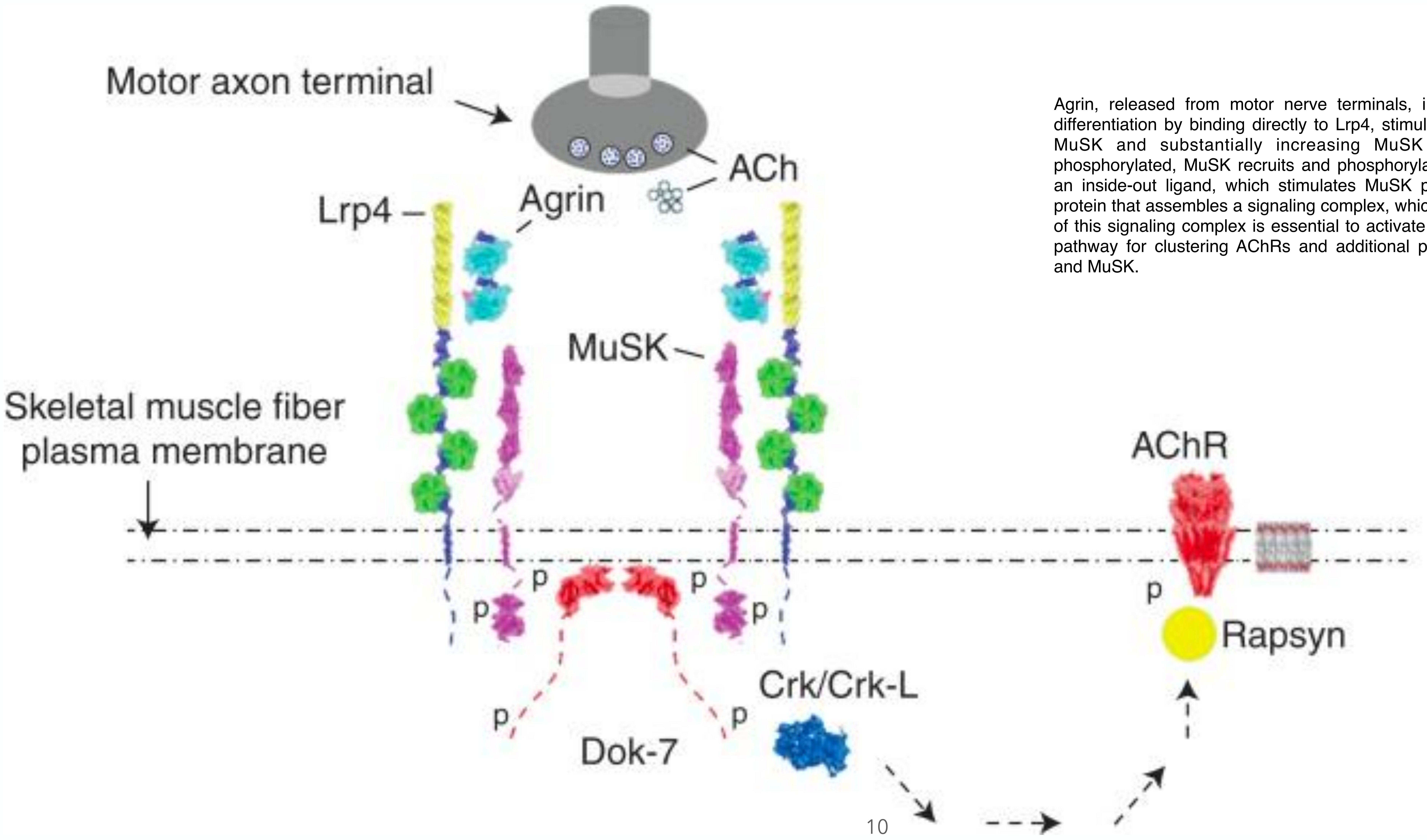
Stochiometry α2βγδ

Adult



Stochiometry α2βεδ

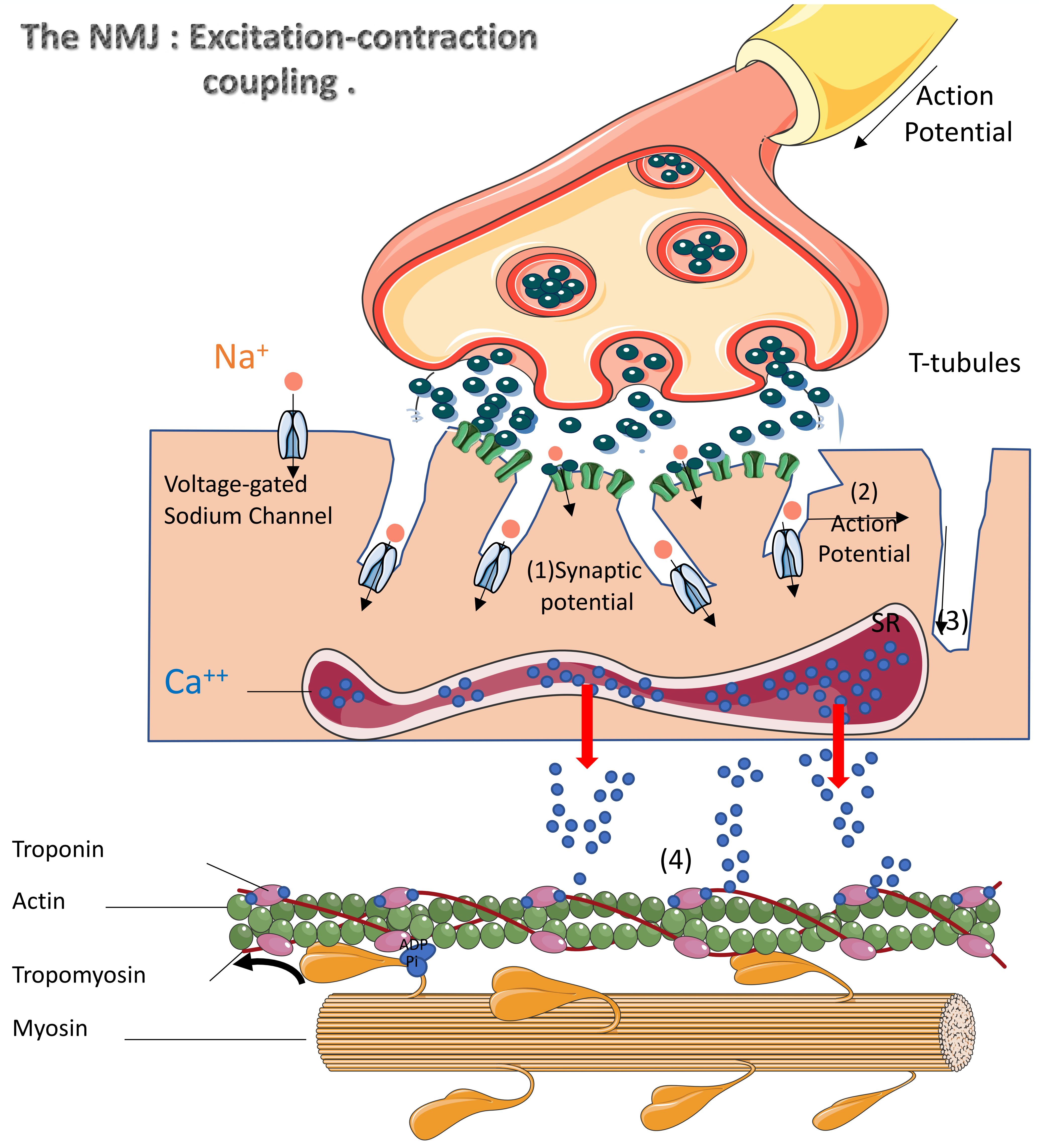
NMJ is extremely complex with elaborate cross talk between the neuron and the muscle



Agrin, released from motor nerve terminals, induces and stabilizes postsynaptic differentiation by binding directly to Lrp4, stimulating association between Lrp4 and MuSK and substantially increasing MuSK phosphorylation. Once tyrosine phosphorylated, MuSK recruits and phosphorylates Dok-7. Dok-7 functions both as an inside-out ligand, which stimulates MuSK phosphorylation, and as an adapter protein that assembles a signaling complex, which includes Crk and Crk-L. Formation of this signaling complex is essential to activate a Rac/Rho- and Rapsyn-dependent pathway for clustering AChRs and additional postsynaptic proteins, including Lrp4 and MuSK.

From Burden Lab Research

The NMJ : Excitation-contraction coupling .



From

> Electrical

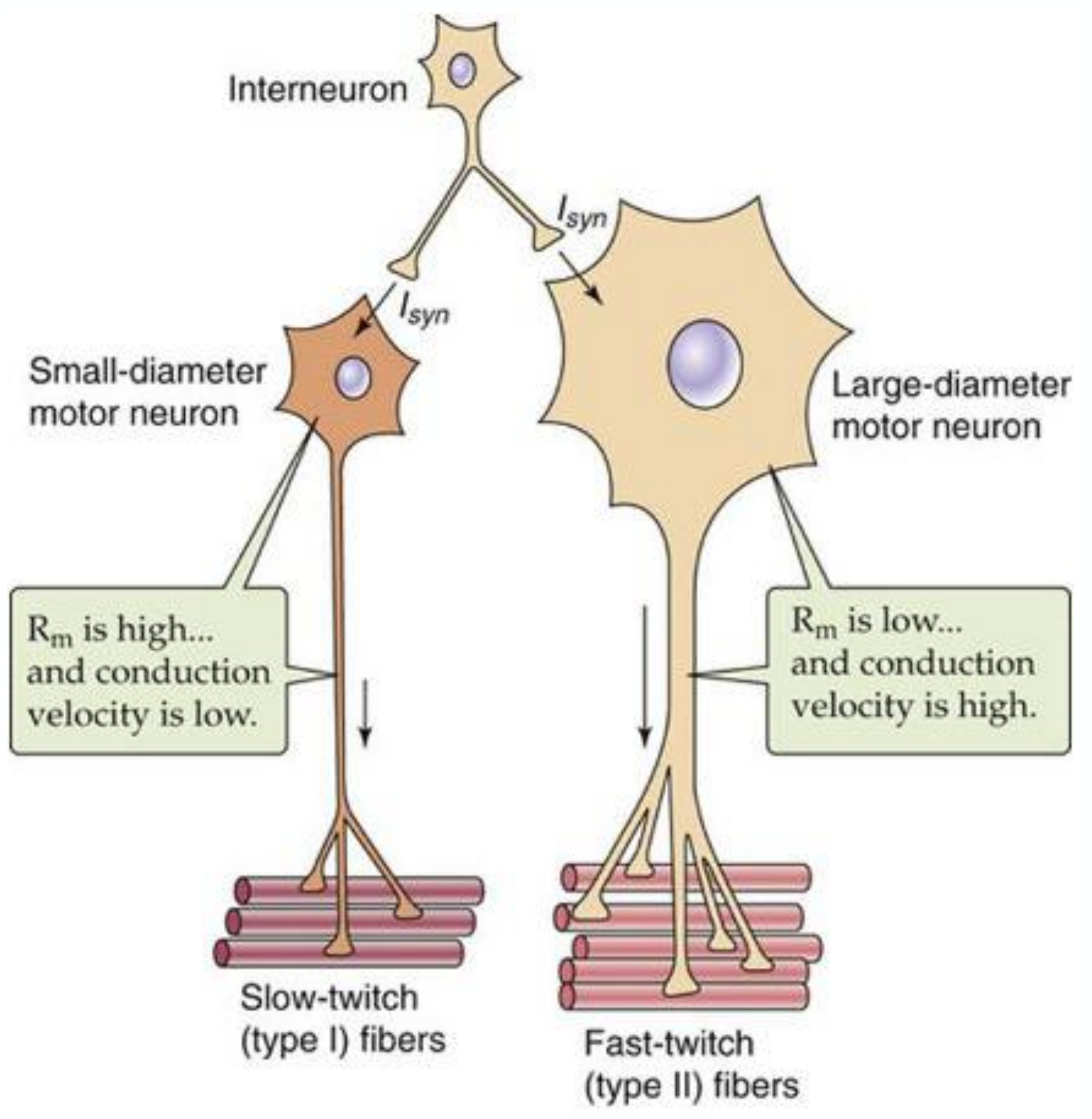
> Mechanical

CONTRACTION

Adapted from Wenxuan and Chakkalakal: Current topics in development Biology, vol 126

NMJ : a matter of « slow » and « fast » fibers

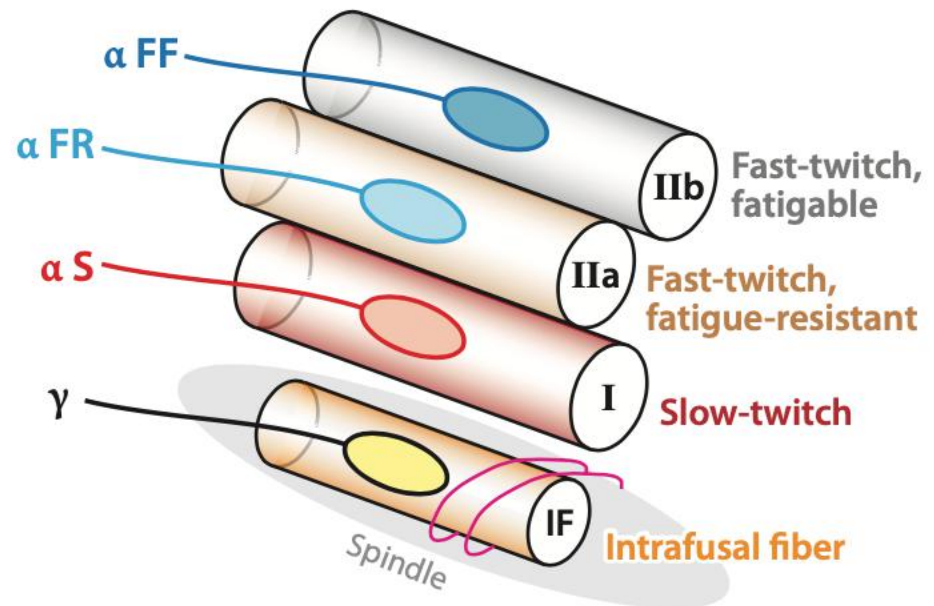
Type of fibers	I	IIA	IIX	IIB
Contraction	slow	Fast		
MyHC	I	IIa	IIX	IIB
ATPasic Activity	weak	Strong		
Metabolism	oxidative	Oxidative-Glycolic		Glycolic
Fatigue Resistance	***	**	*	*
Mitochondria numbers	***	**	*	*



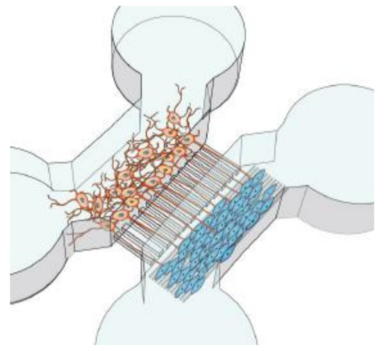
Adapted from Kandel et al., : Principles of Neural Science 4th ed. New York, Mc Graw-Hill, 2000

Type of alpha MNs	S	FR	FF
Axon conduction Velocity	Slower	Faster	Faster
size	Small	Big	Very Big
Excitability	High	Average	Low

Henneman's size principle describes relationships between properties of motor neurons and the muscle fibers they innervate and thus control, which together are called motor units. Motor neurons with large cell bodies tend to innervate fast-twitch, high-force, less fatigue-resistant muscle fibers, whereas motor neurons with small cell bodies tend to innervate slow-twitch, low-force, fatigue-resistant muscle fibers.



Annu. Rev. Neurosci. 2010.33 : 409-440



NMJ : From Rodents >>> Human Co-Culture

1972

Co-culture : adult rodent and human skeletal fibers – rodent spinal cord explants.

Démonstration of the needed of innervation for muscle regeneration

2010

Rat primary culture for MN and muscle

Demonstration of NMJ formation by IF but not functional evidences

2014

Stem cell from mouse (mESC) with primary chick myotubes

Comparison with primary models and functional test with recording of NMJ Action Potential

2010

NMJ :h spinal cord stem cells derived MN and rat skeletal muscle

IF : Yes

Functional No

2011

NMJ :h spinal cord stem cells derived MN and human skeletal muscle

IF yes

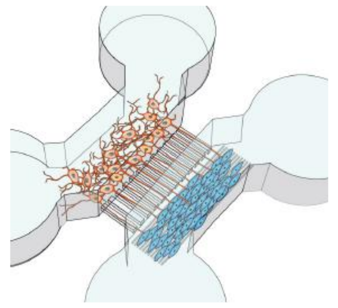
Functional : recording muscle contraction following agonists

2015

hNMJ from iPSC

IF : Yes

Functional No



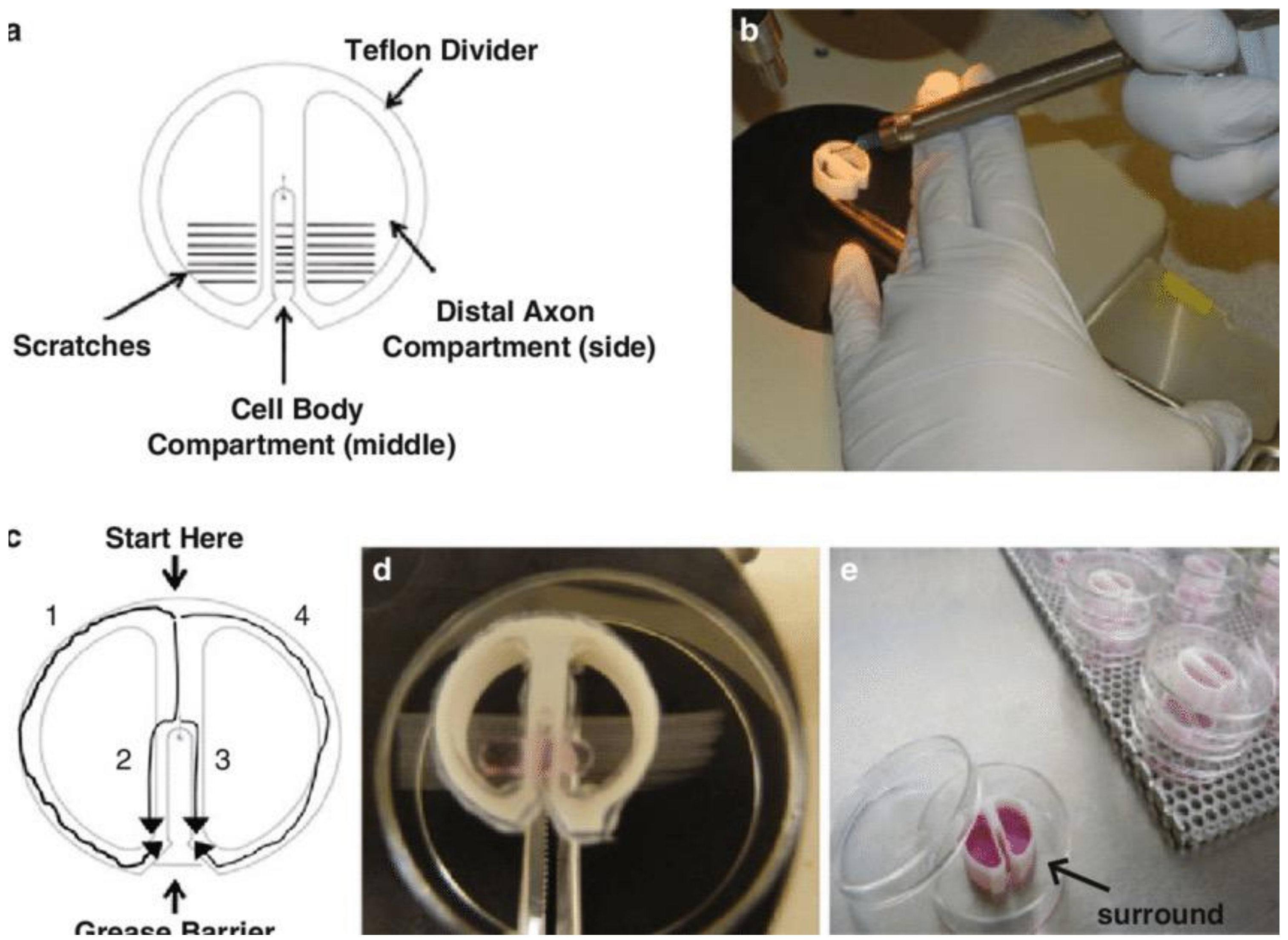
COMPARTIMENTALIZATION : From Teflon >>> PDMS

