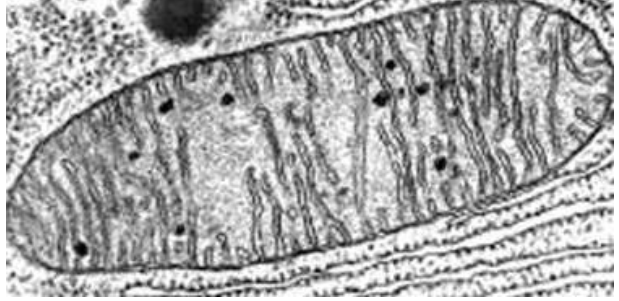
FINDING OF MYELIN ENERGETIC FUNCTION SUPPORTING THE AXONAL NERVOUS CONDUCTION: PERSPECTIVES IN NEUROLOGY.

Neurology 2015 – Rome, july 2015 – 28

Alessandro Morelli – University of Genova (www.biochemlab.it)

1)

ATP Production in the mitochondria and in the myelin



Mitochondria (and thylacoid disks) are considered the exclusive site of aerobic ATP synthesis. Is it true?

It is well known that in vitro mitochondria produce few ATP and that there is the need to energize them with pyruvate (and to add cyclosporin-A, etc.), which seems anomalous.

Moreover other cellular processes (DNA duplication, proteic synthesis, etc.) can procede *in vitro* with the same if not higher efficiency than *in vivo*.

The ATP production in mitochondria, isolated from several cell types wich, in the best experimental conditions, is about

7- 8 nmol ATP /min / mg protein

Overall Oxygen (atomic) consumption for a tissue (muscle at rest) is

3 nmole/min/mg prot.

With a P/O ratio = 2,36 the ATP production is:

~7 nmol ATP /min / mg protein

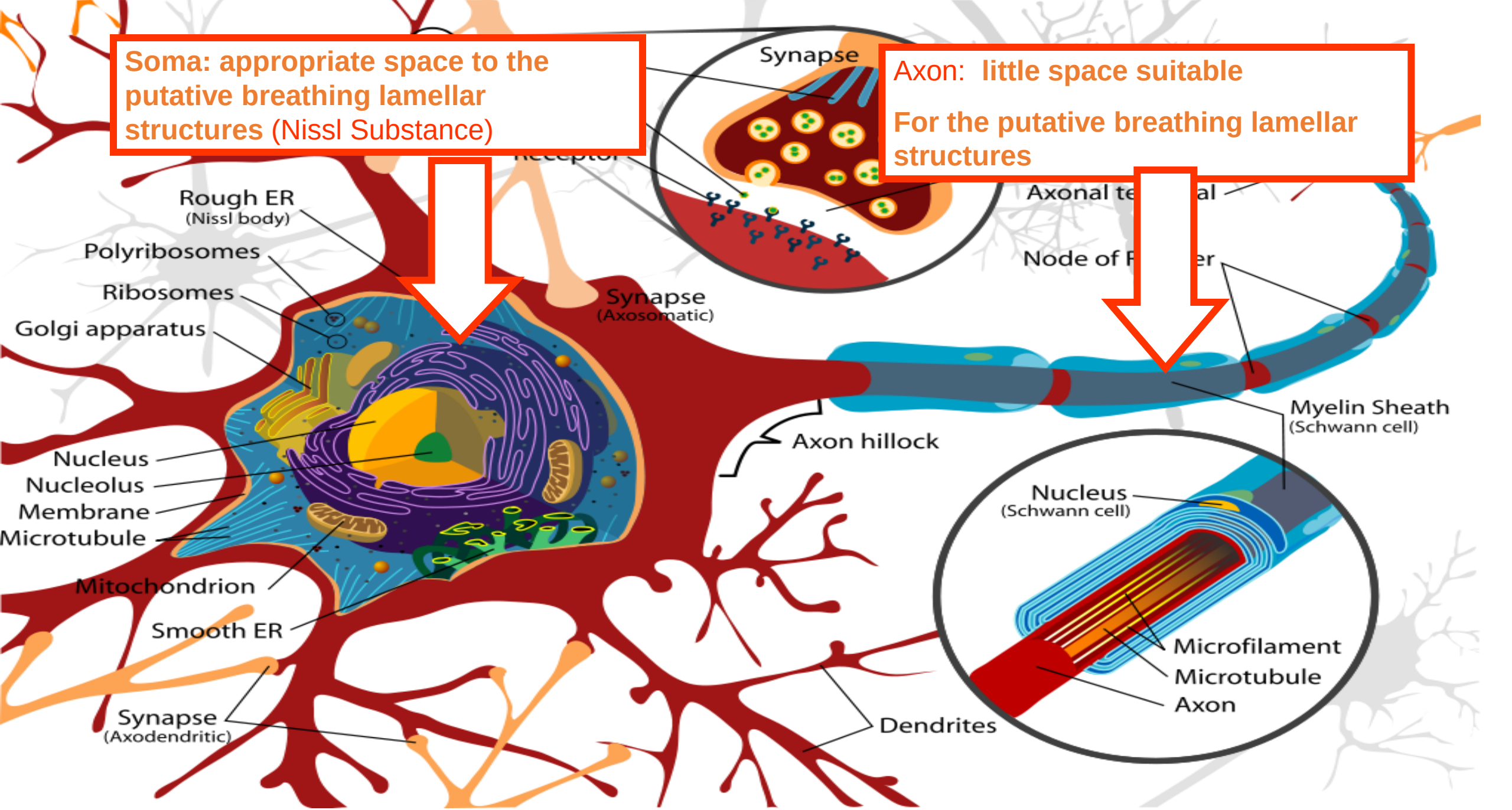
So muscle shuld be made only by mitochondria…