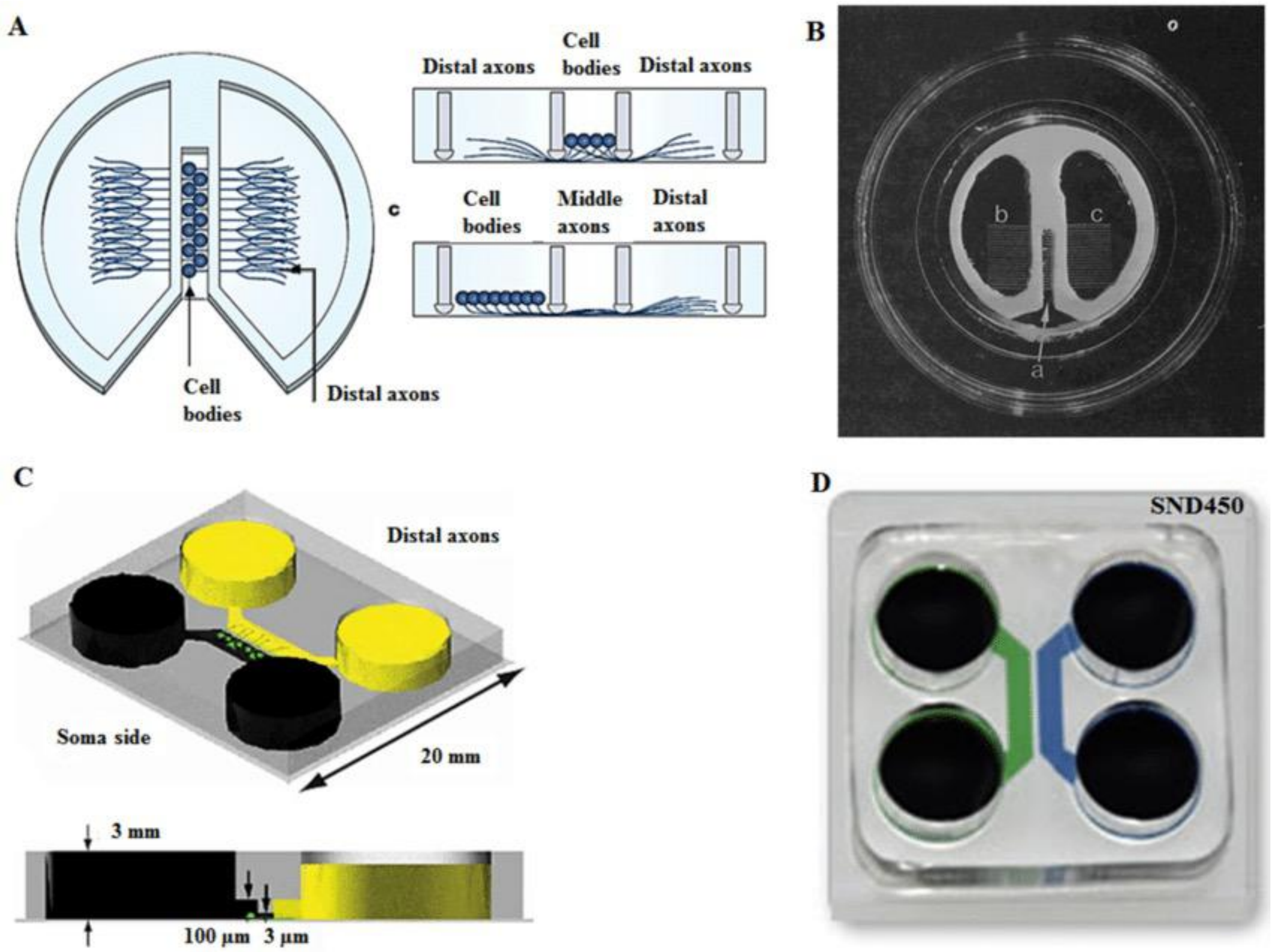
1977

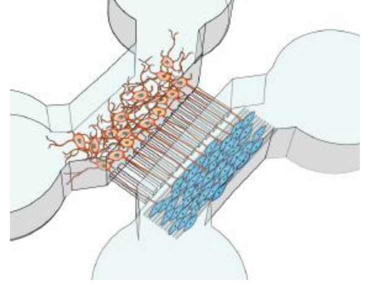
2013

Campenot Chambers

Microfabrication using PDMS
New design
CHIPS







NMJ : From Rodents >>> Human Compartimentalized

2013

First model of rNMJ on Chip

Physiology relevance

2015

First model of rNMJ on Chip

Biological relevance

2018

First model of hNMJ on Chip

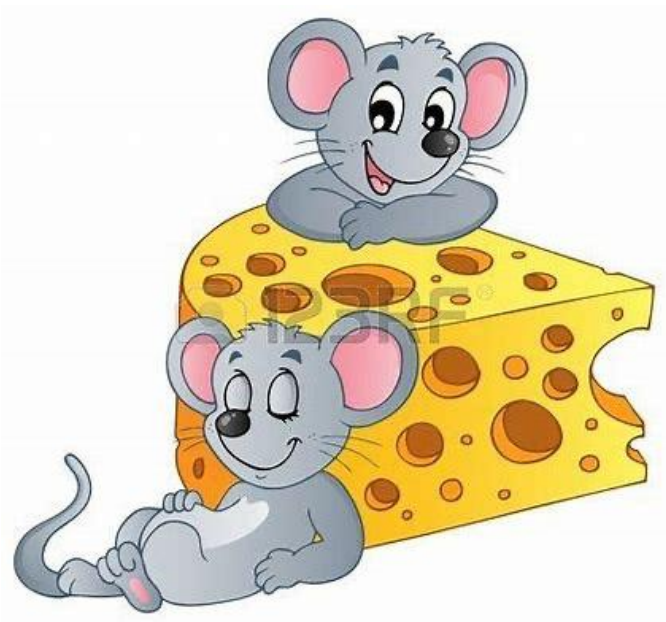
Action Potential induction and contraction recording

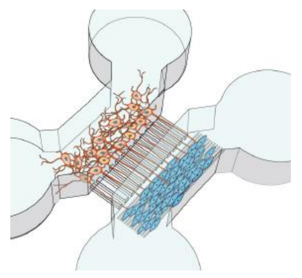
2021

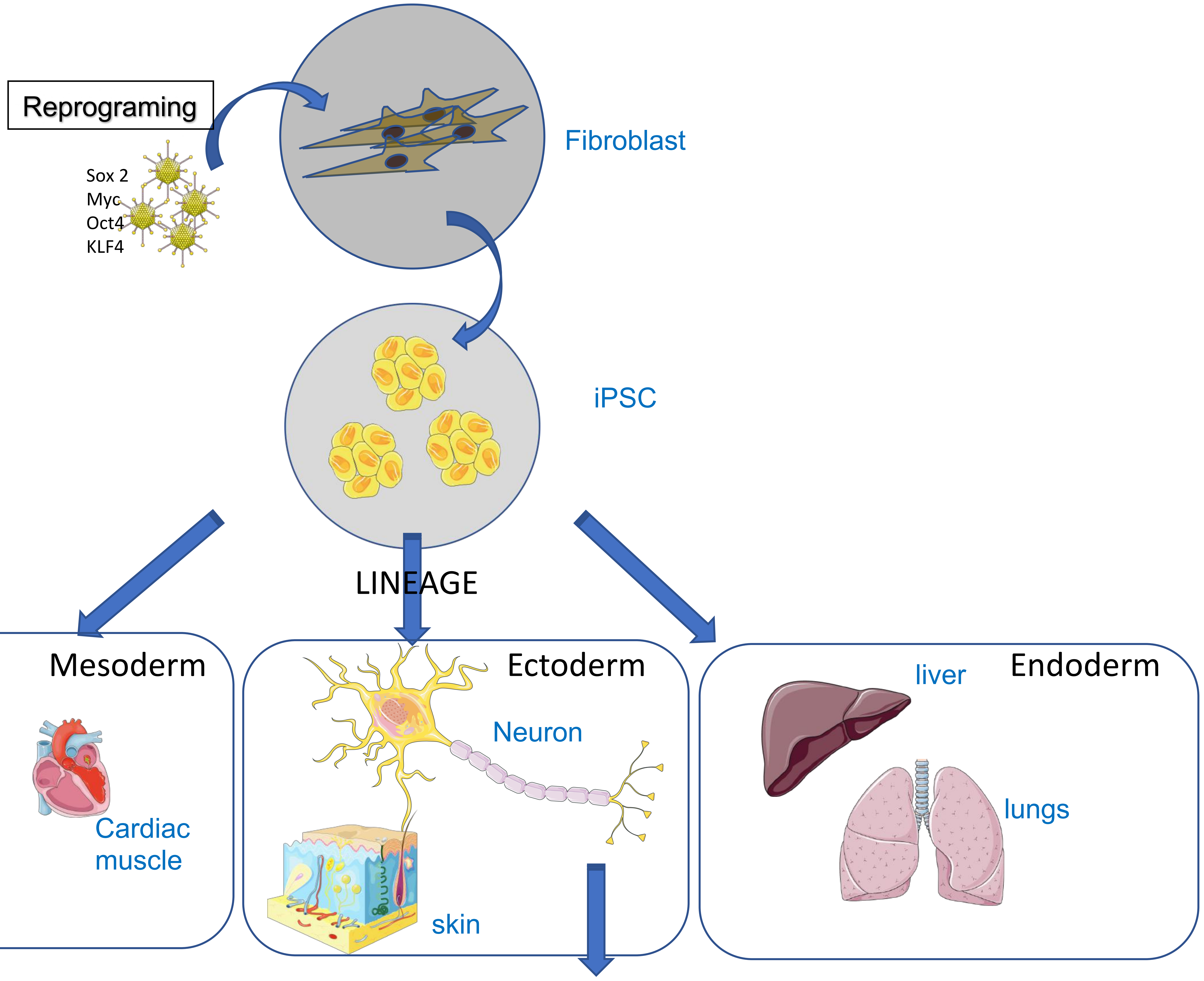
hNMJ on Chip

Action Potential induction and muscle Action Potential recording

MEA







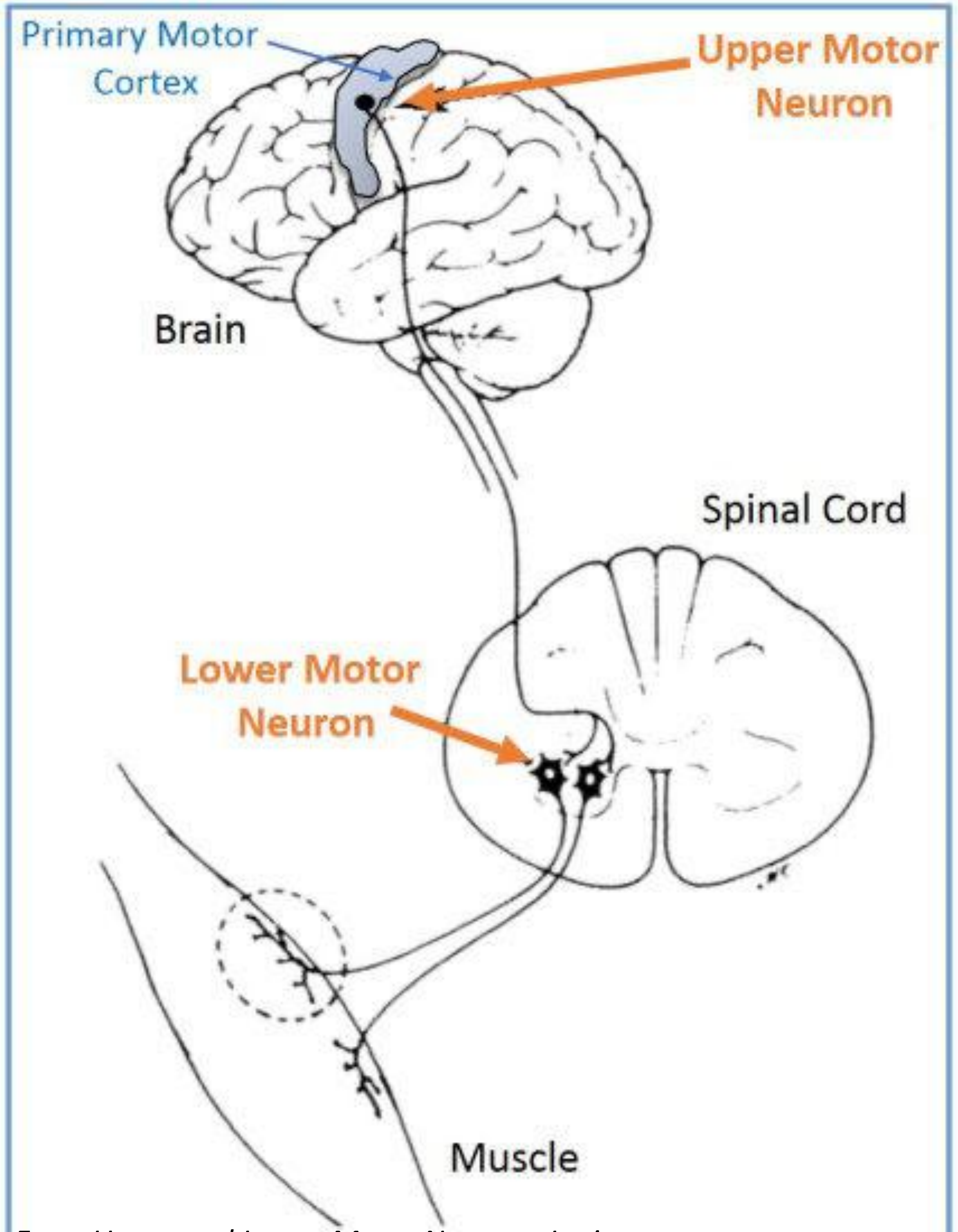
Recapitulate Neurodegenerative diseases

Motoneuron Diseases

- ✓ Progressive loss/degeneration of motoneurons
  - o Weakness
  - o Atrophy
  - o No sensory or autonomic symptoms

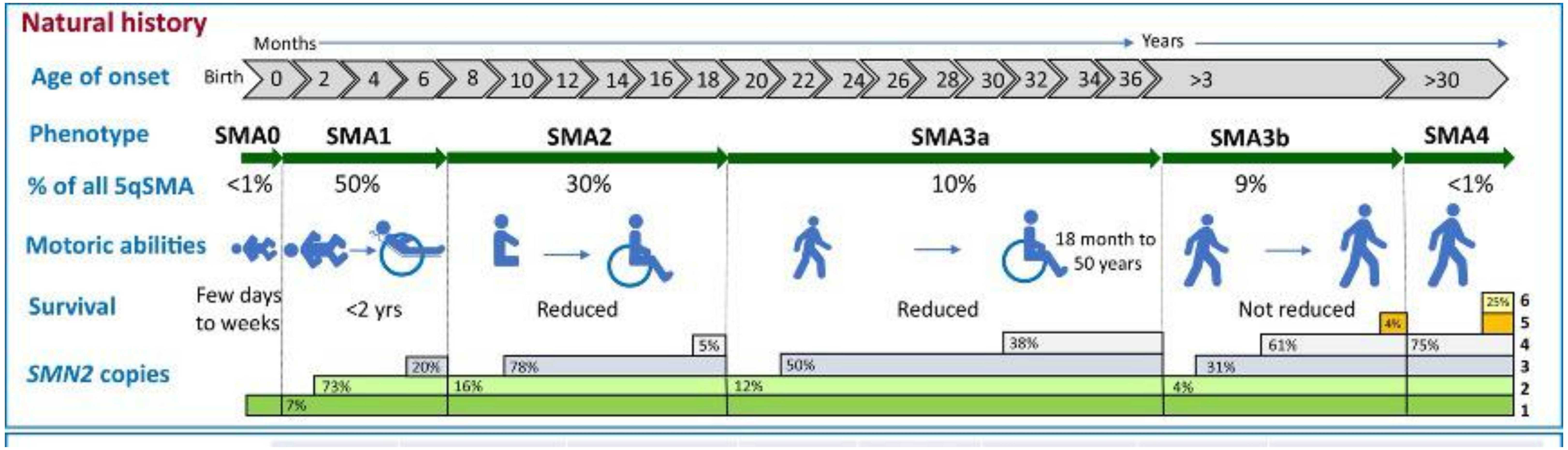
- ✓ Two Major Types
  - o Amyotrophic Latereral Sclerosis (ALS) both upper and lower MNs affected
  - o Spinal Muscular Atrophy (SMA) Lower MNs syndromes only

- ✓ ALS : 90% Sporadic-10% genetic
- ✓ SMA: 100% genetic

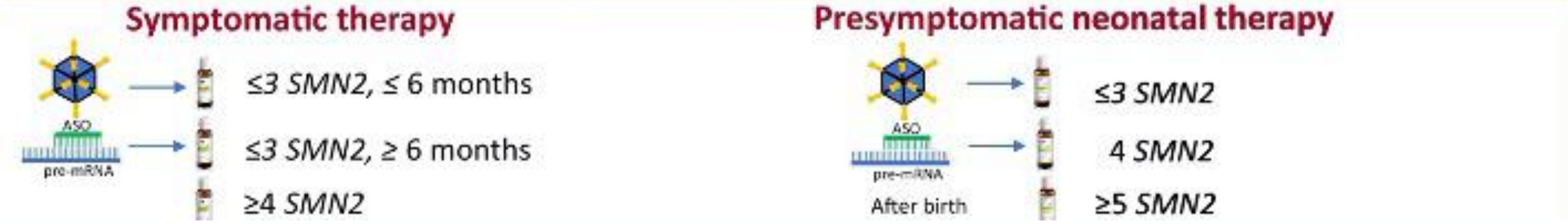


From Upper and Lower Motor Neuron - Lesions

SMA

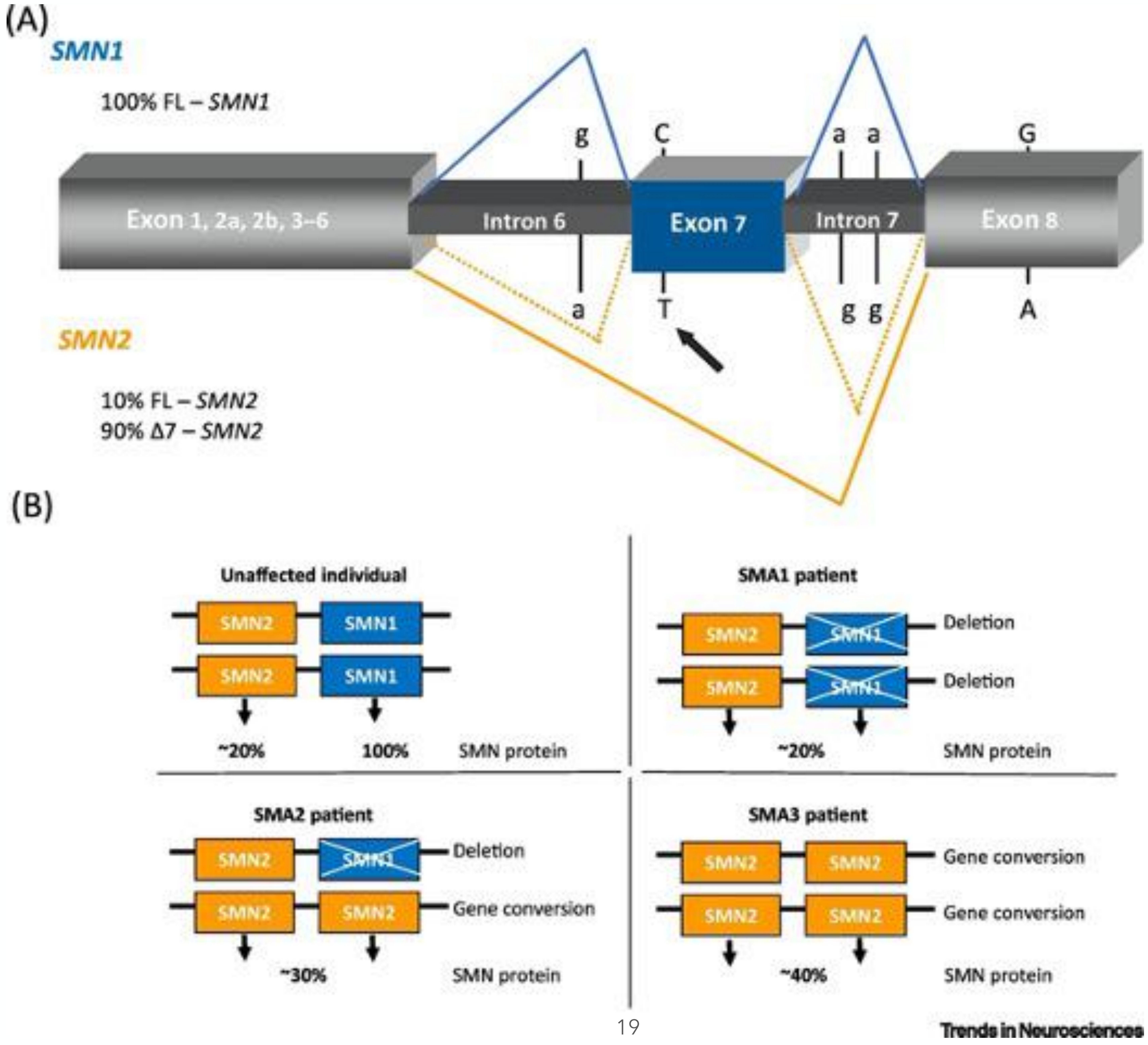


Therapy	Therapy	Type	Administration	SMA type	SMN2 copies	Age	Weight (kg)	Dosing
CNS only	Spinraza	ASO	Intrathecal	All	All	No limitation		5x 1st year; 3x/year lifetime
Systemic	Zolgensma	Gene therapy	Intravenous	All	≤3 in EU	<2 years in US	13.5 US; 21 EU	1x
	Evrysdi	Small molecule	Oral	All	All	> 2 months		Daily, lifetime

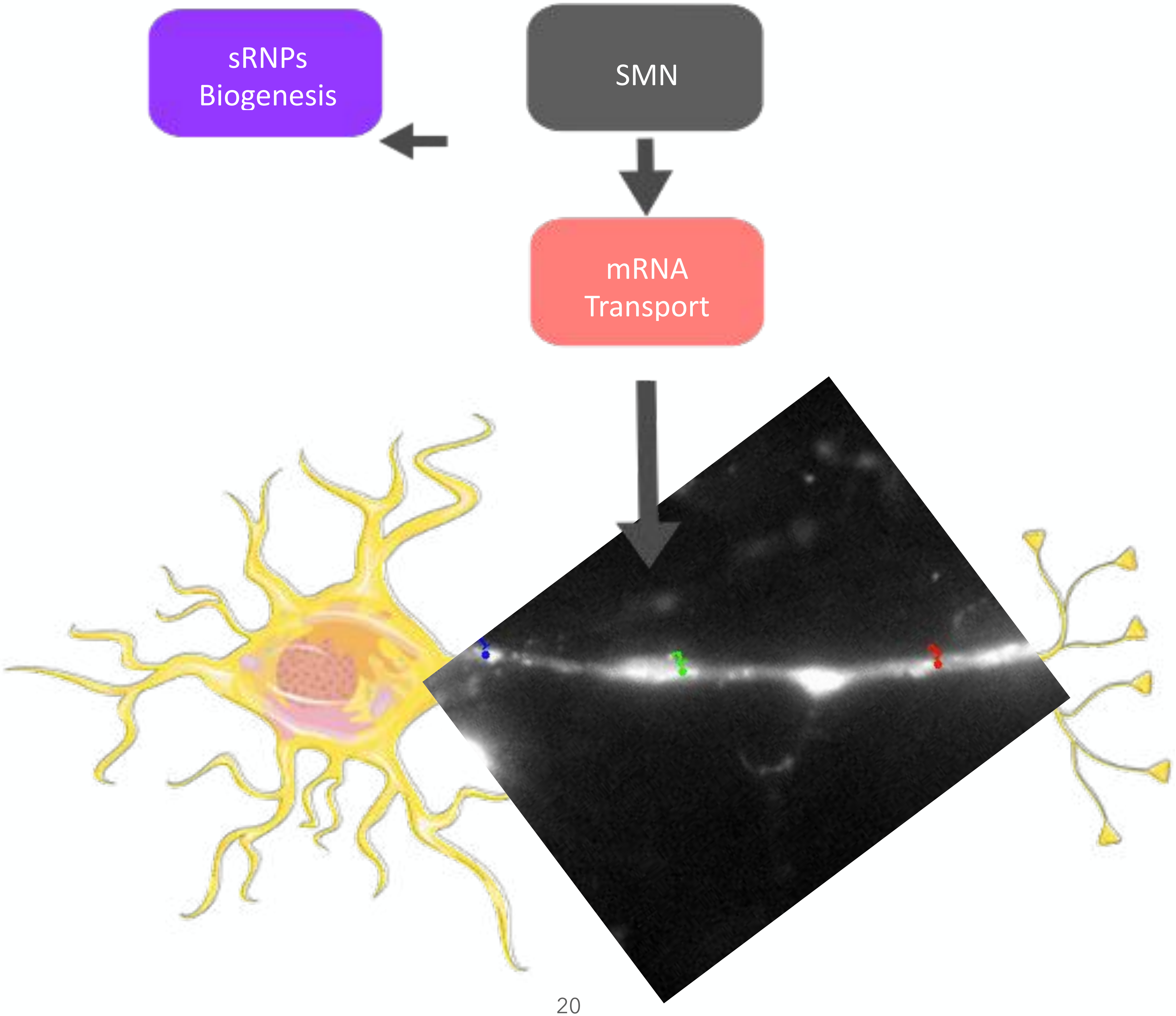


Wirth B. Trends Neurosci. 2021 Apr;44(4):306-322.

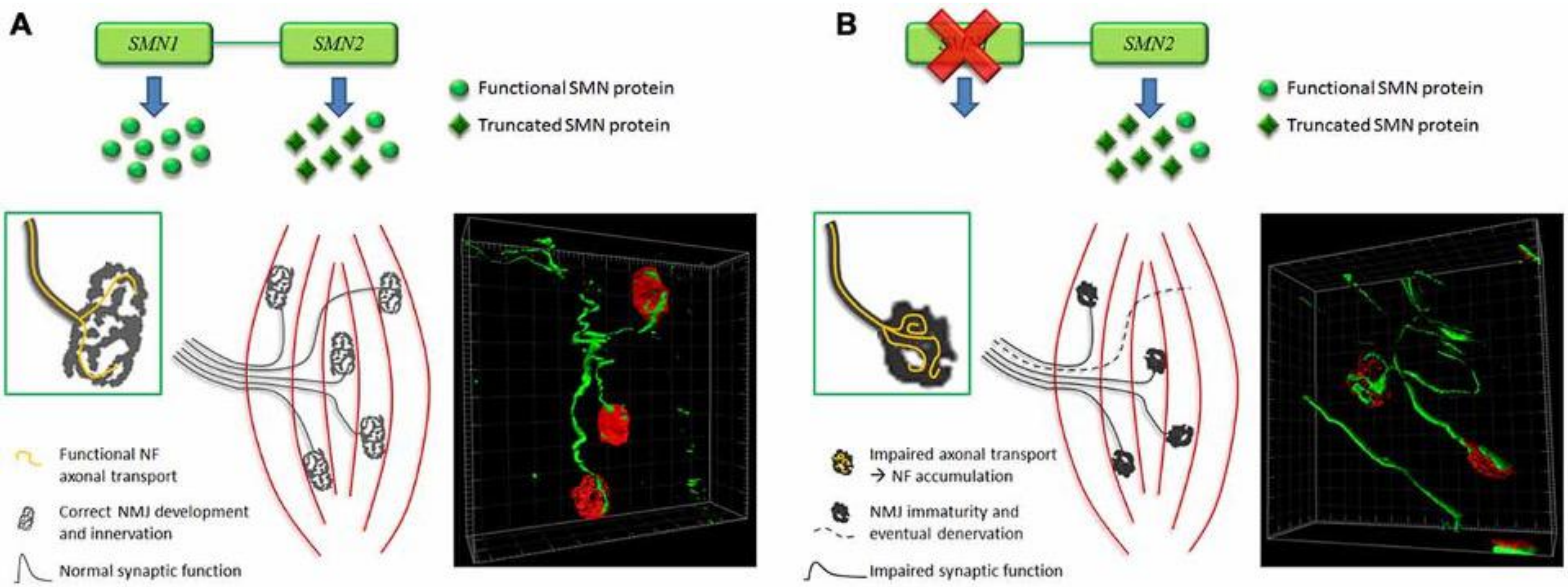
Genetic Causes of SMA



Role of SMN

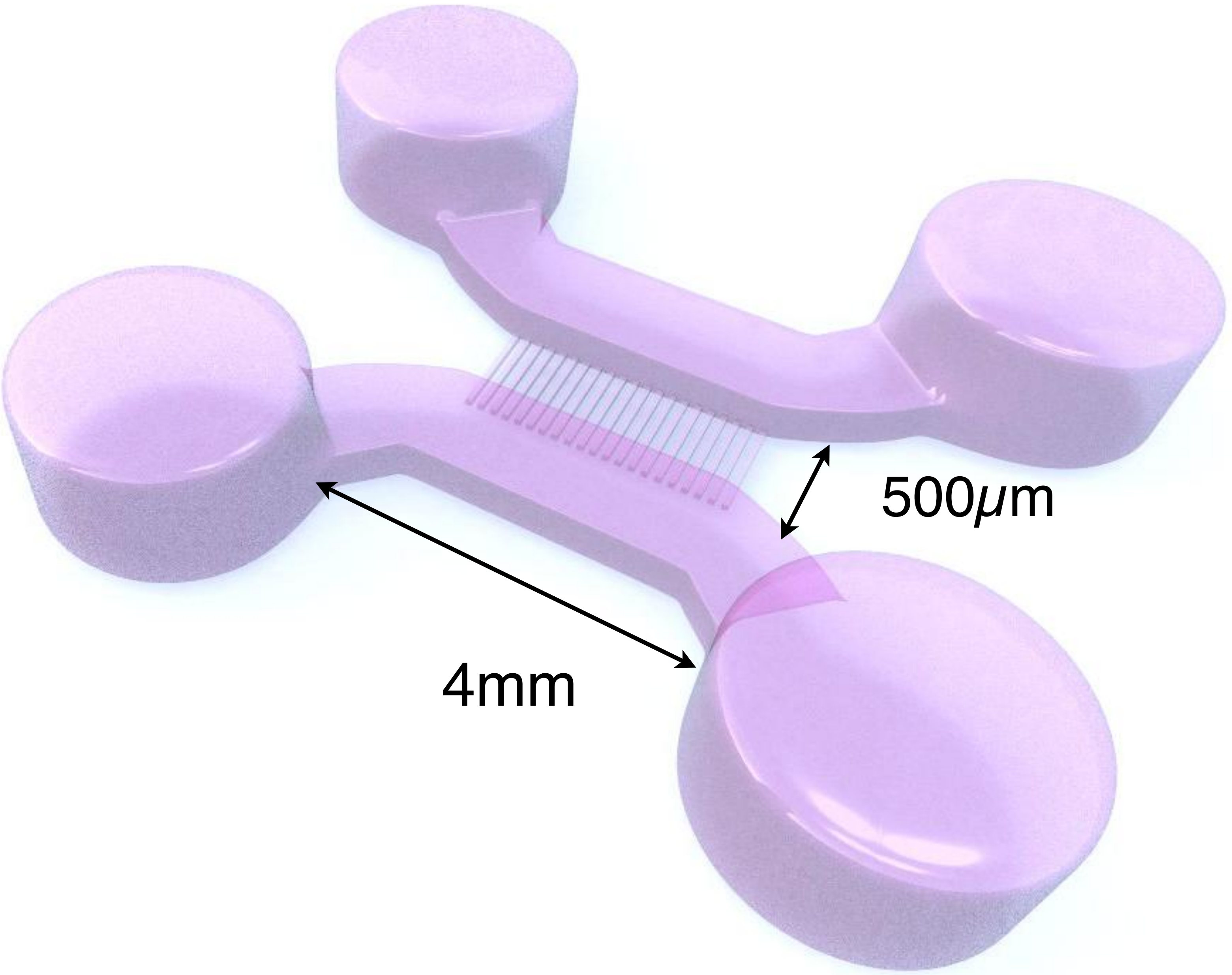


Impact of the lack of SMN on the NMJ



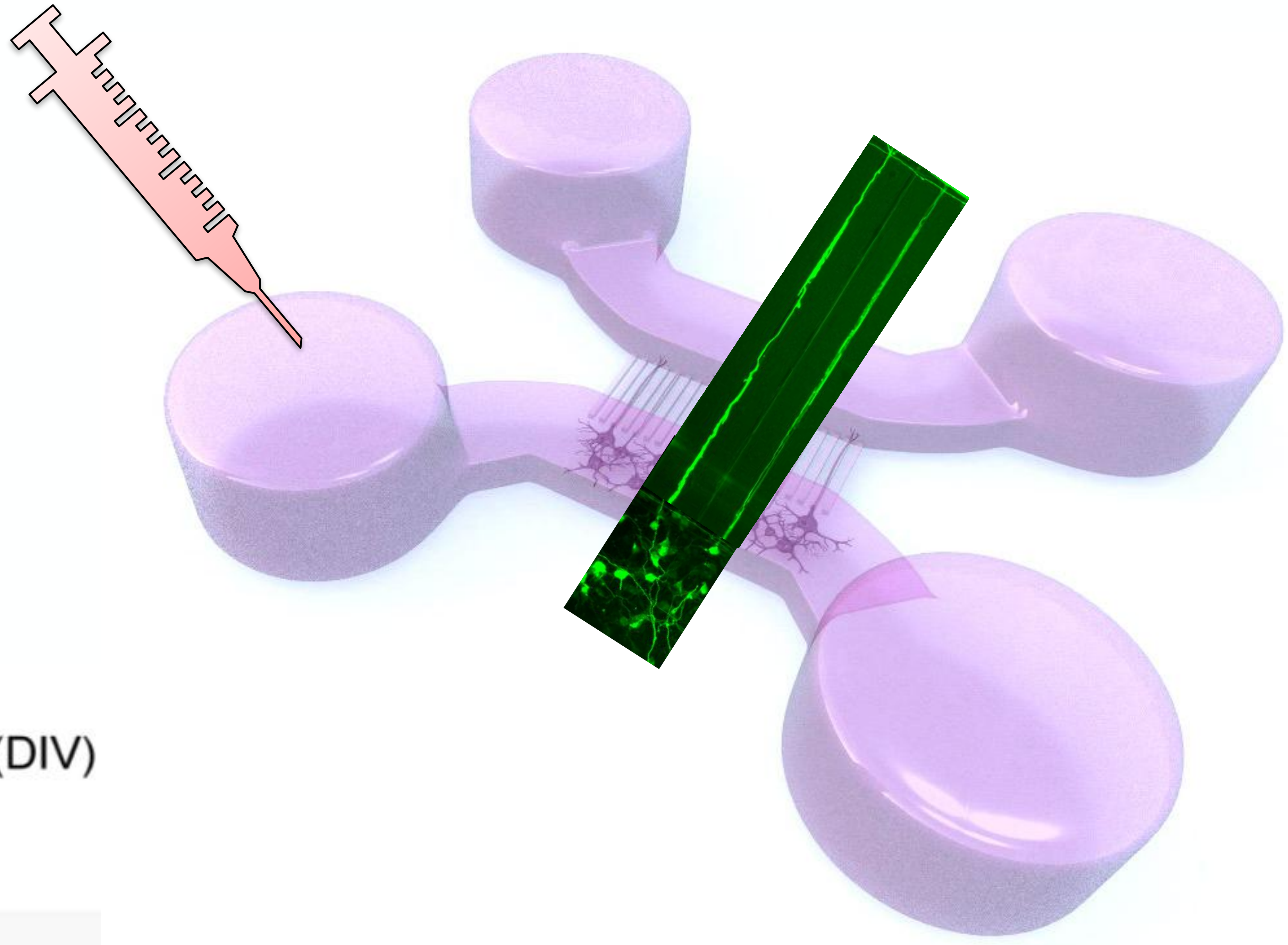
Front. Neuroanat., 03 February 2016

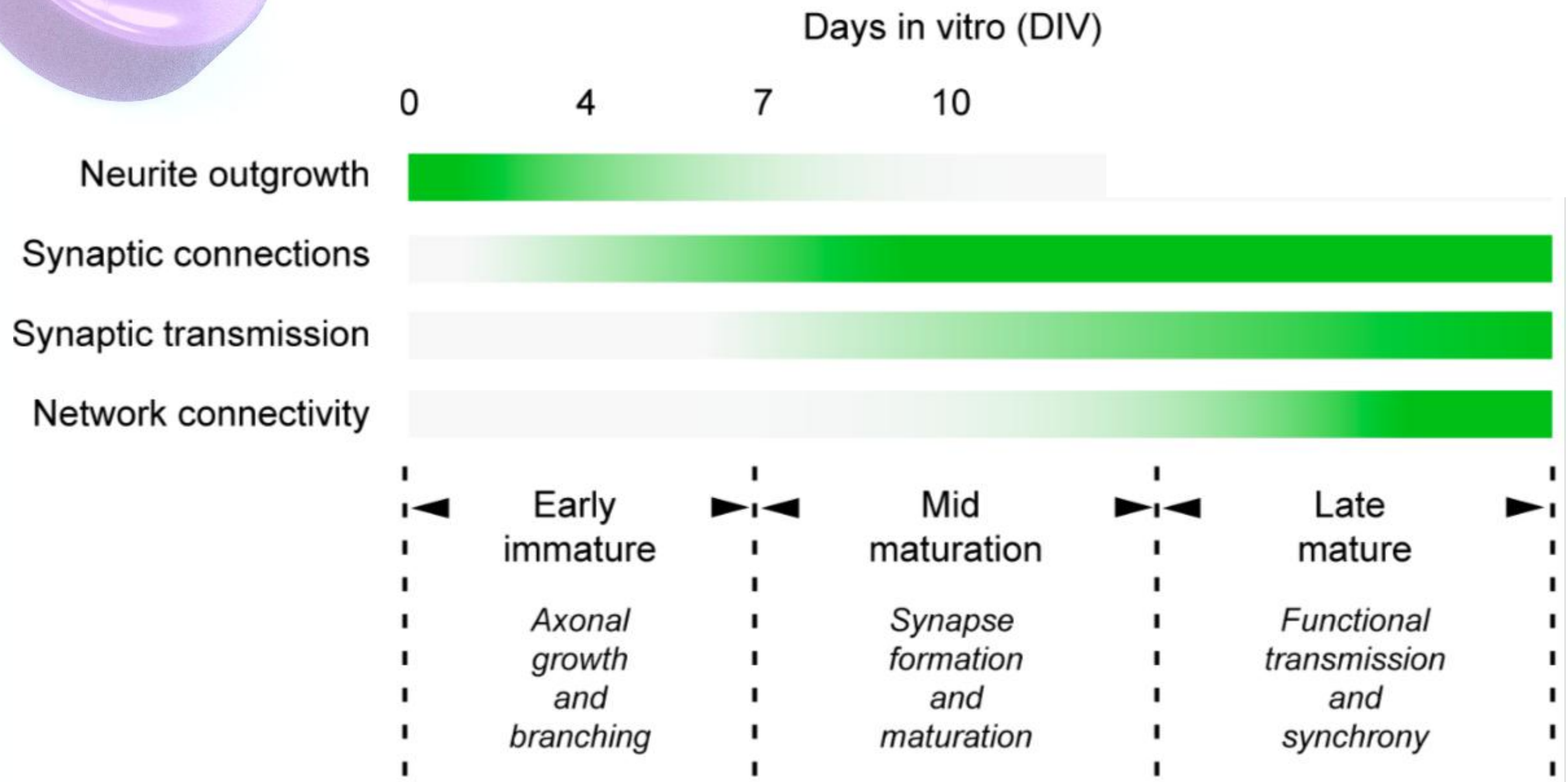
Microfluidics SAGA

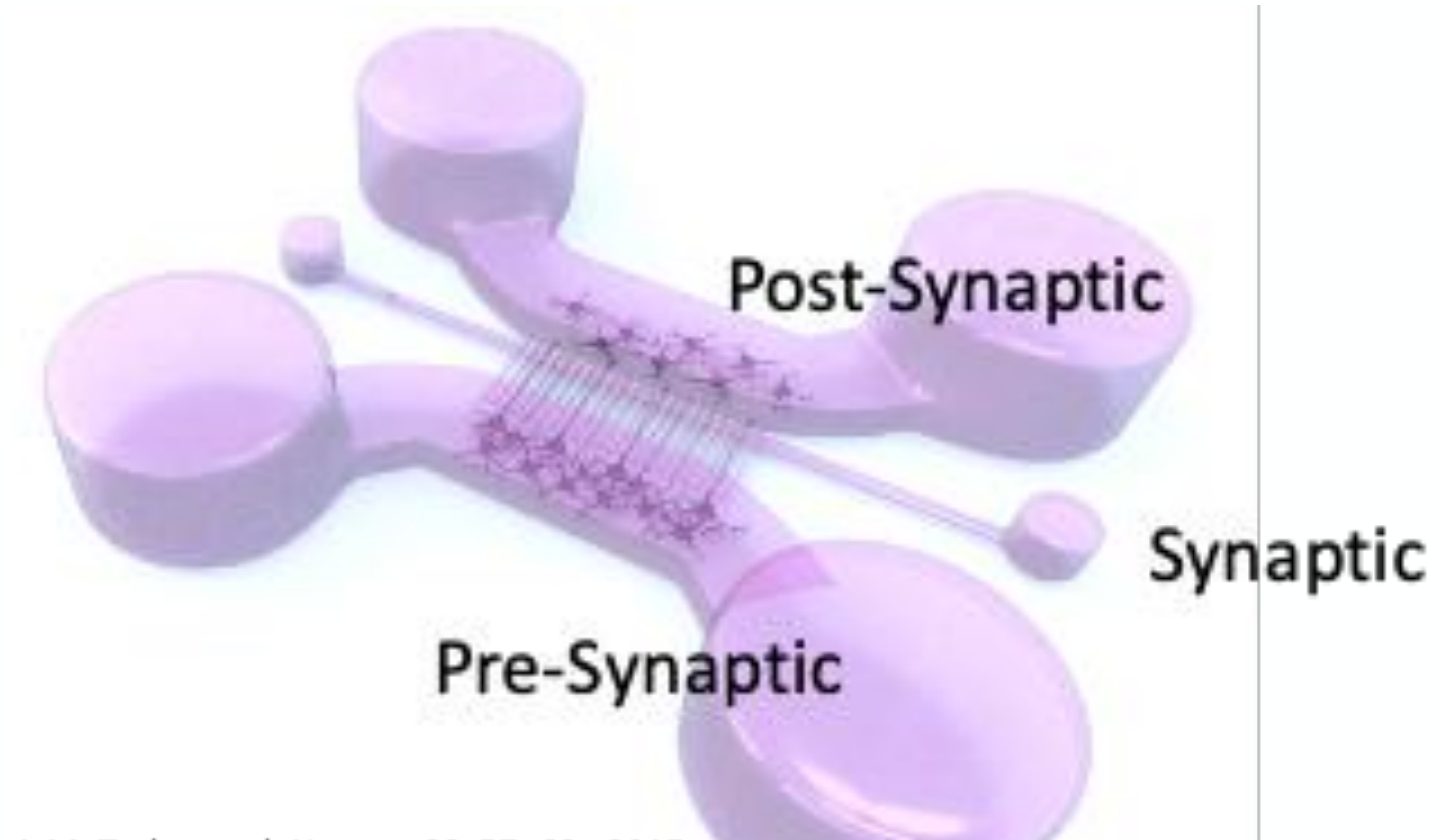


A.M.Taylor et al. Langmuir 19, 2003
A.M.Taylor et al. Nat. Methods 2, 2005

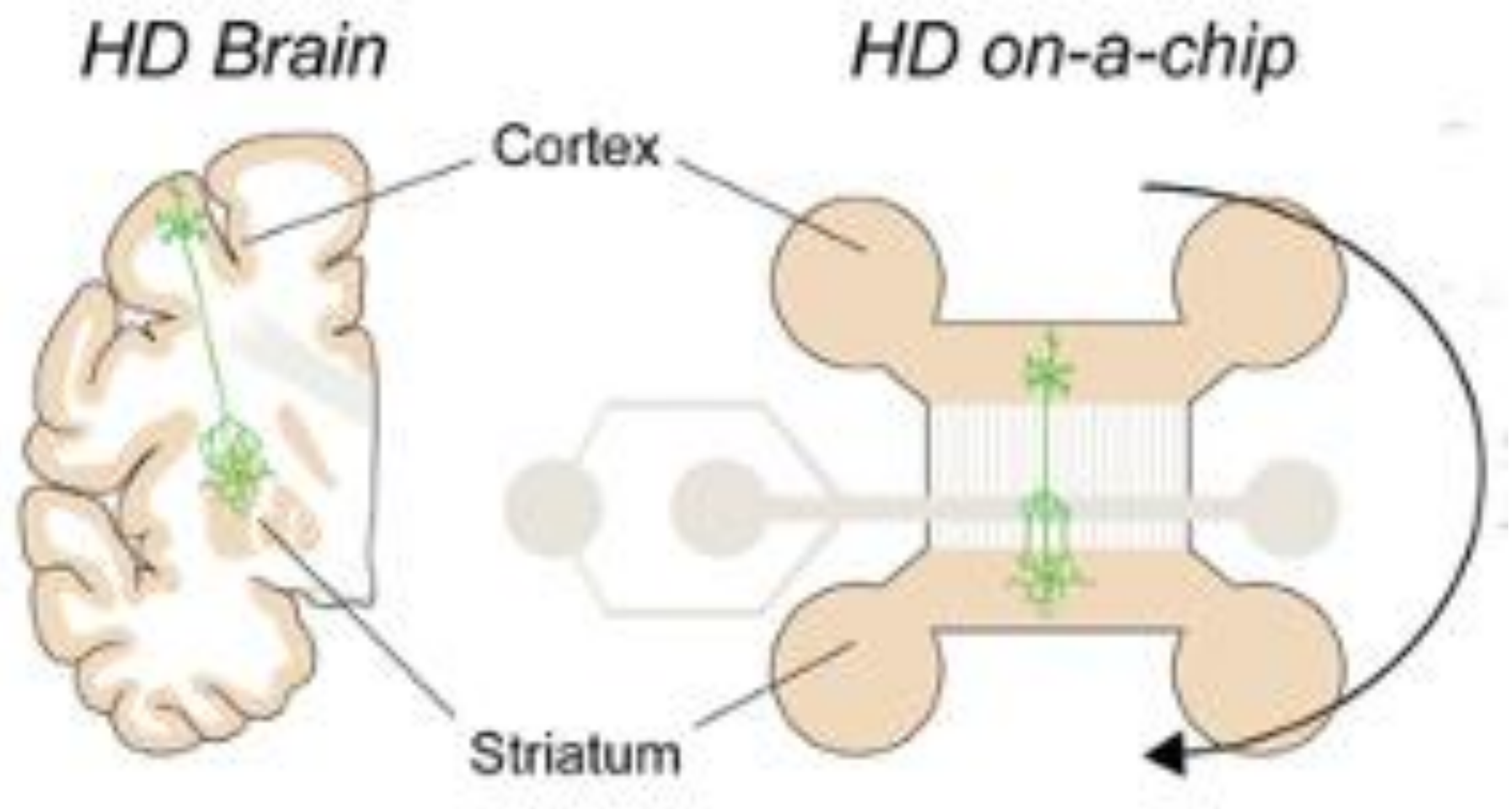
2 compartments



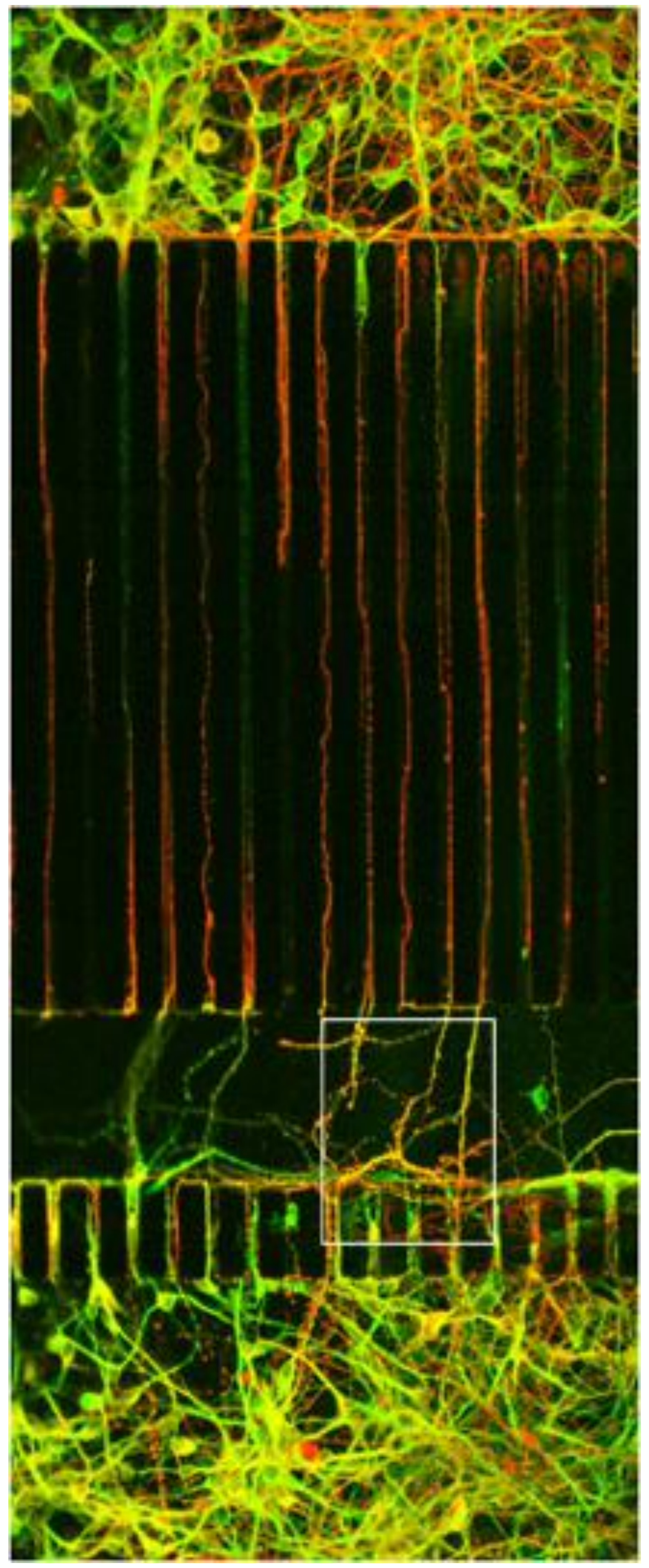




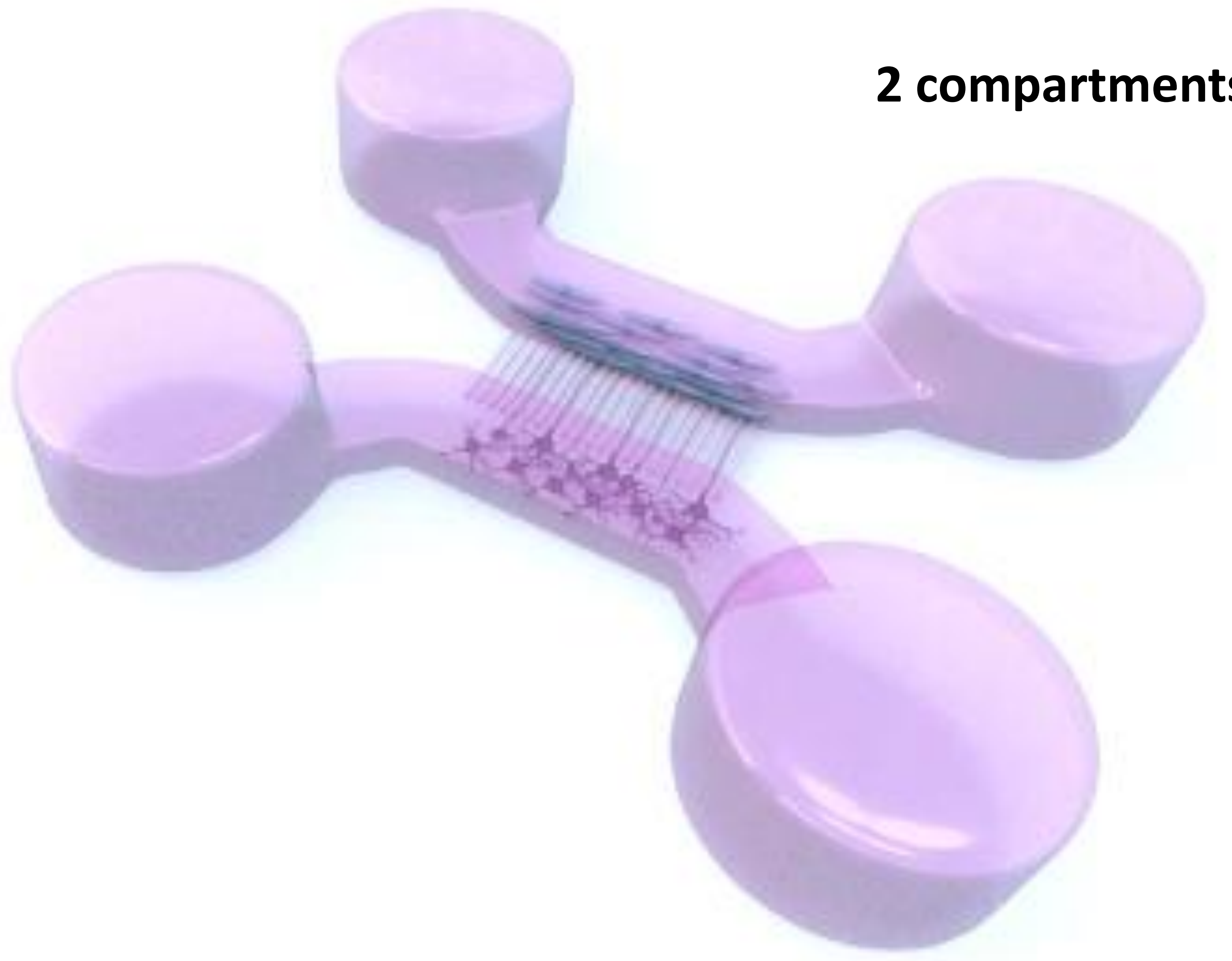
A.M. Taylor et al. Neuron 66, 57–68, 2015



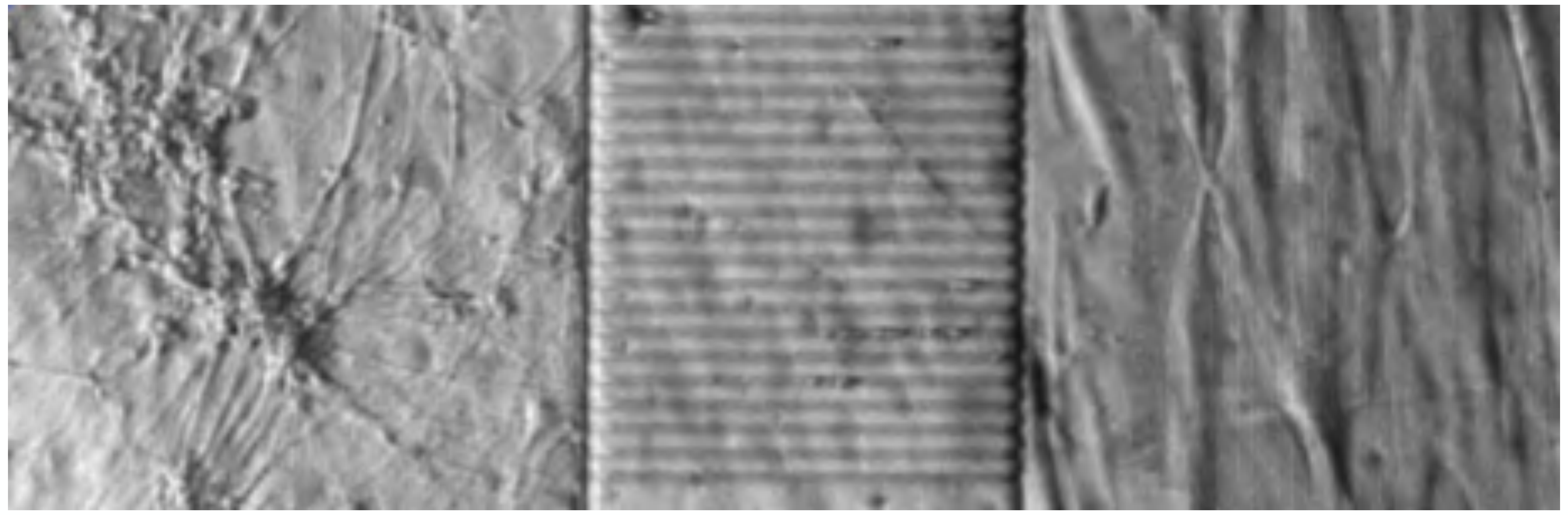
3 compartments



A.Virlogeux et al. Cell Reports 22-1 (2018)