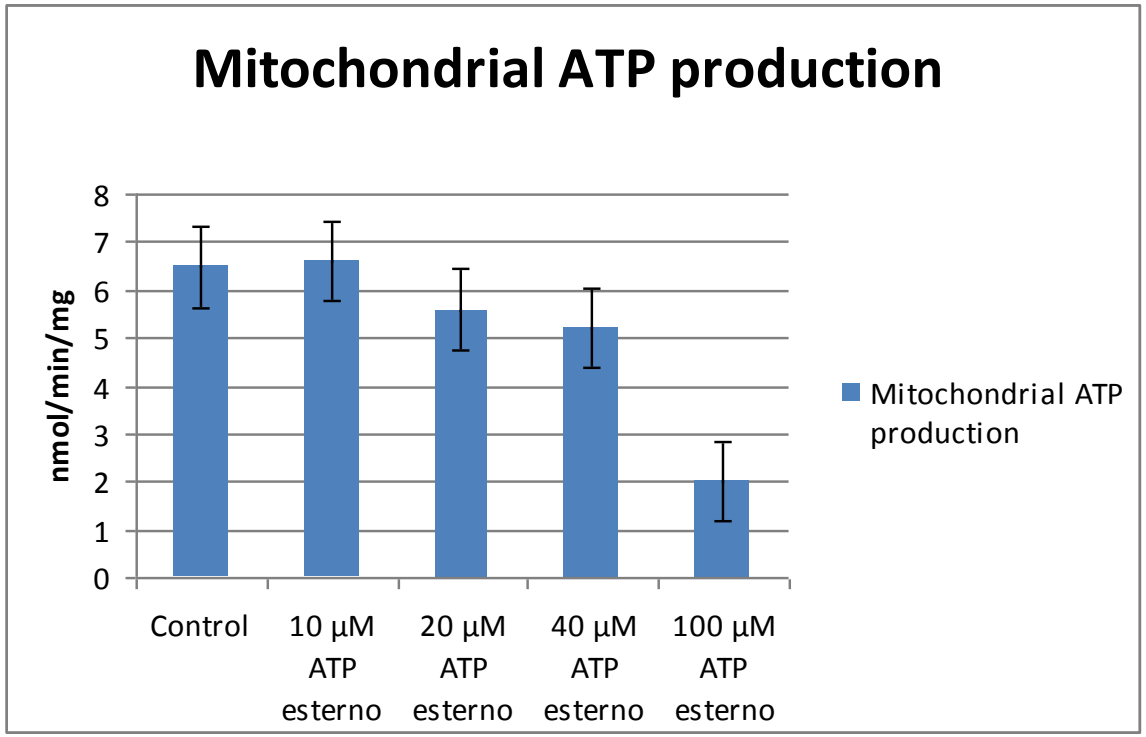
Otherwise…

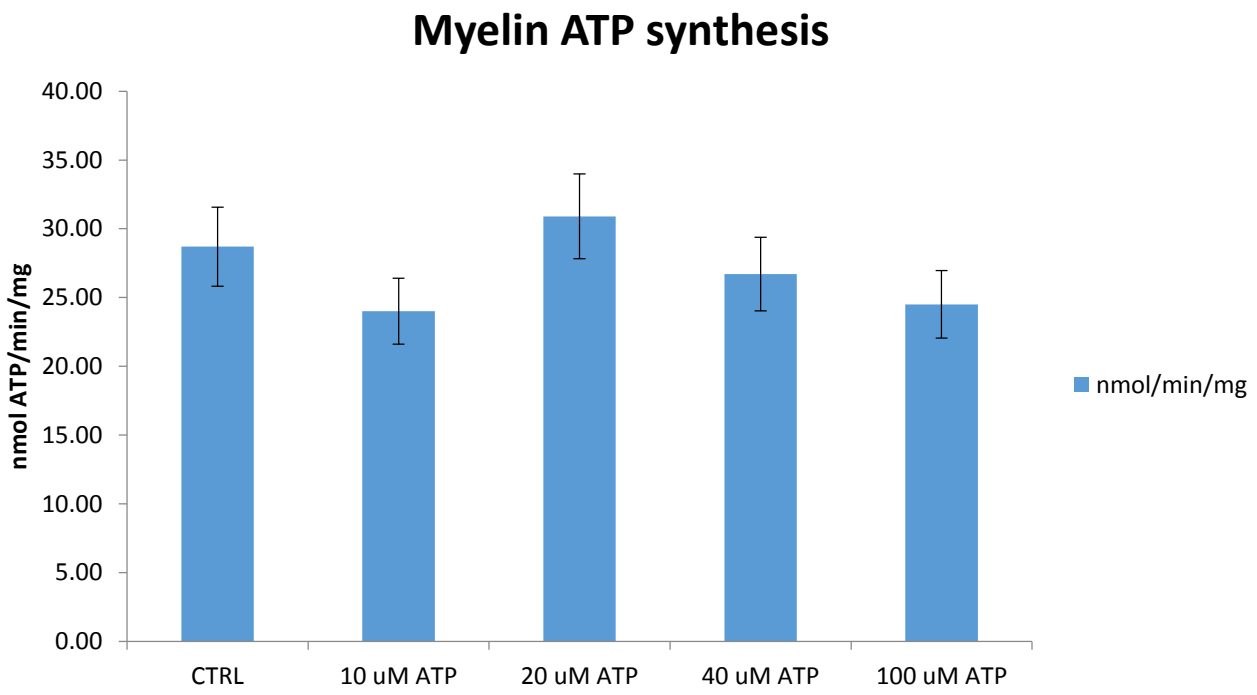
- **Considering that brain consumes much more oxygen than the other tissues it must have a great mitochondrial number. Instead…:**

Neuronal mitochondria:

- **Are *fewer* than in the other tissues.**
- **Have *smaller* dimensions.**
- **Have *smaller* cristal surface.**