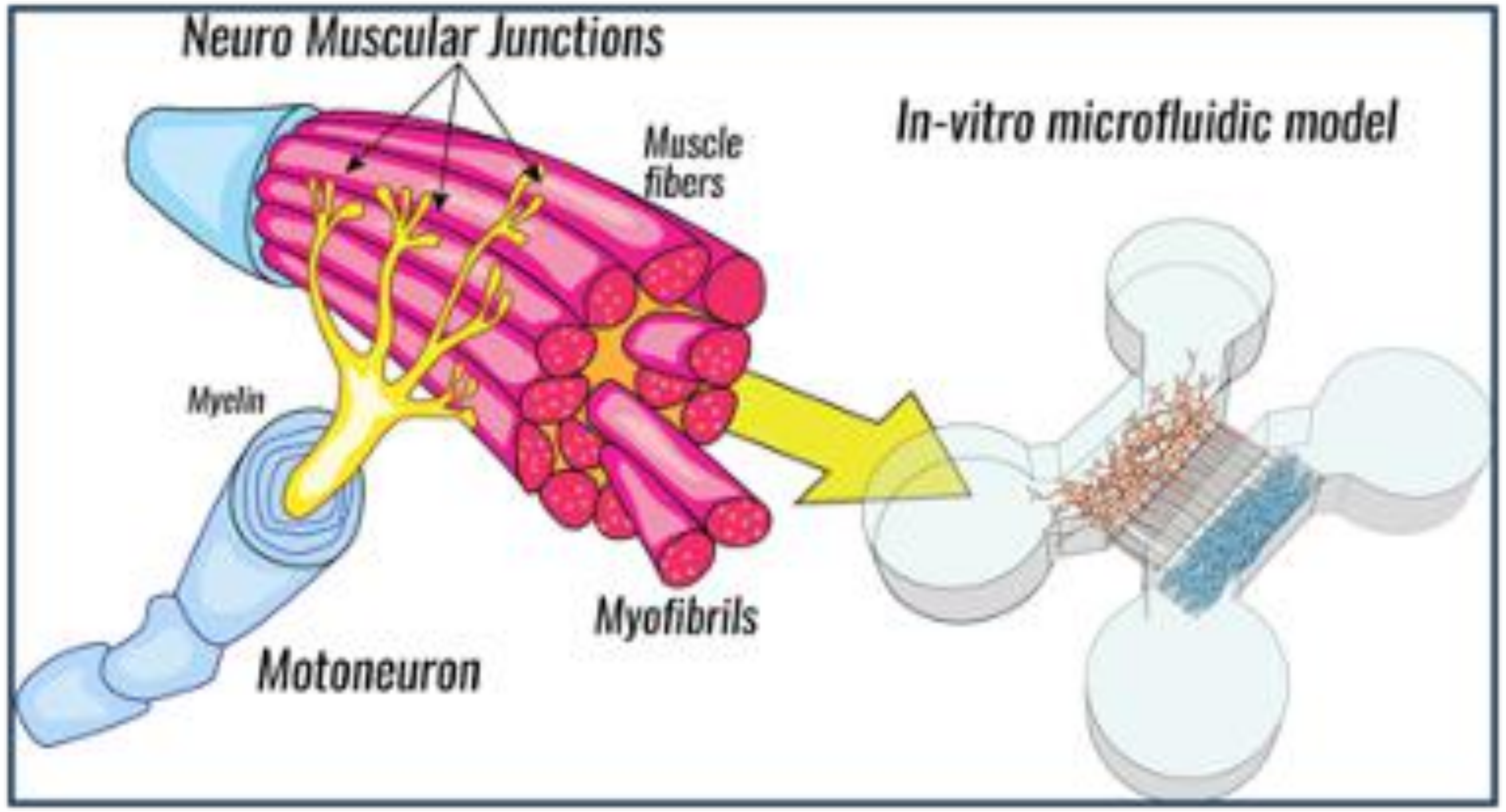
2 compartments and microstructuration

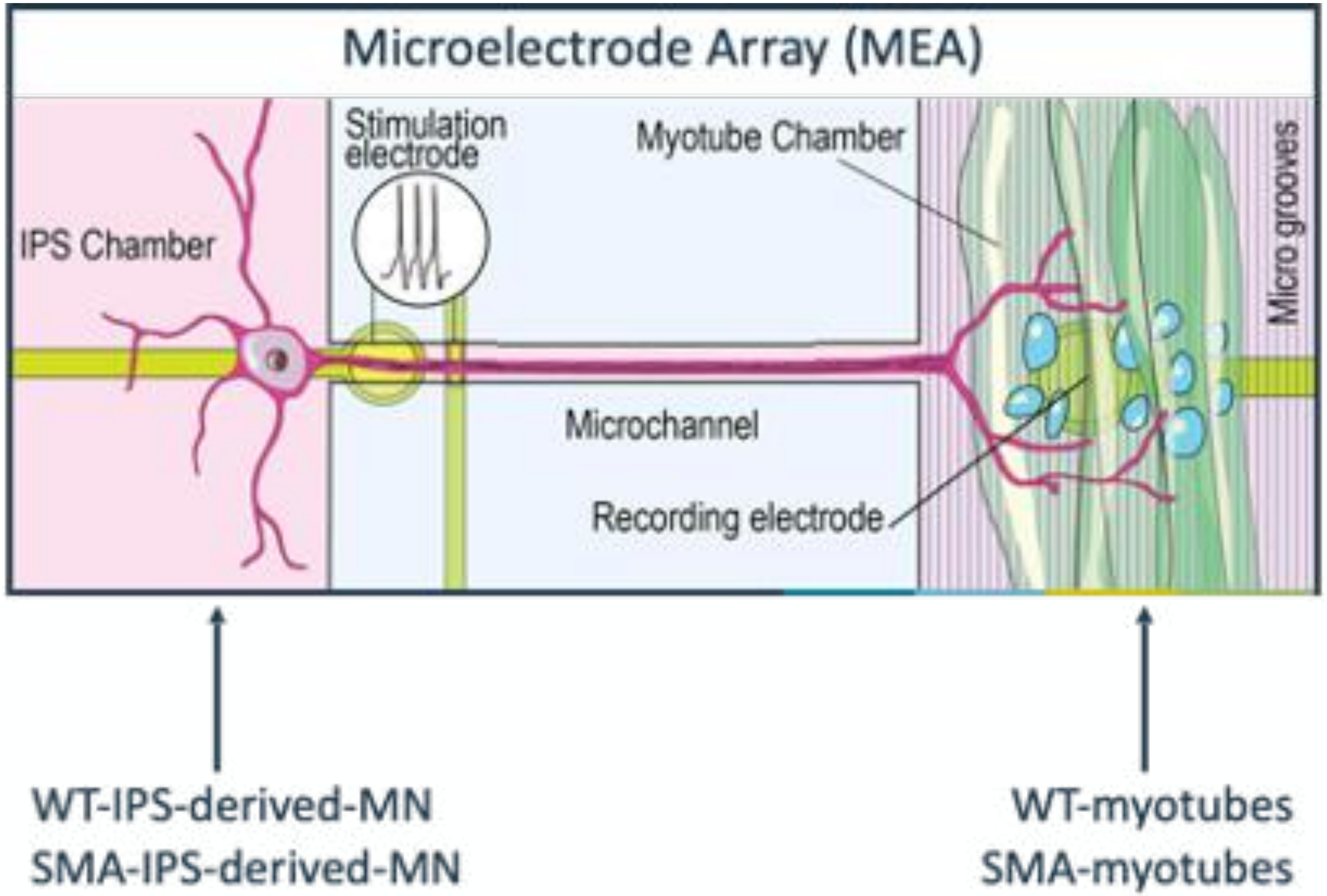


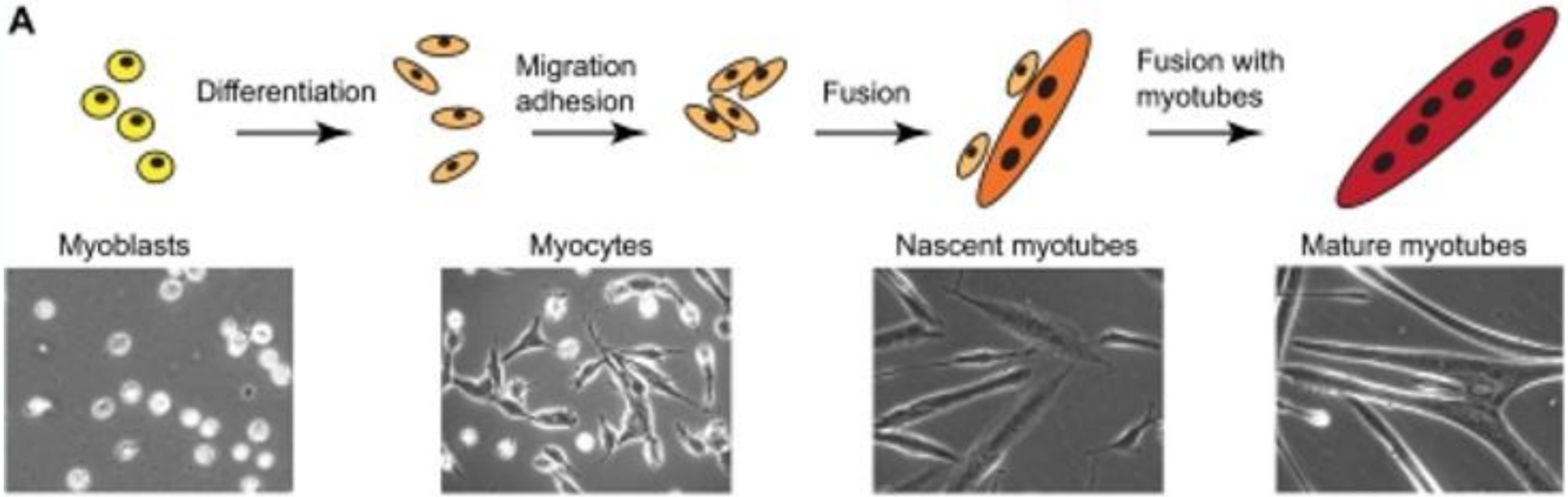
Motoneurons Axons extension Myotubes

Pauline Duc, IGMM
Benoit Charlot IES

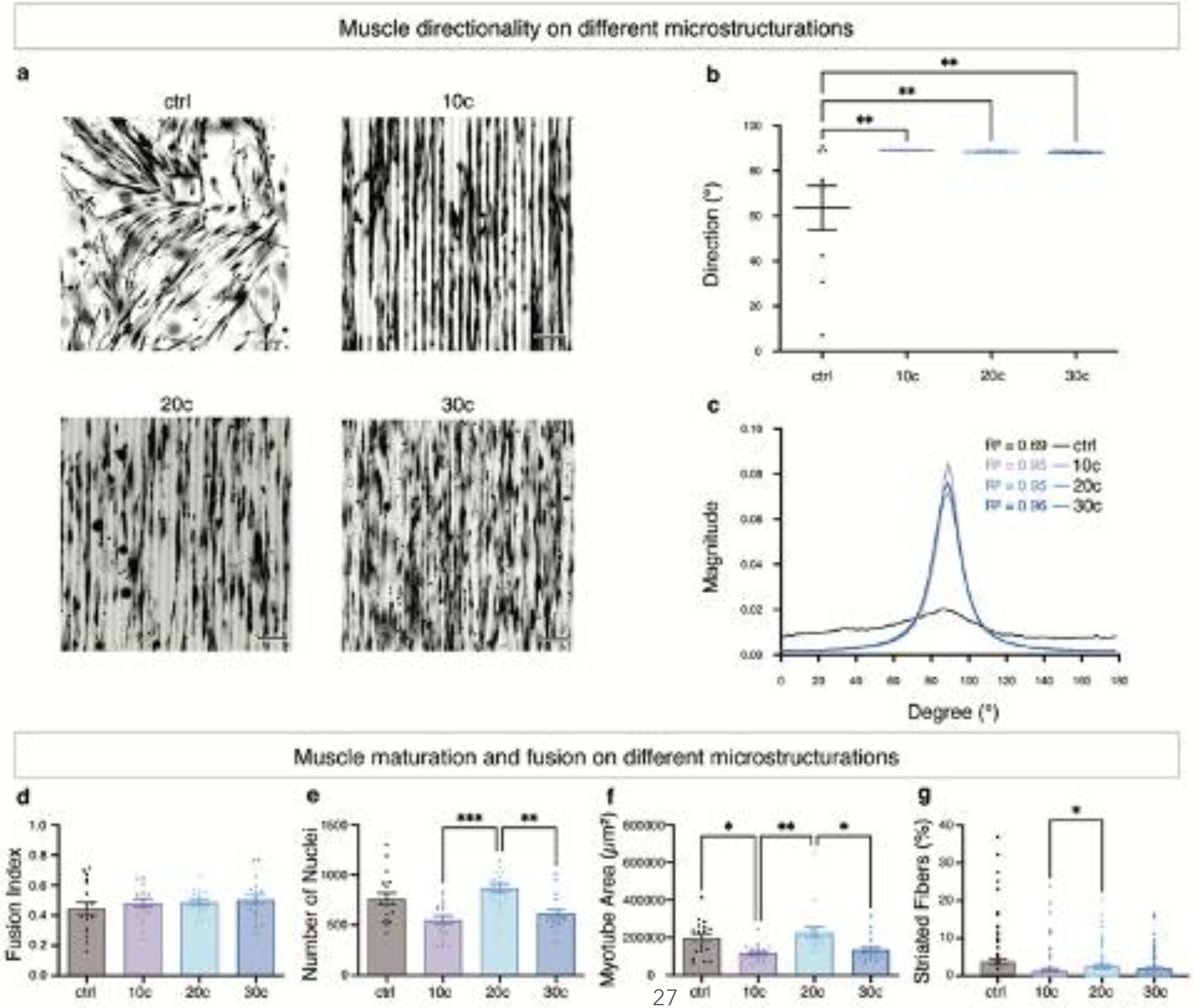
How to build a human NMJ





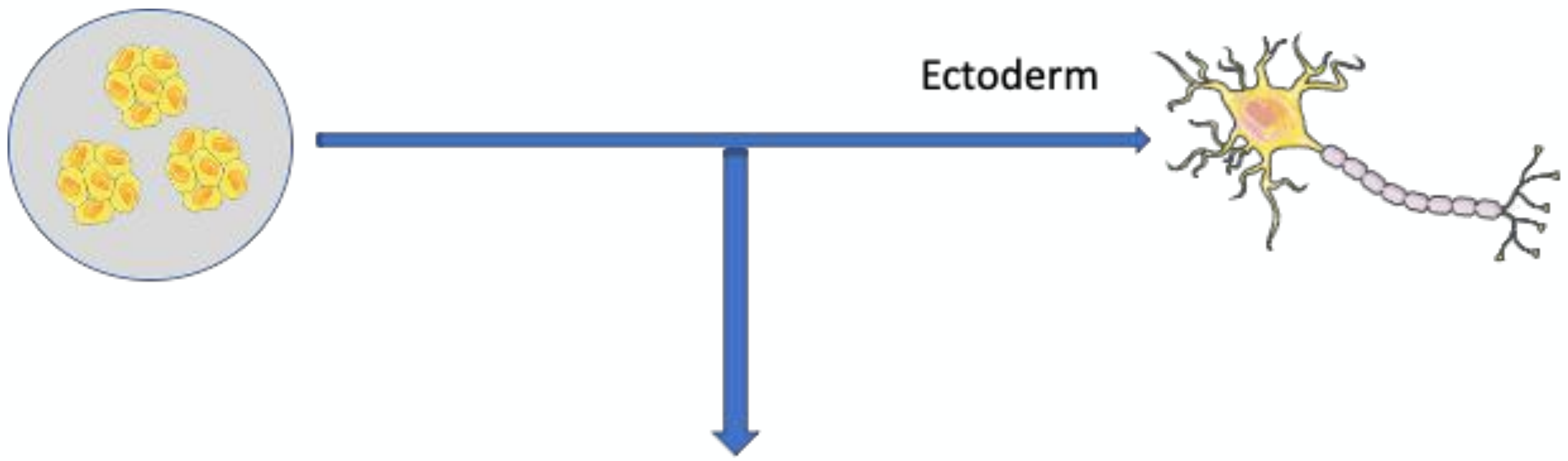


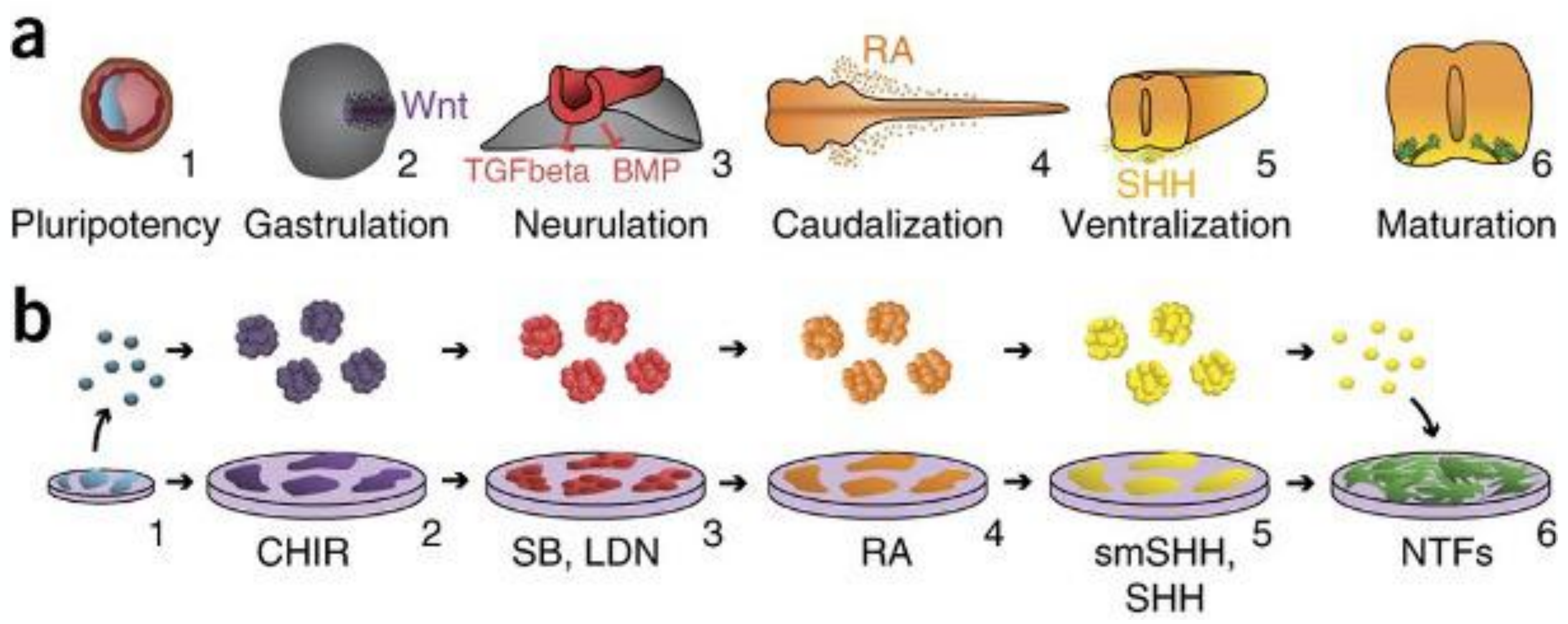
From Abmayr and Pavlath, Development. 2012



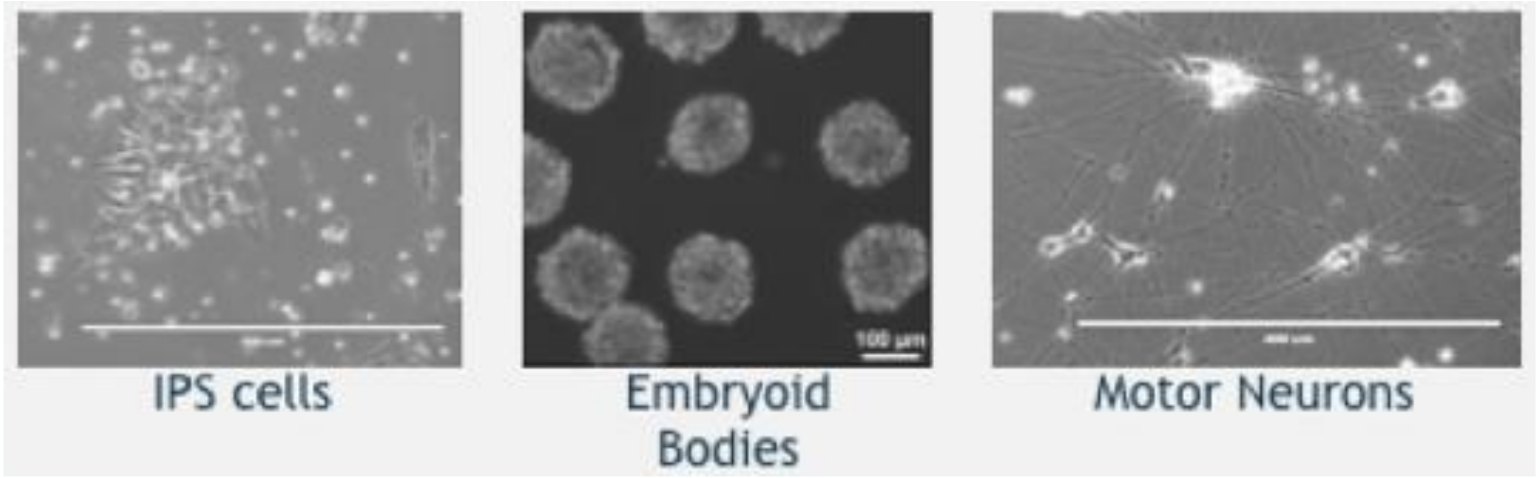
Duc et al.,2021 Sep 24. doi: 10.1039/d1lc00497b. Online ahead of print.

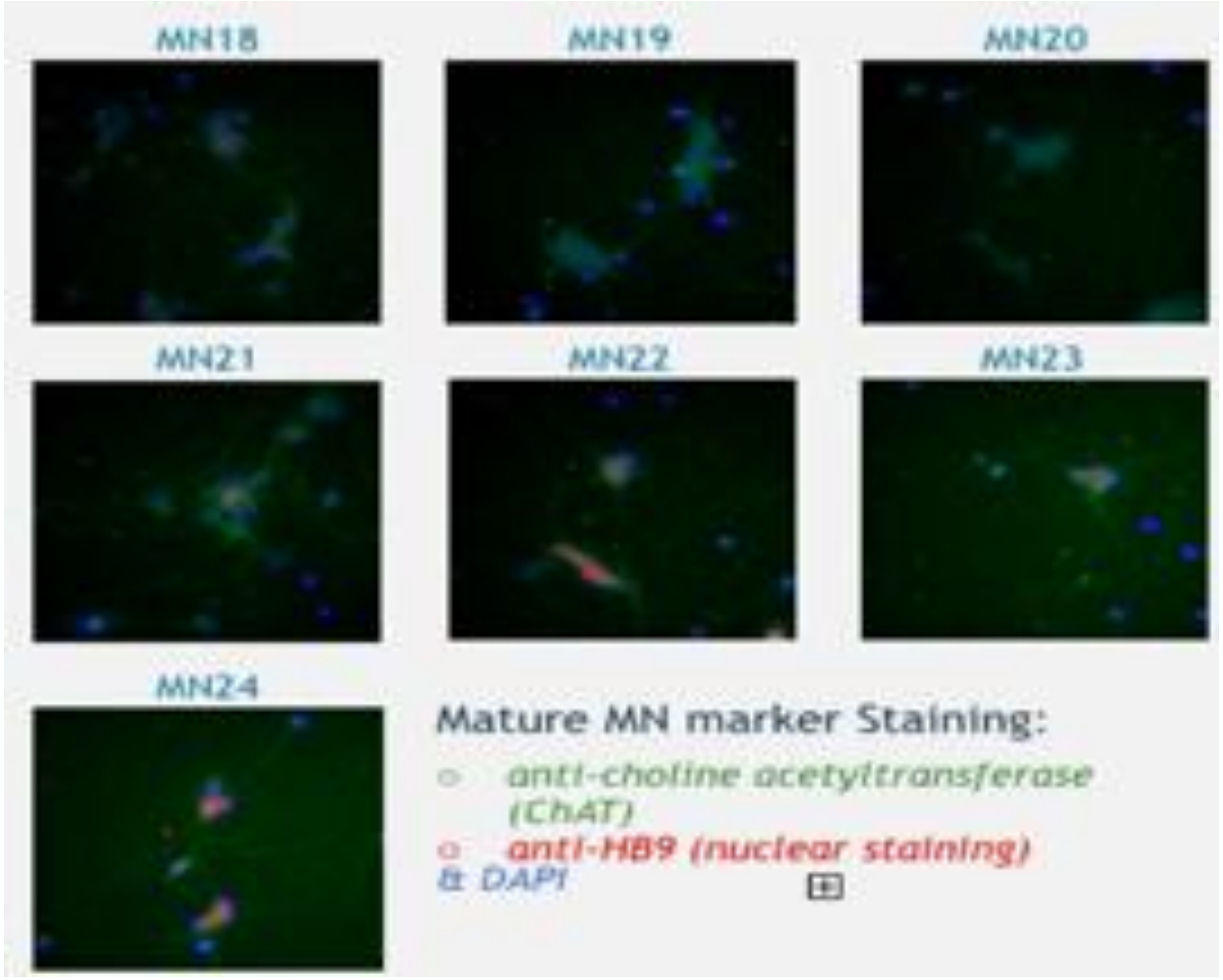
Generating Lower Motor Neurons from Pluripotent Stem Cells





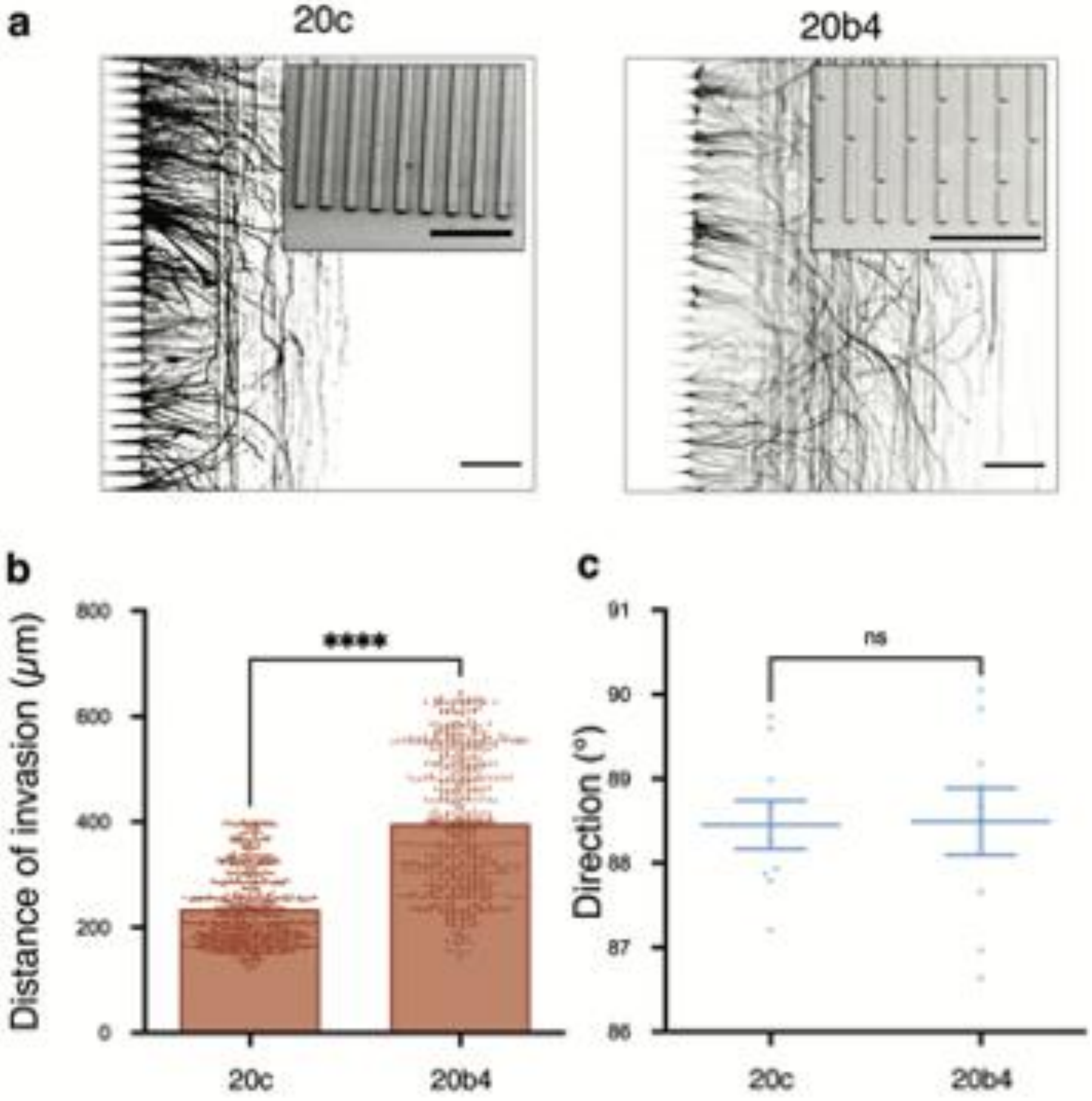
Sances et al., Nat Neurosci. 2016



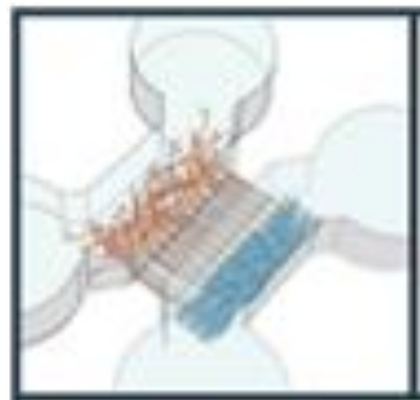


Pauline Duc, IGMM, unpublished data

Lets Axons extend



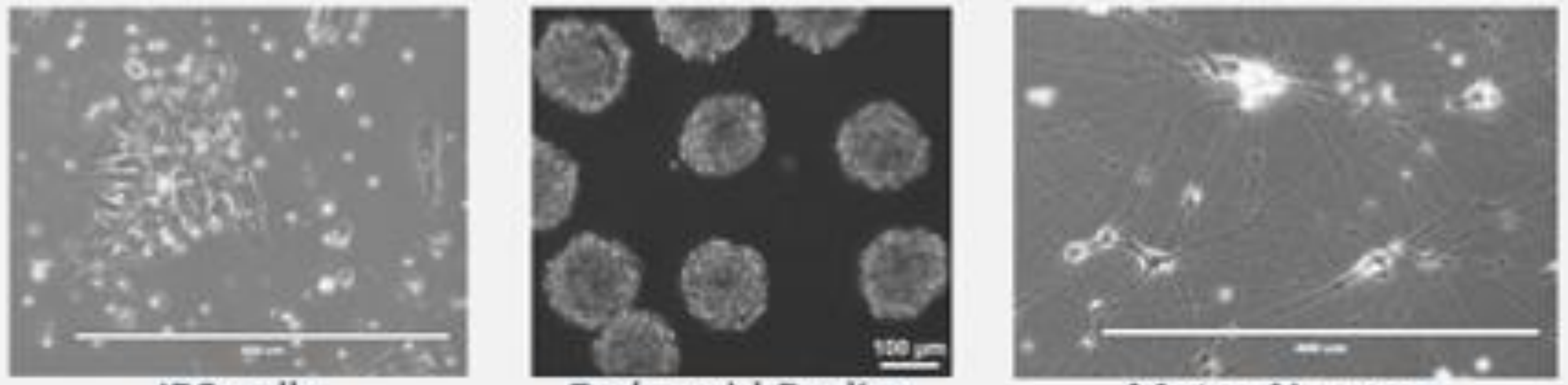
Duc et al.,2021 Sep 24. doi: 10.1039/d1lc00497b. Online ahead of print.