Soma: appropriate space to the putative breathing lamellar structures (Nissl Substance)
Axon: little space suitable
For the putative breathing lamellar structures
Synapse
Rough ER
(Nissl body)
Polyribosomes
Ribosomes
Golgi apparatus
Synapse
(Axosomatic)
Axonal te al
Node of F er
Myelin Sheath
(Schwann cell)
Axon hillock
Nucleus
Nucleolus
Membrane
Microtubule
Mitochondrion
Smooth ER
Synapse
(Axodendritic)
Dendrites
Nucleus
(Schwann cell)
Microfilament
Microtubule
Axon





→Myelin produce ~ 30 nmol ATP/min/mg (purified mitochondria only ~ 6 nmol ATP/min/mg .

→ATP production in myelin is'nt inbited by external ATP. Otherwise at very low concentration external ATP inibite strongly ATP production by mitochondria.

Biochimie. - 2013 Nov;95(11):1991-8. doi: 10.1016/j.biochi.2013.07.003.

Biochimie xxx (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

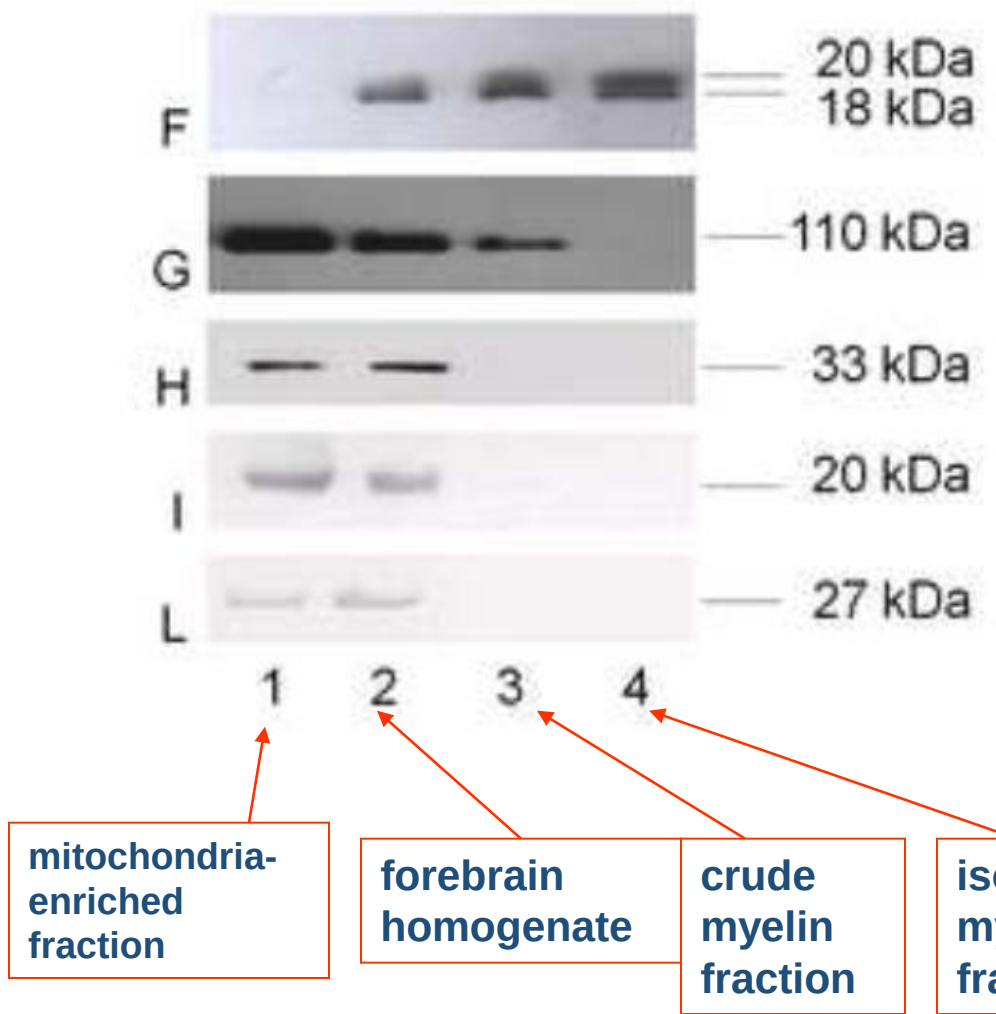
Research paper

Tricarboxylic acid cycle-sustained oxidative phosphorylation in isolated myelin vesicles

Silvia Ravera [a,*], Martina Bartolucci [a], Daniela Calzia [a], Maria Grazia Aluigi [b], Paola Ramoino [b], Alessandro Morelli [a], Isabella Panfoli [a]

	Mitochondria enriched fraction	Forebrain homogenate	Crude myelin	Isolated myelin
Citrate Synthase	220 ± 20	60 ± 6	80 ± 9	140 ± 20
Aconitase	30 ± 5	60 ± 7	80 ± 10	90 ± 10
Isocitric dehydrogenase	13 ± 2	11 ± 1	8 ± 1	11 ± 2
αChetoglutarate dehydrogenase	7 ± 1	4 ± 0,5	5 ± 0,5	6 ± 1
Succinil CoA synthetase	320 ± 30	115 ± 11	155 ± 16	210 ± 23
Succinic dehydrogenase	12 ± 2	9 ± 1	6 ± 0,7	6 ± 0,7
Fumarase	92 ± 9	42 ± 4	39 ± 4	33 ± 5
Malate dehydrogenase	900 ± 87	556 ± 55	753 ± 73	820 ± 86

Mitochondrial contamination excluded in purified myelin



MBP

Na+/K+ ATPase

ANT

TIM

AK3

Cyclic Nucleotide Phosphodiesterase (CNP) abundant in myelin born with sequence for importation in mitochondria

Journal of Neurochemistry

JNC

JOURNAL OF NEUROCHEMISTRY | 2015

doi: 10.1111/jnc.13142

ORIGINAL ARTICLE

Proteomics-level analysis of myelin formation and regeneration in a mouse model for Vanishing White Matter disease