Cell Culture Optimization

Motor Neuron Differentiation Protocol

November to December 2018



IPS cells | Embryoid Bodies | Motor Neurons

IPS Amplification — *EB Differentiation* — *Maturation*

D-10 Thawing or Seeding

D0 Diff. Start Point

D11 Diff. End Point

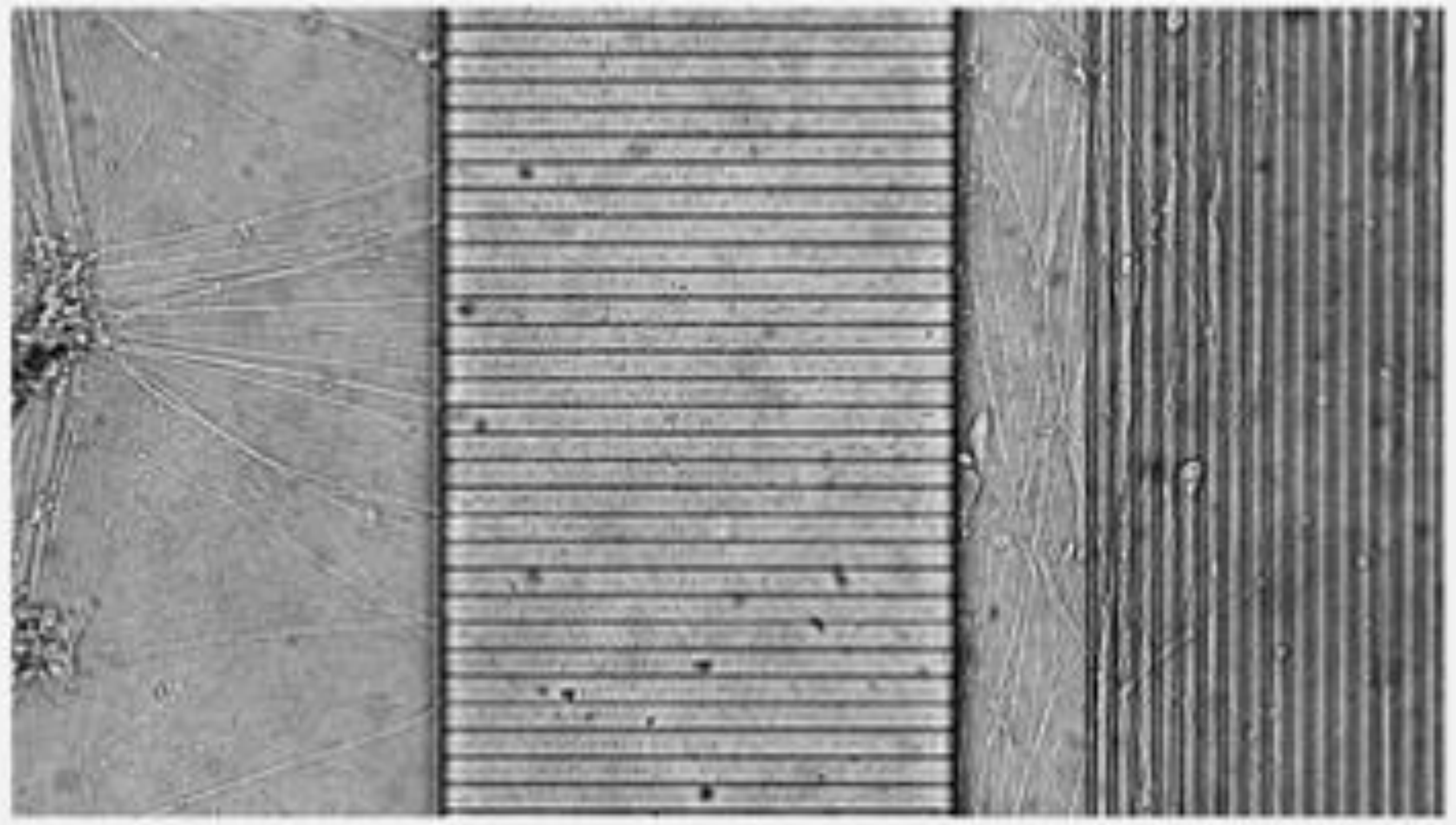
D23/24

Prolif. D13 — D15 *Diff.*

plating on chips

Protocol adapted from Maury

Compartmentalized Microfluidics co-culture



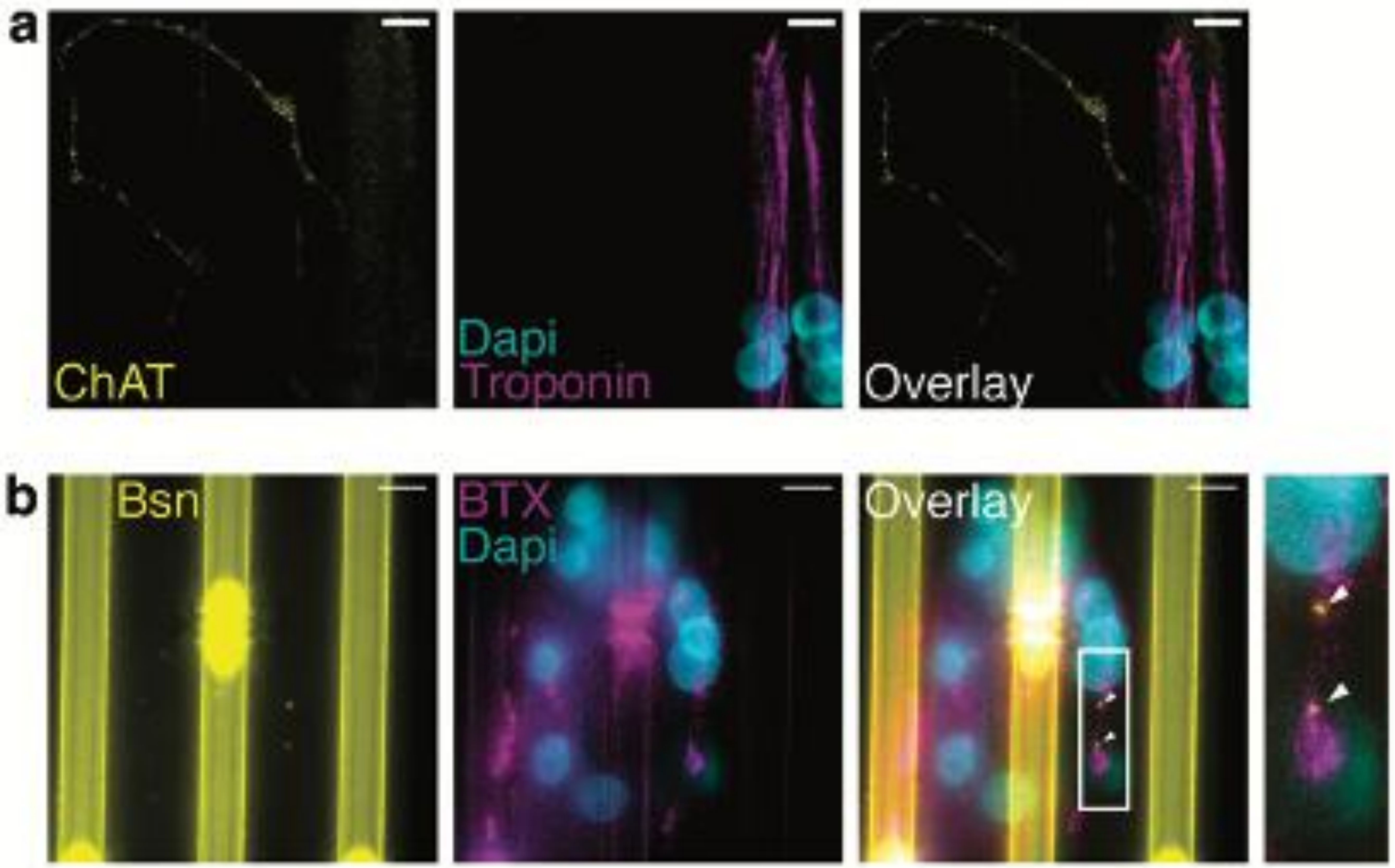
WT-IPS-derived-MN
or
SMA-IPS-derivedMN

WT-myoblasts
or
SMA-myoblasts

X

Pauline Duc, IGMM,

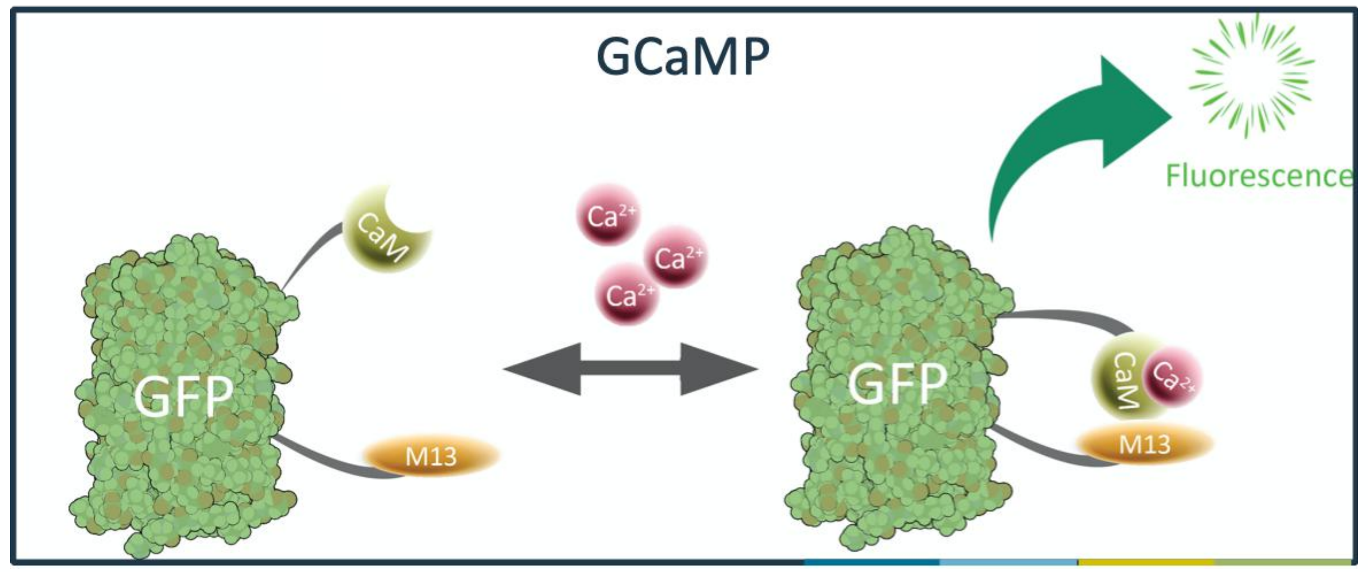
Specific Markers for NMJ maturation

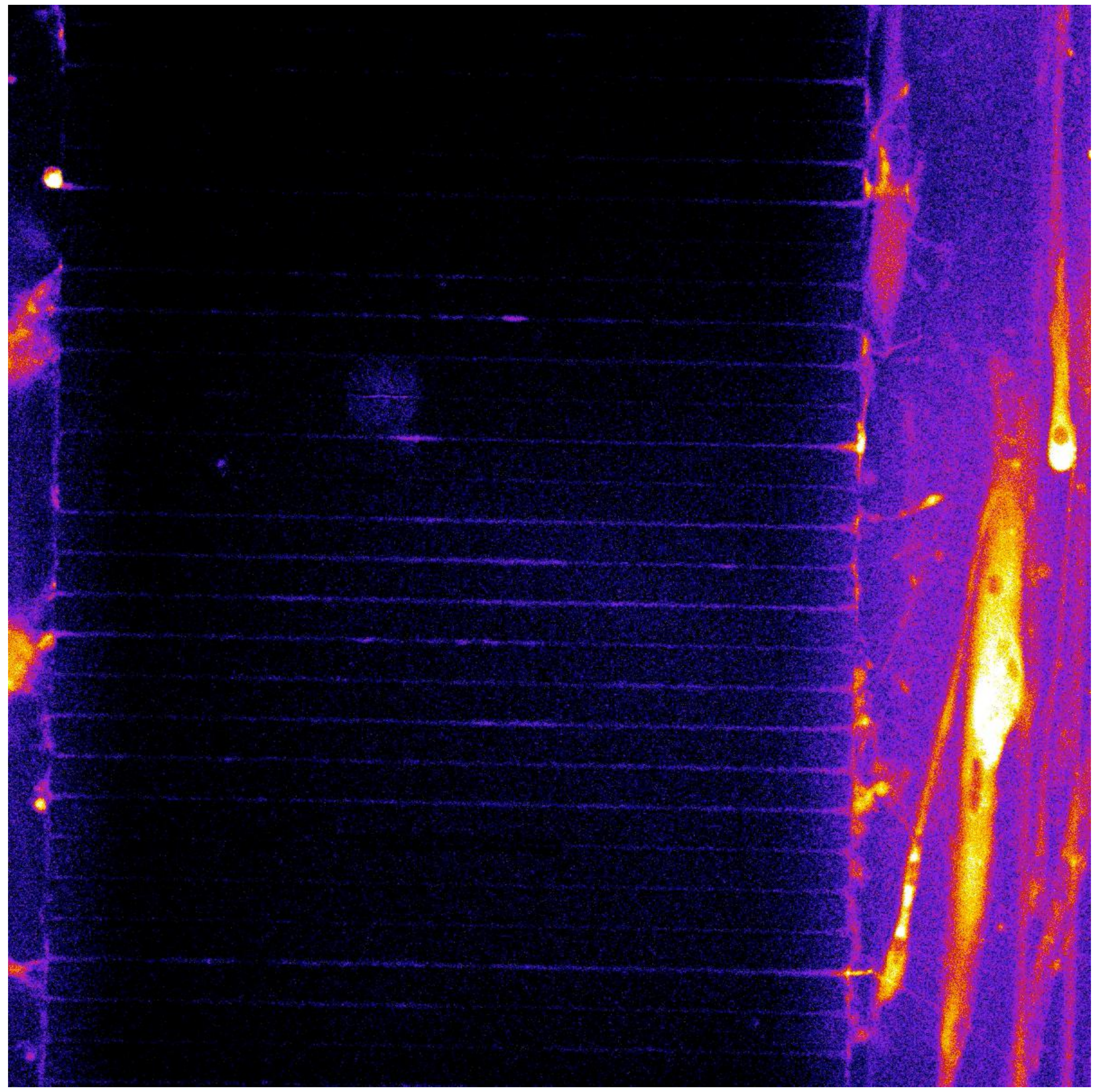


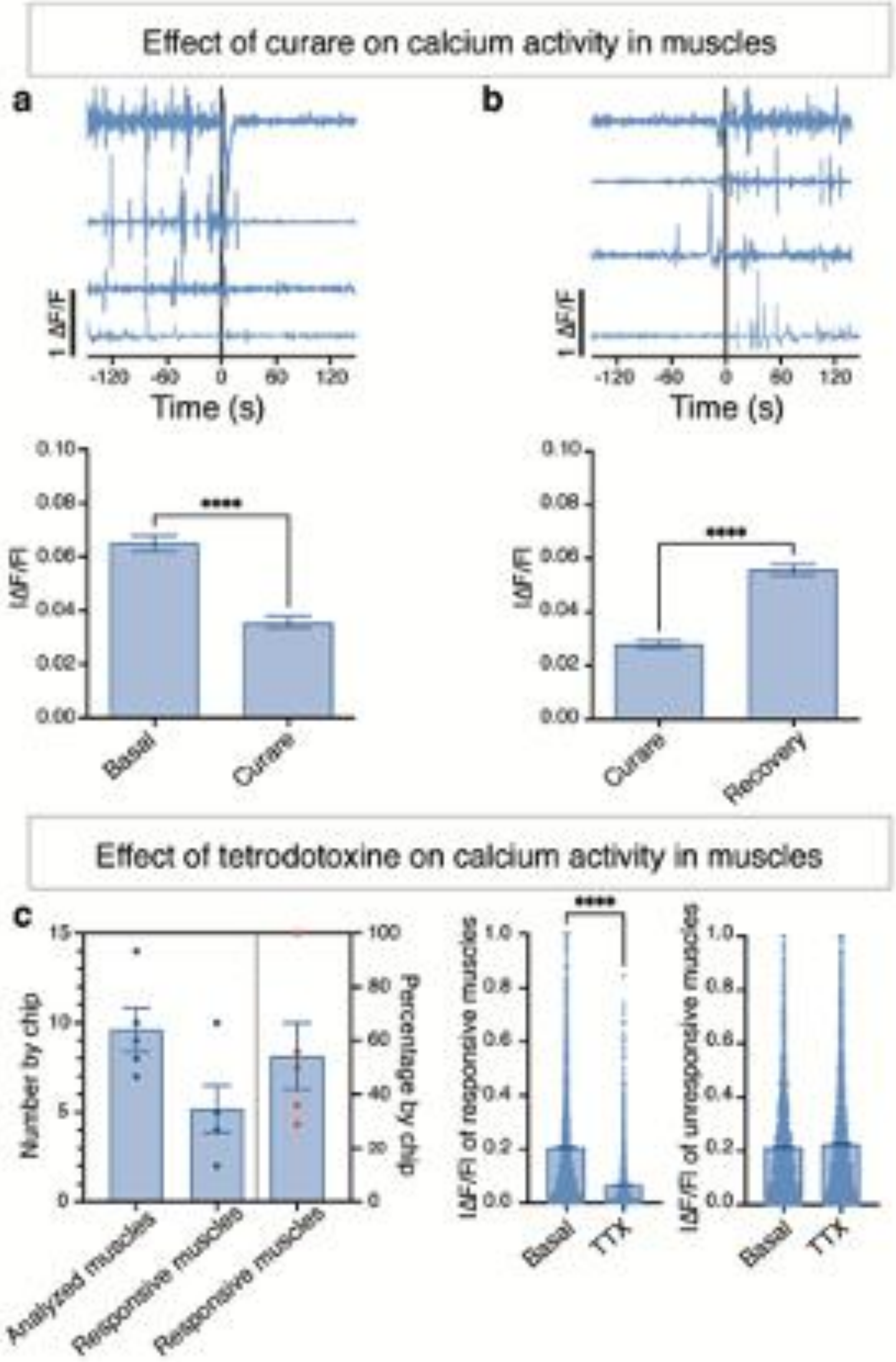
Duc et al.,2021 Sep 24. doi: 10.1039/d1lc00497b. Online ahead of print.

Functionality of the NMJ : calcium imaging activity

Genetically Encoded indicators

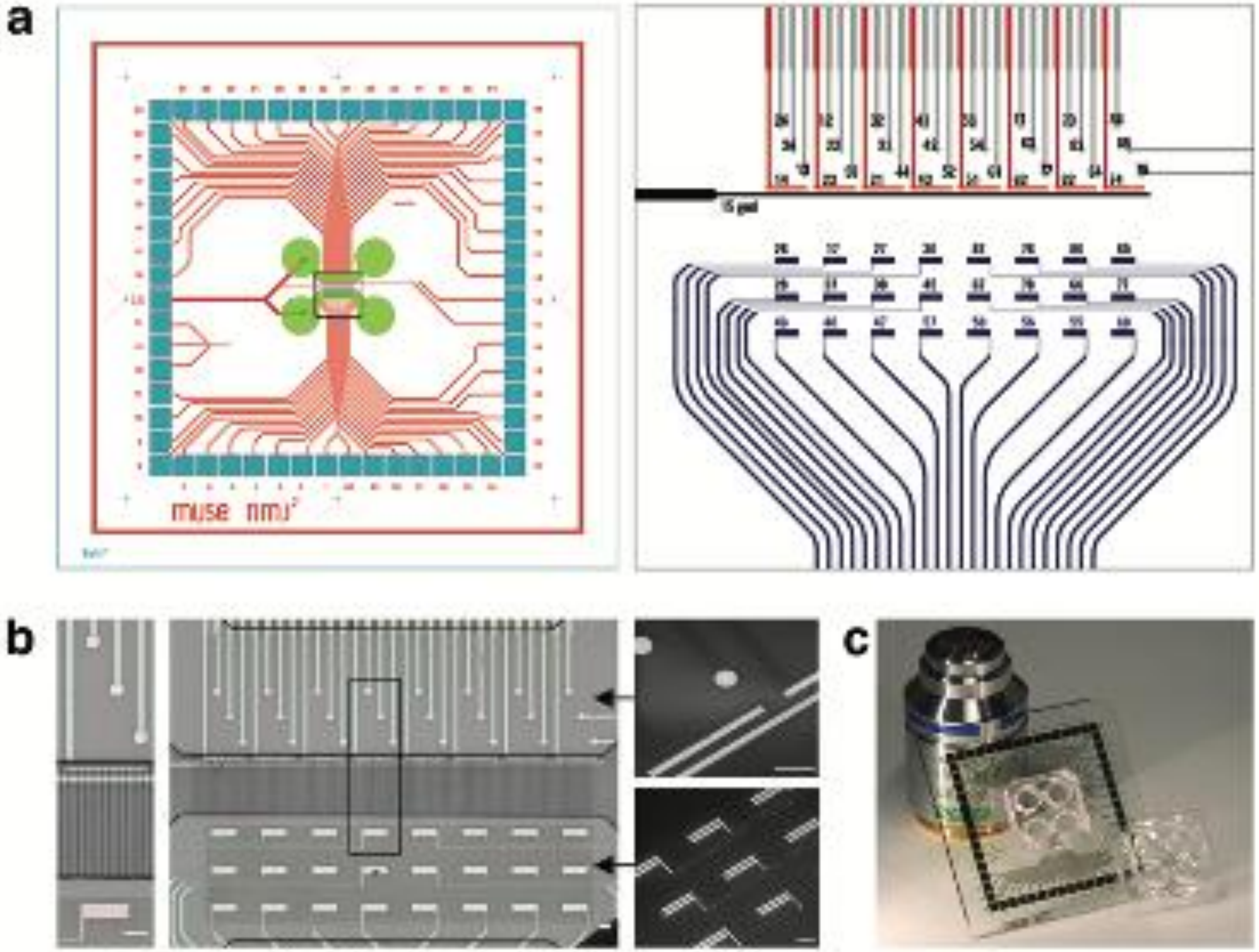




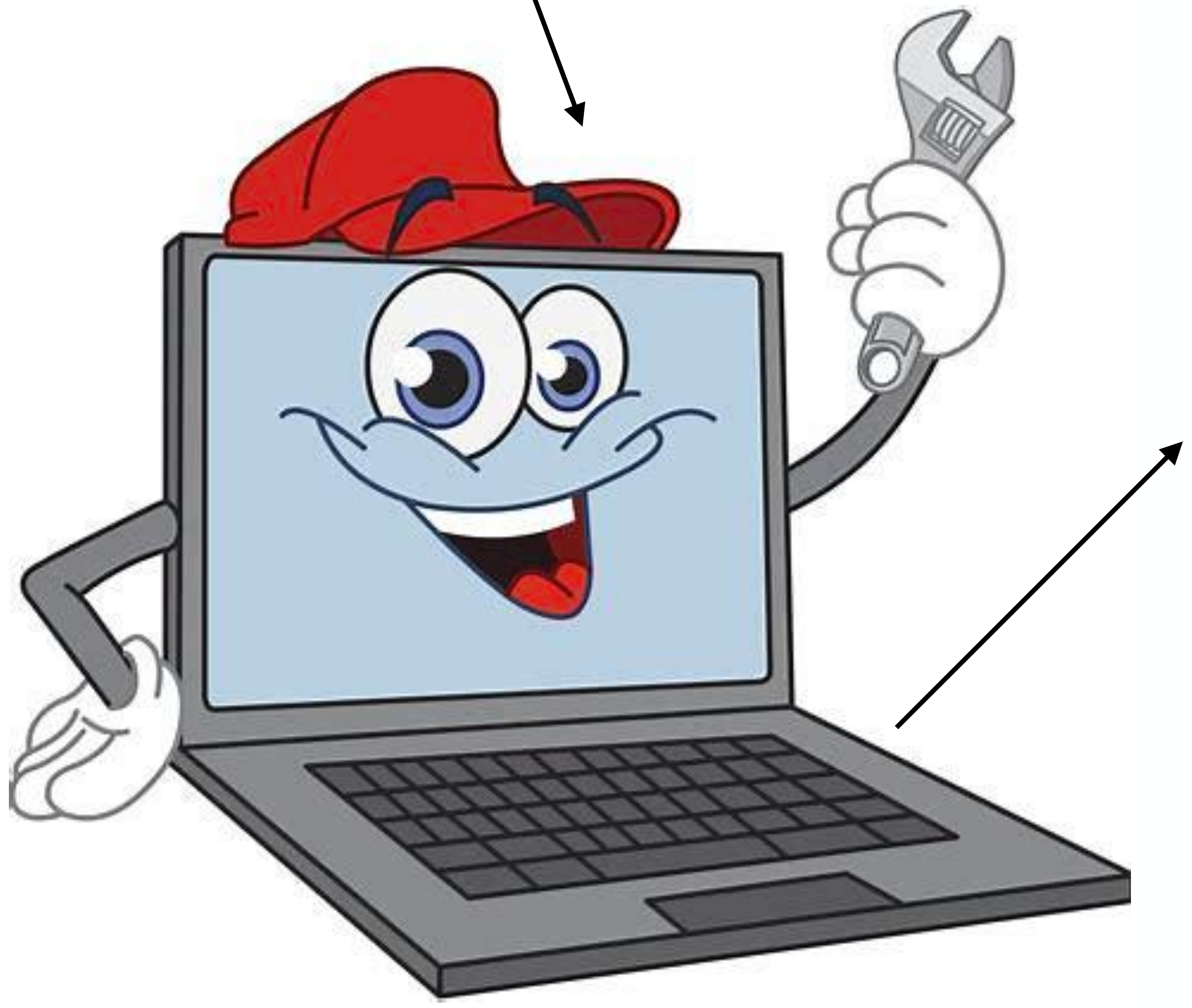


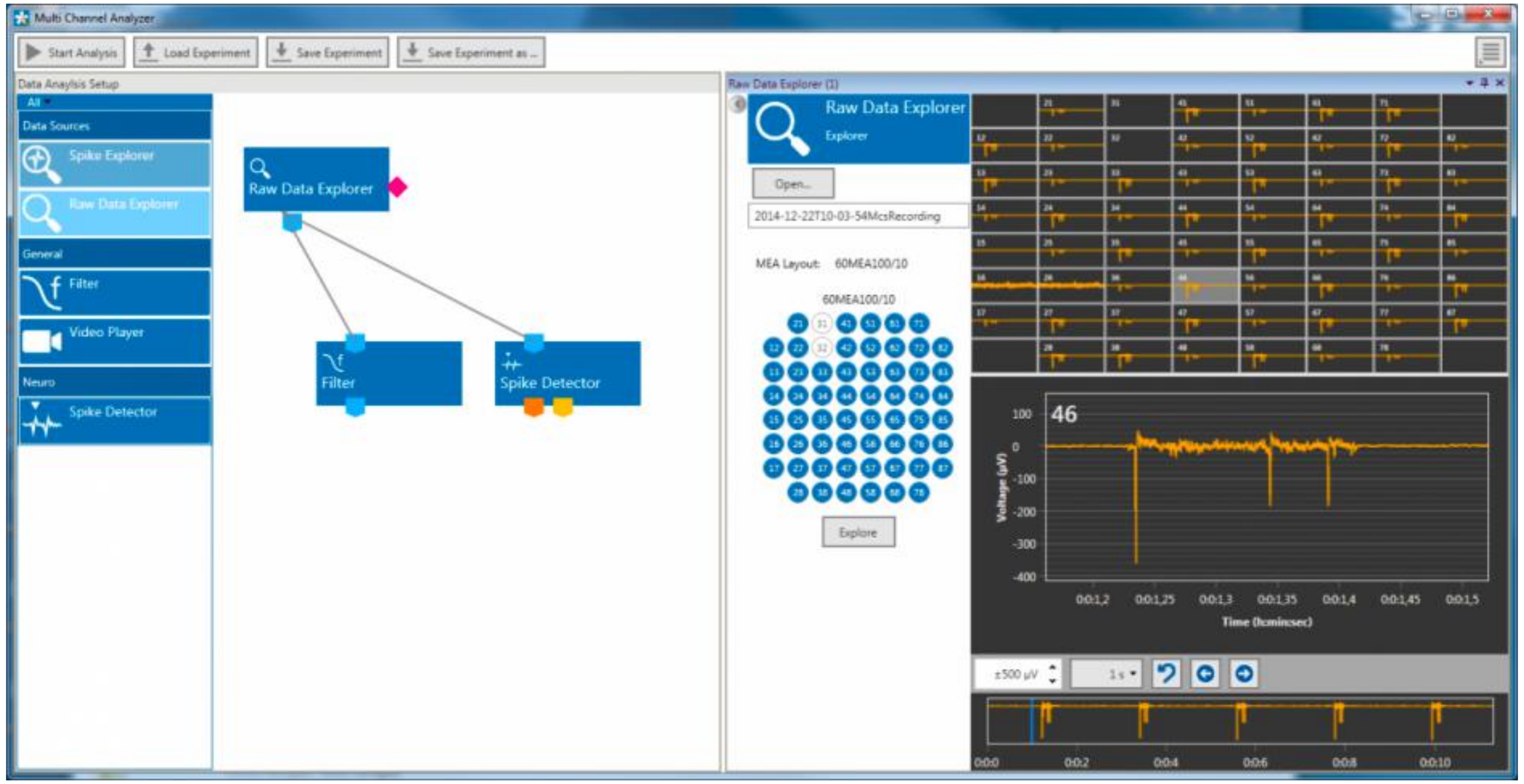
Duc et al.,2021 Sep 24. doi: 10.1039/d1lc00497b. Online ahead of print.

Functionality of the NMJ : Electrical recording activity



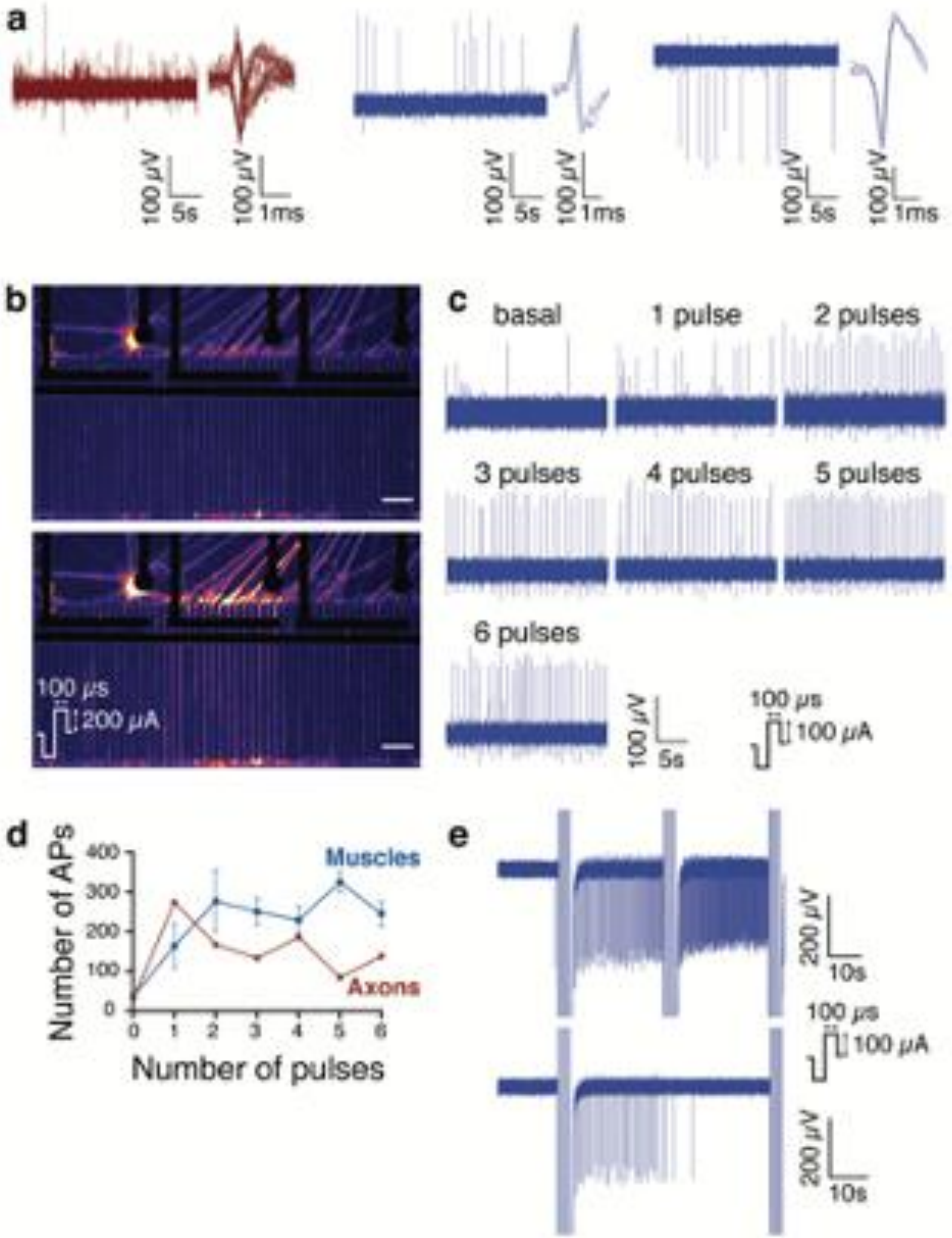
Duc et al.,2021 Sep 24. doi: 10.1039/d1lc00497b. Online ahead of print.



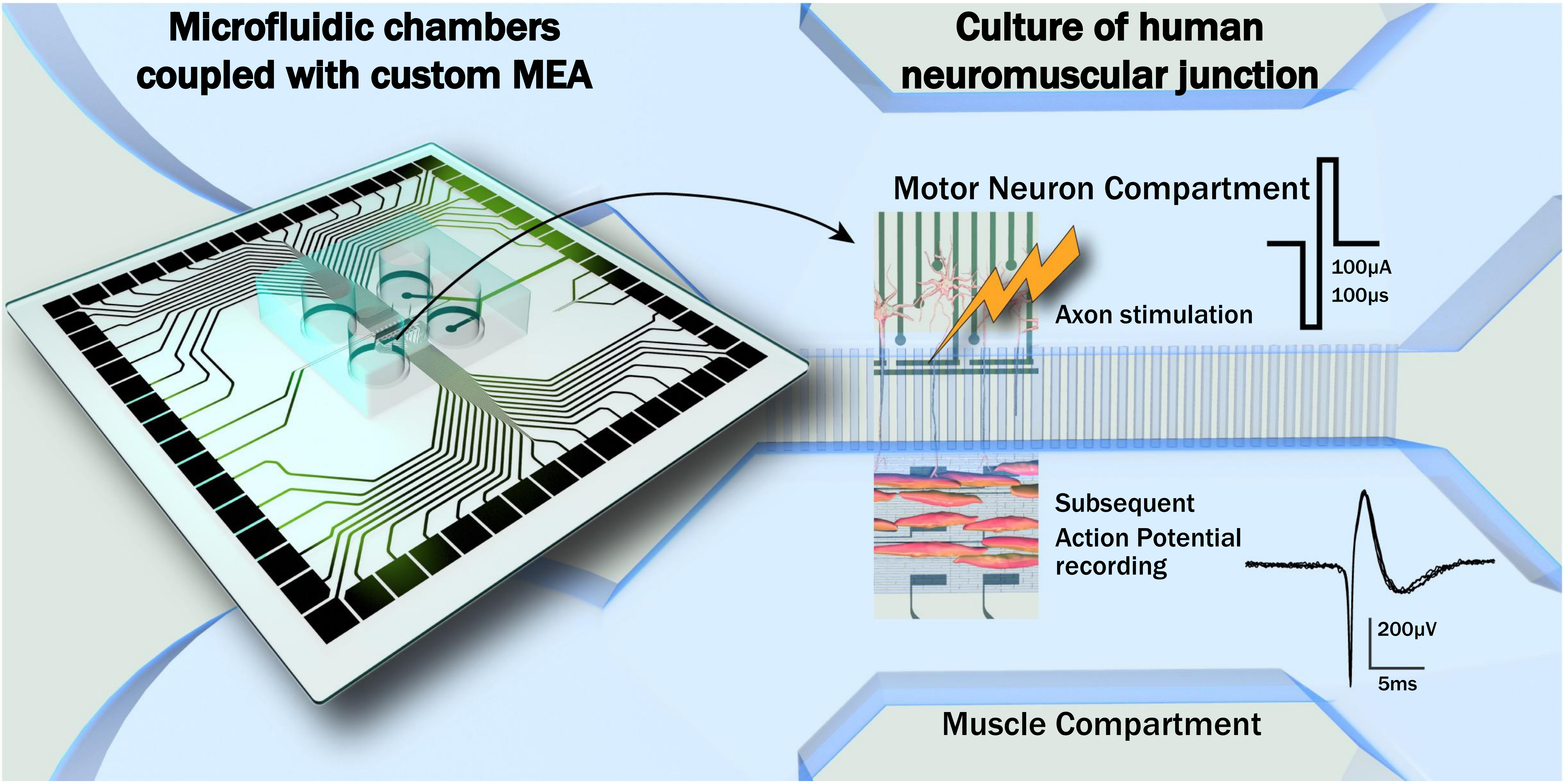


https://www.multielectrodearrays.com/

Action Potential recording

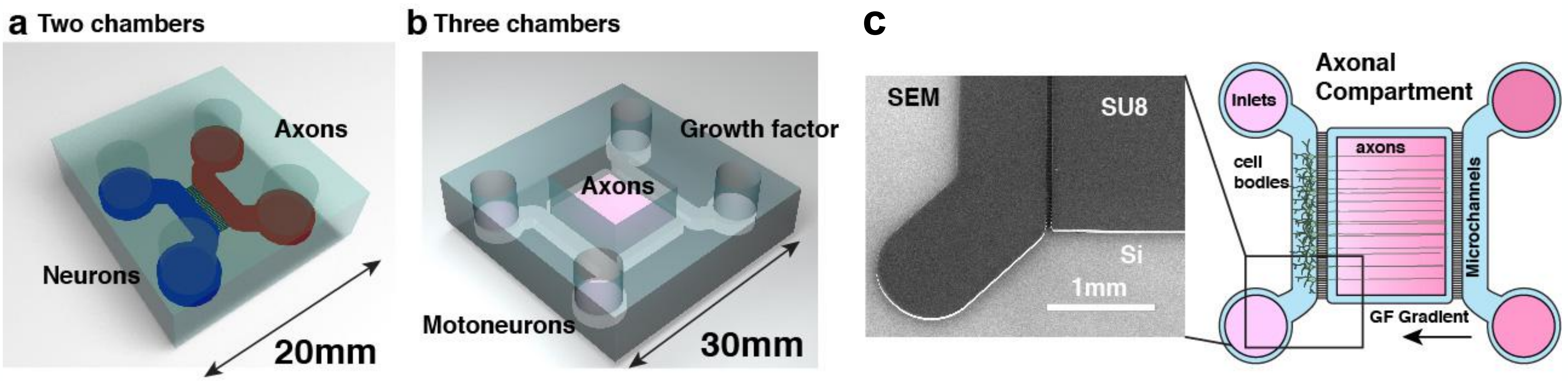


Duc et al.,2021 Sep 24. doi: 10.1039/d1lc00497b. Online ahead of print.



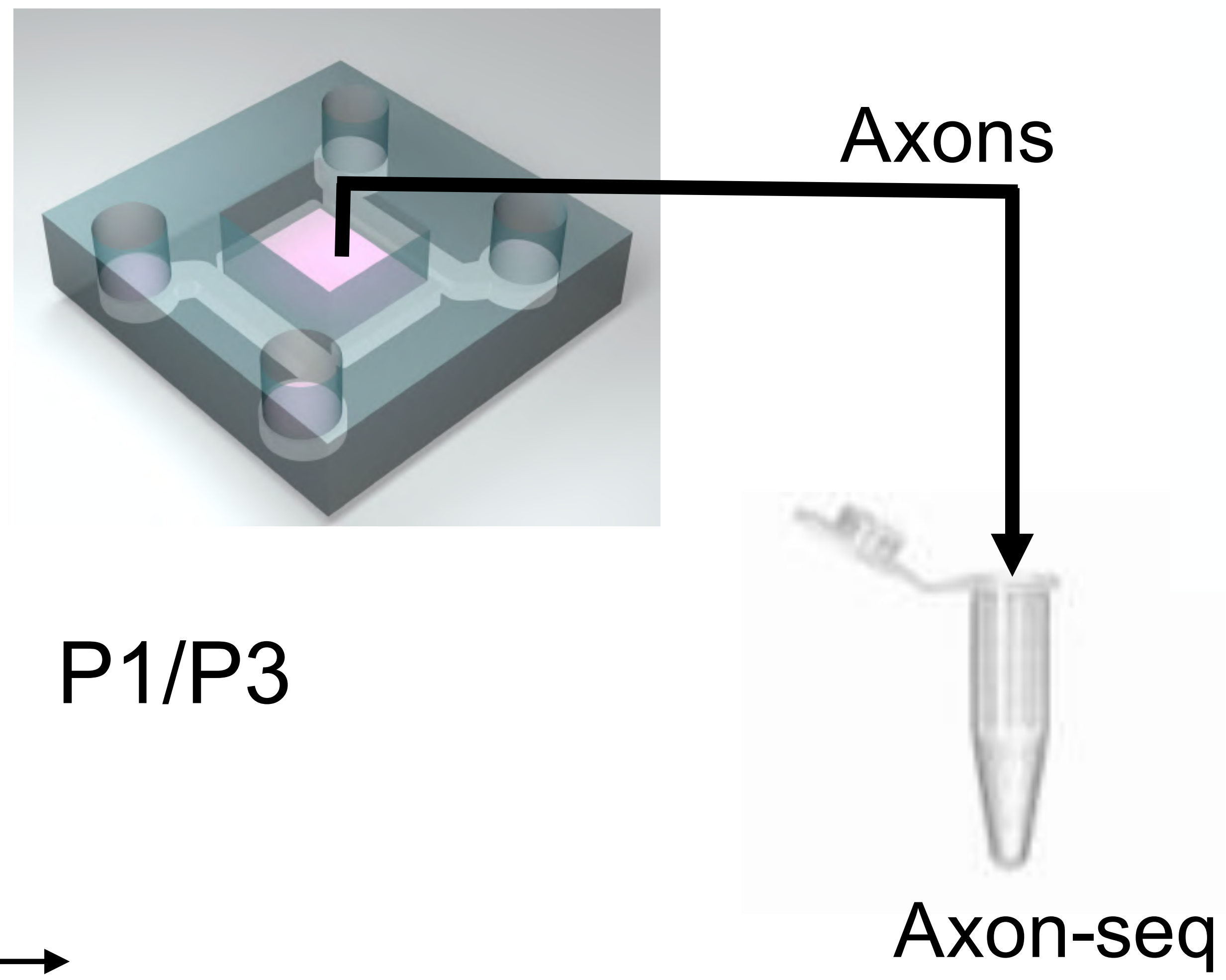
Duc et al.,2021 Sep 24. doi: 10.1039/d1lc00497b. Online ahead of print.

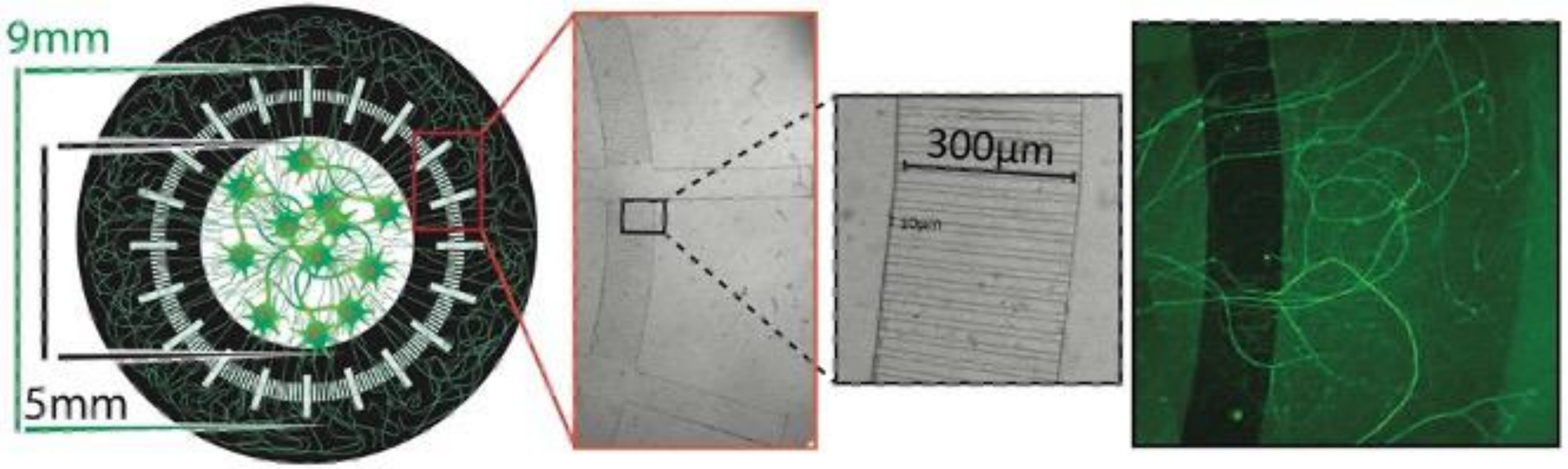
Applications



Benoit Charlot IES, Montpellier

RNA harvesting and RNA seq Analysis

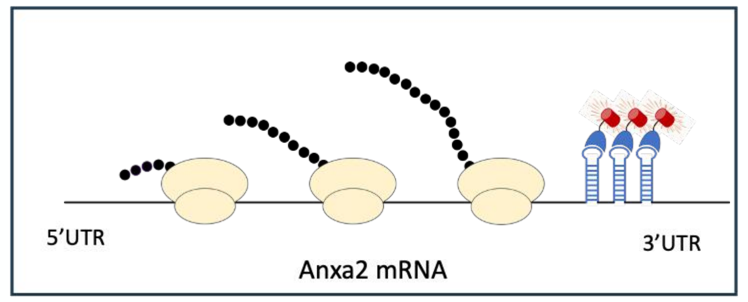




Eran Perlson, Université de Tel Aviv

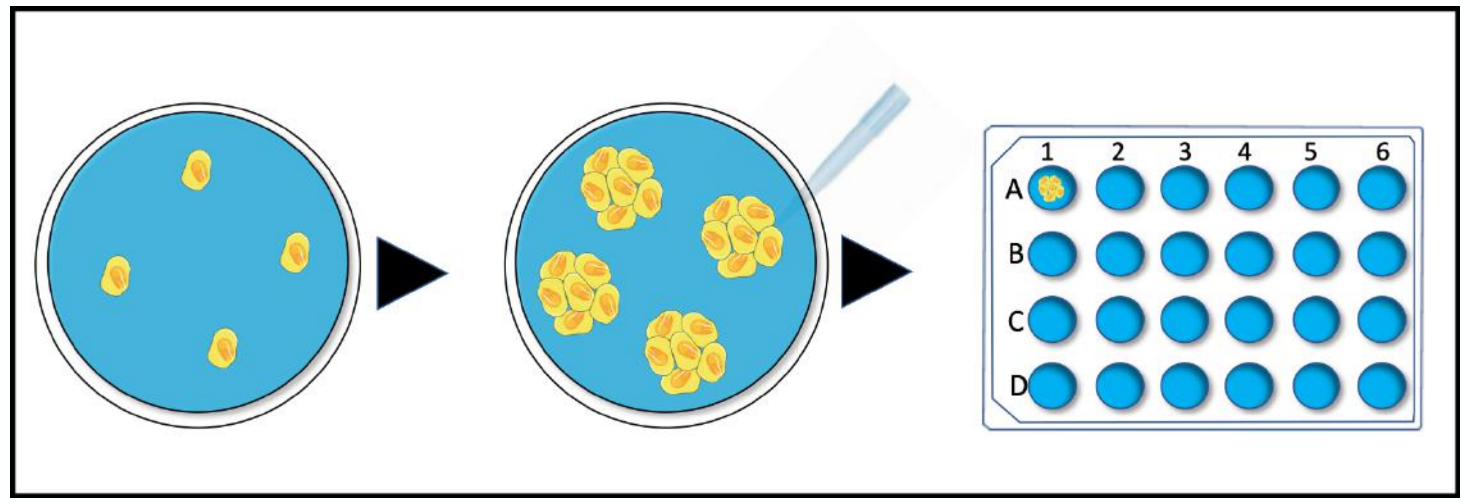
Studying RNA transport

Edition of gene using CRISPR-Cas9 technology

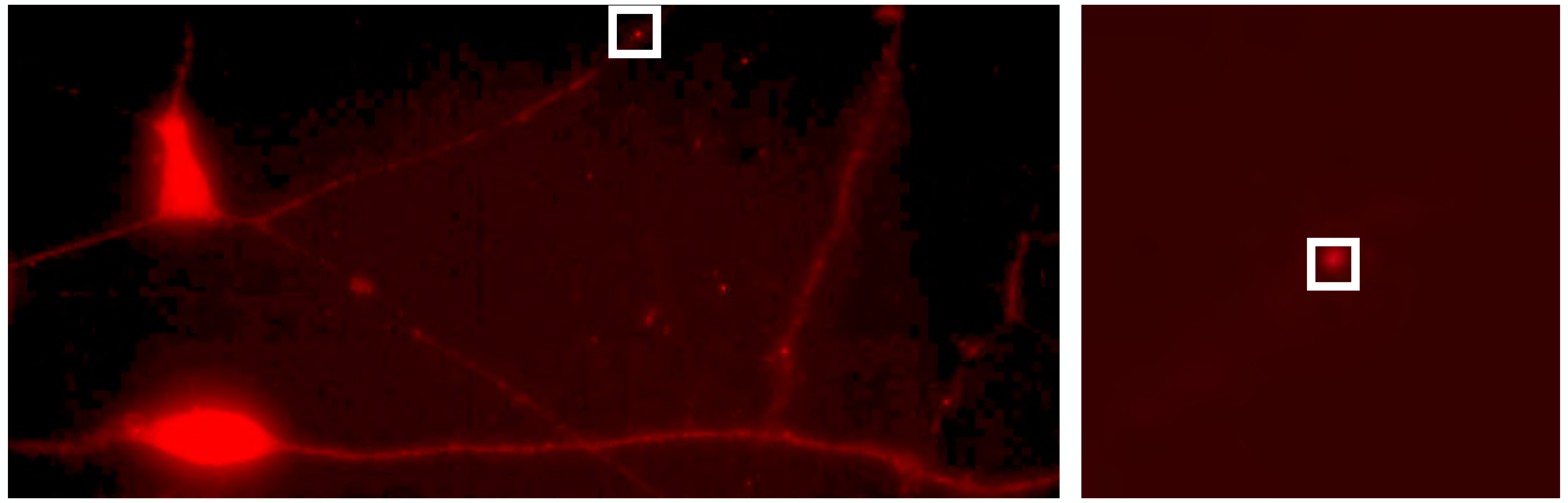


Insertion of MS2 Tag

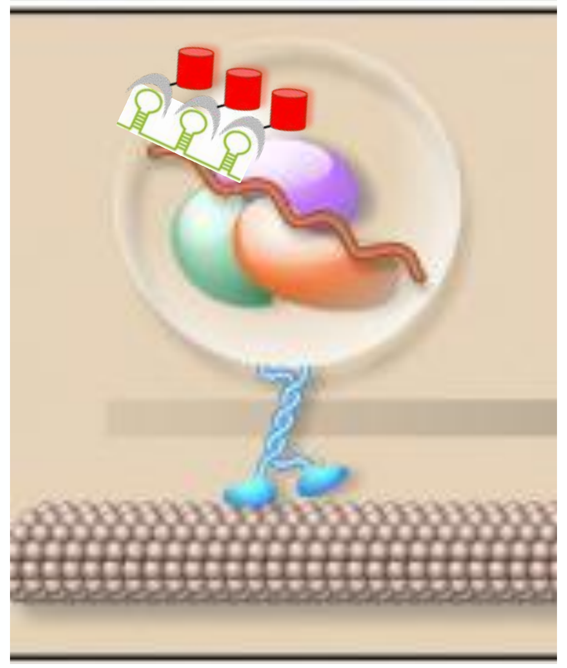
MS2-Tag



iPSC cloning/selection/differentiation

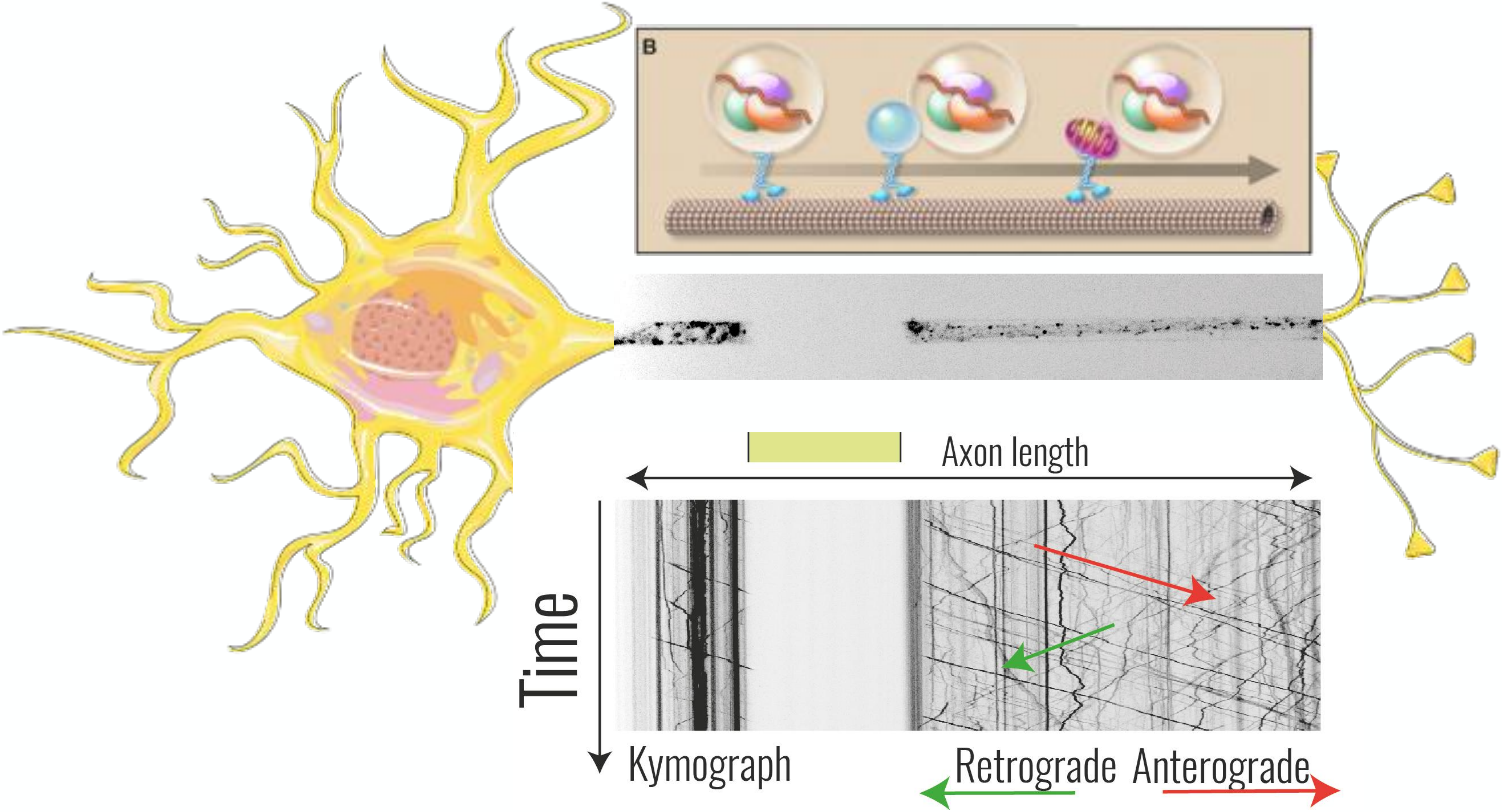


Identification of mRNA by smFISH



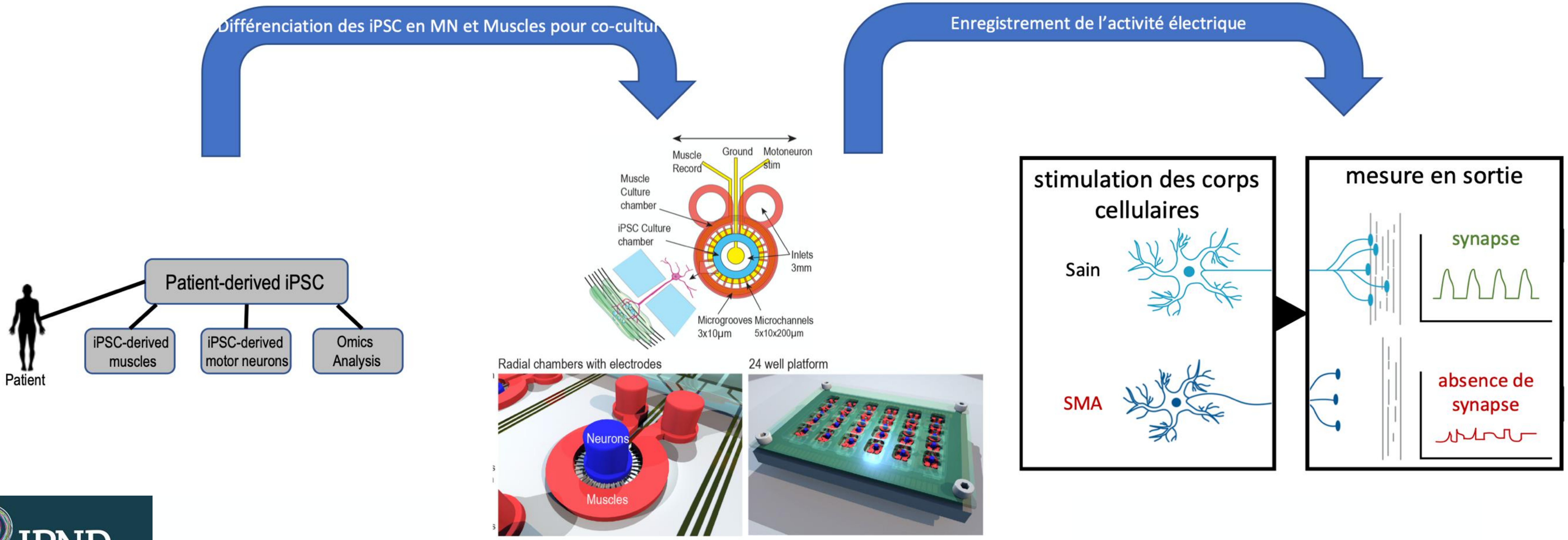
Dynamic Study

Duc, IGMM, unpublished data



E.Moutaux, M.Cazorla, Frederic Saudou , GIN Grenoble

Personalized medecine



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Marcus Krüger (Allemagne)
Silvestro Conticello (Italie)
Jozef Dulak (Pologne)
Duygu Uckan Cetinkaya (Turquie)


Thank you for
your
attention!

Annexe 1

Overview of the advanced *in vitro* models of NMJ and their applications for drug testing and disease modeling.

Muscle source	Neuron source	Special features	Validation of NMJ function	Disease model	Drug testing	Ref
Rat	Rat	Compartmentalized, glial cells	No	No	No	Southam *et al.*, 2013 [43]
Mouse C2C12	Mouse	Compartmentalized	No	No	No	Park *et al.*, 2013 [44]
Mouse	Mouse	Compartmentalized	Glutamate stimulation, drug response	No	TTX	Zahavi *et al.*, 2015 [45]
Human	human NSC, iPSCs	Compartmentalized, BioMEMs	Electrical stimulation, drug response	No	TC, BoNT, BTX	Santhanam *et al.*, 2018 [46]
Mouse C2C12	Mouse, ESCs	3D skeletal tissue, cantilevers	Glutamate stimulation, drug response	No	TC	Morimoto *et al.*, 2013 [54]
Rat	Rat	Cantilevers, photodetector	Glutamate stimulation, drug response	No	TC	Smith *et al.*, 2013 [56]
Human	Human ESCs	3D culture	Glutamate stimulation	MG	TC, BoT, WTX	Afshar Bakooshli *et al.*, 2019 [57]
Human	Human ESCs	Optogenetic	Optical stimulation	MG	PYR	Steinbeck *et al.*, 2016 [59]
Mouse C2C12	Mouse ESCs	Compartmentalized, 3D, optogenetic	Optical stimulation, drug response	No	BTX	Uzel *et al.*, 2016 [60]
Human	Human iPSCs	Compartmentalized, 3D, optogenetic, automated	Optical stimulation, drug response	MG	BTX	Vila *et al.*, 2019 [62]
Human iPSCs	Human iPSCs	Compartmentalized, optogenetic	Optical stimulation, drug response	ALS	BTX, rapamycin, bosutinib	Osaki *et al.*, 2018 [61]

ALS: amyotrophic lateral sclerosis, ATX: Agatoxin, BoNT: Botulinum toxin, TC: Tubocurarine, ESC: embryonic stem cells, iPSCs: induced pluripotent stem cells, MG: myasthenia gravis, NSC: Neural stem cells, PYR: pyridostigmine; TTX: Tetrodotoxin.

From Vila et al., Expert Opin Drug Discov. 2020 March ; 15(3): 307–317.

Overview of the *in vitro* co-culture models of NMJ and their use for drug testing.

Muscle source	Neuron source	Achievement	Validation of NMJ function	Disease model	Drug testing	Ref
Rat/mouse	Rat/mouse	First *in vitro* NMJs	No	No	No	Peterson *et al.*, 1972 [33]
Rat	Rat	long-term culture, defined system	No	No	No	Das *et al.*, 2010 [34]
Mouse ESCs	Chicken	stem cell derived	Drug response	No	ATX,TC, dynasore, nifedipine, TTX	Chipman et al, 2014 [35]
Mouse, transdifferentiated fibroblasts	Mouse ESCs	electrical stimulation	Glutamate Stim, drug response	No	BoNT, neostigmine, MEChMAz, TTX, vesamicol	Charoensook *et al.*, 2017 [68]
Mouse C2C12	Human ESCs	human MN	No	No	No	Li *et al.*, 2005 [36]
Rat	Human spinal cord stem cells	human MN, defined system	Glutamate Stim, drug response	No	TC	Guo *et al.*, 2010 [37]
Human	Human spinal cord stem cells	patterned surface for myotube alignment	Drug response	No	TC	Guo *et al.*, 2011[38]
Mouse C2C12	Human iPSCs	First disease NMJ model	No	SMA	VPA, ASO	Yoshida *et al.*, 2015 [65]
Human iPSCs	Human iPSCs	all iPSC-derived	No	No	No	Demestre *et al.*, 2015 [39]

ASO: antisense oligonucleotides, ATX: Agatoxin, BoNT: Botulinum toxin, TC: Tubocurarine, ESC: embryonic stem cells, MEChMAz: Acetylethylcholine mustard hydrochloride, iPSCs: induced pluripotent stem cells, TTX: Tetrodotoxin., VPA: valproic acid.

From Vila et al., Expert Opin Drug Discov. 2020 March ; 15(3): 307–317.