Irit Gat-Viks,* Tamar Geiger,† Mali Barbi,* Gali Raini* and Orna Elroy-Stein*,‡

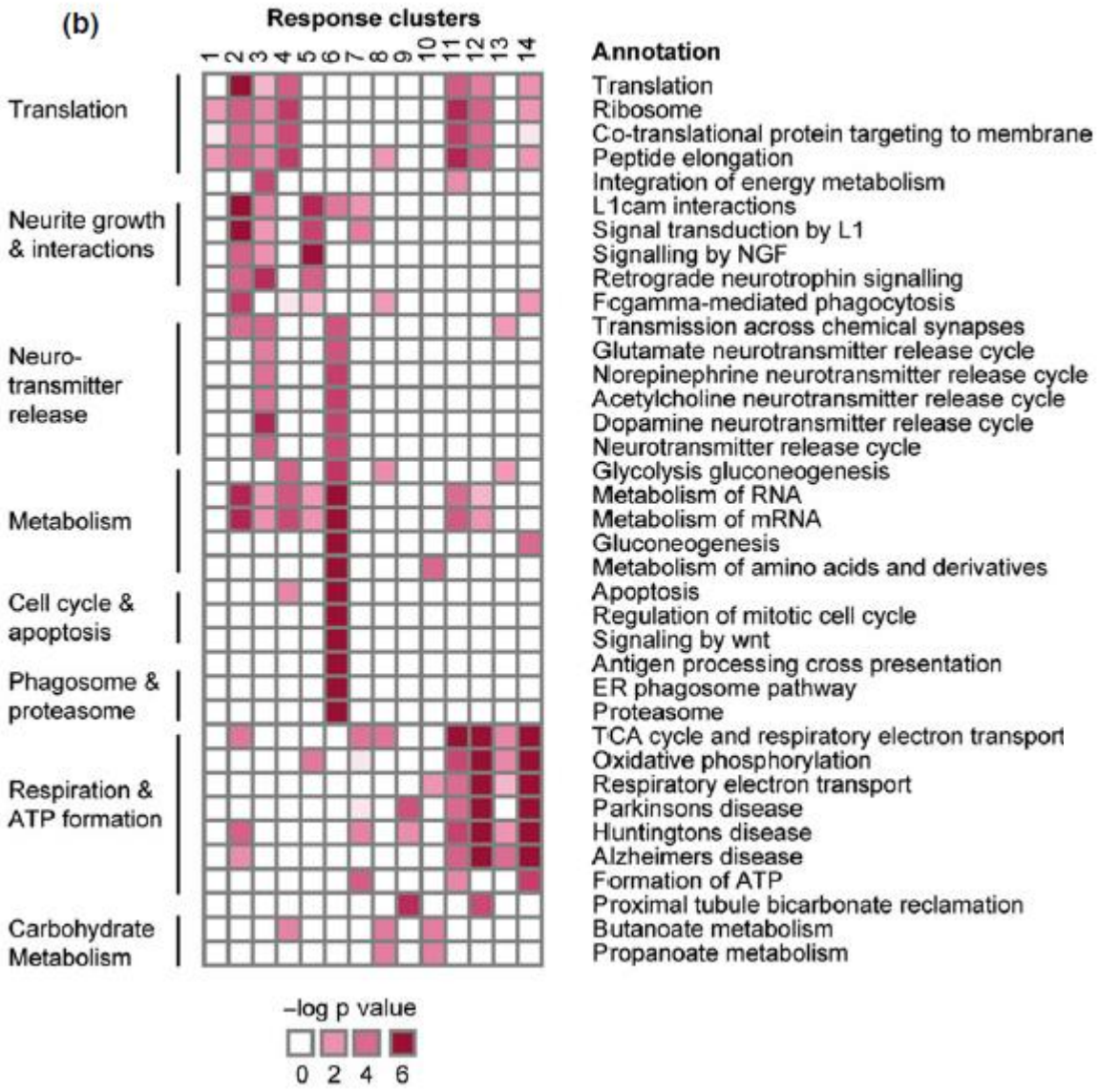
*Department of Cell Research & Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

†Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

‡Sagol school of Neuroscience, Tel Aviv University, Tel Aviv, Israel

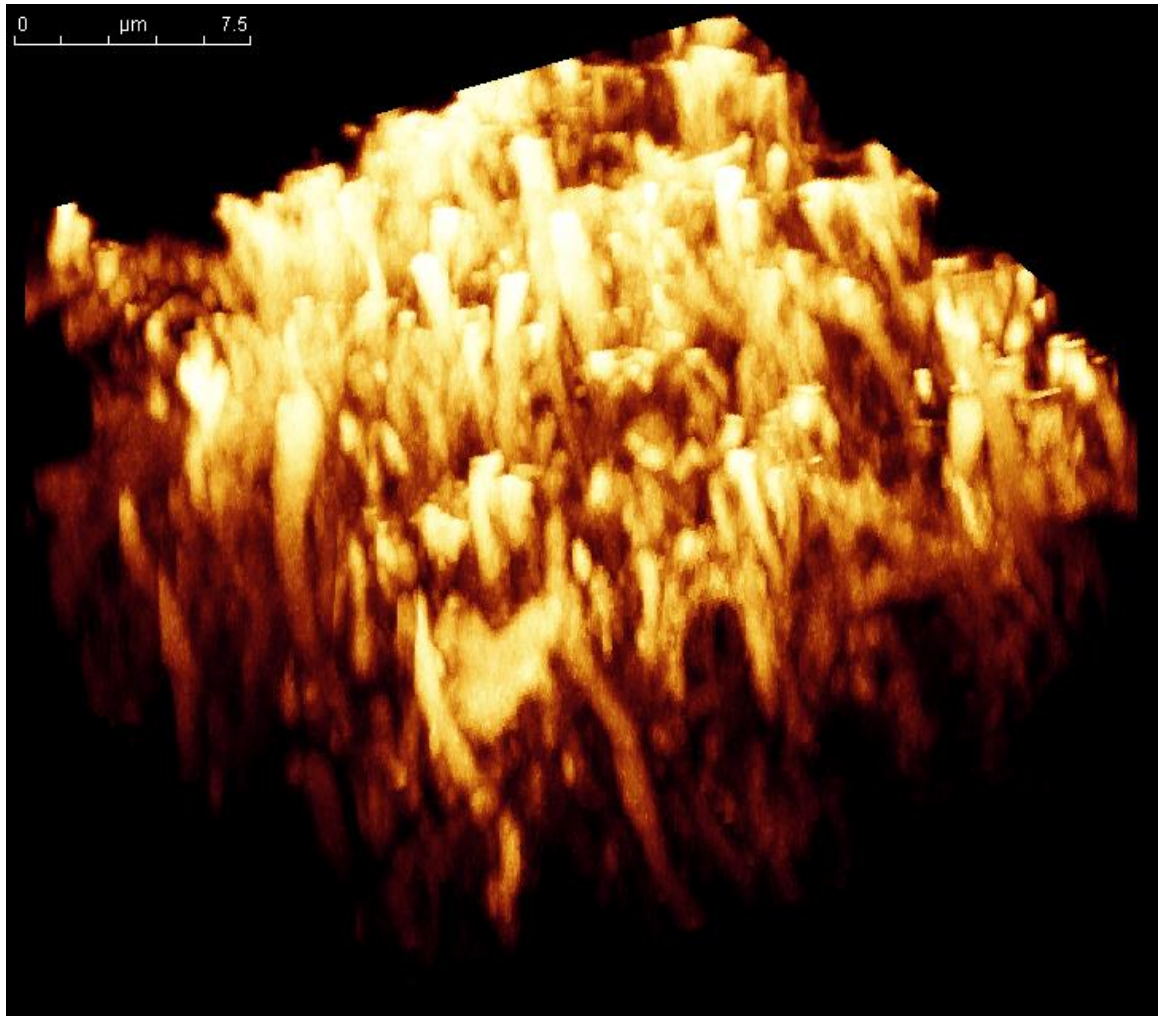
… The absence of other mitochondrial proteins in myelin vesicles ruled out mitochondrial contamination and led to an intriguing hypothesis that myelin sheaths may be able to perform aerobic metabolism by extra-mitochondrial oxidative phosphorylation, to produce ATP and provide it to axons via gap junctions (Morelli et al. 2011).**

** Morelli A., Ravera S. and Panfoli I. (2011) Hypothesis of an energetic function for myelin. *Cell Biochem. Biophys.* **61**, 179–187.



The possible energetic role of myelin sheaths may explain the degeneration of chronically demyelinated axons and suggest that VWM disease could result from malfunction of energy supply by myelin. The relative contribution of defective mitochondrial function versus possible faulty extra-mitochondrial oxidative phosphorylation to VWM pathogenesis will be addressed in future studies.

By a new technique that we have developed (Bianchini, 2008), myelin vesicles and optical nerves were incubated with MitoTracker Deep Red 633, a fluorescent dye that stains actively respiring membranes. After incubation, the samples were analyzed by CLSM.



(2)

Aerobic ATP synthesis in peripheric nervous system

Journal of Neurochemistry

JNC

JOURNAL OF NEUROCHEMISTRY | 2013

doi: 10.1111/jnc.12253

May 2013

ORIGINAL ARTICLE

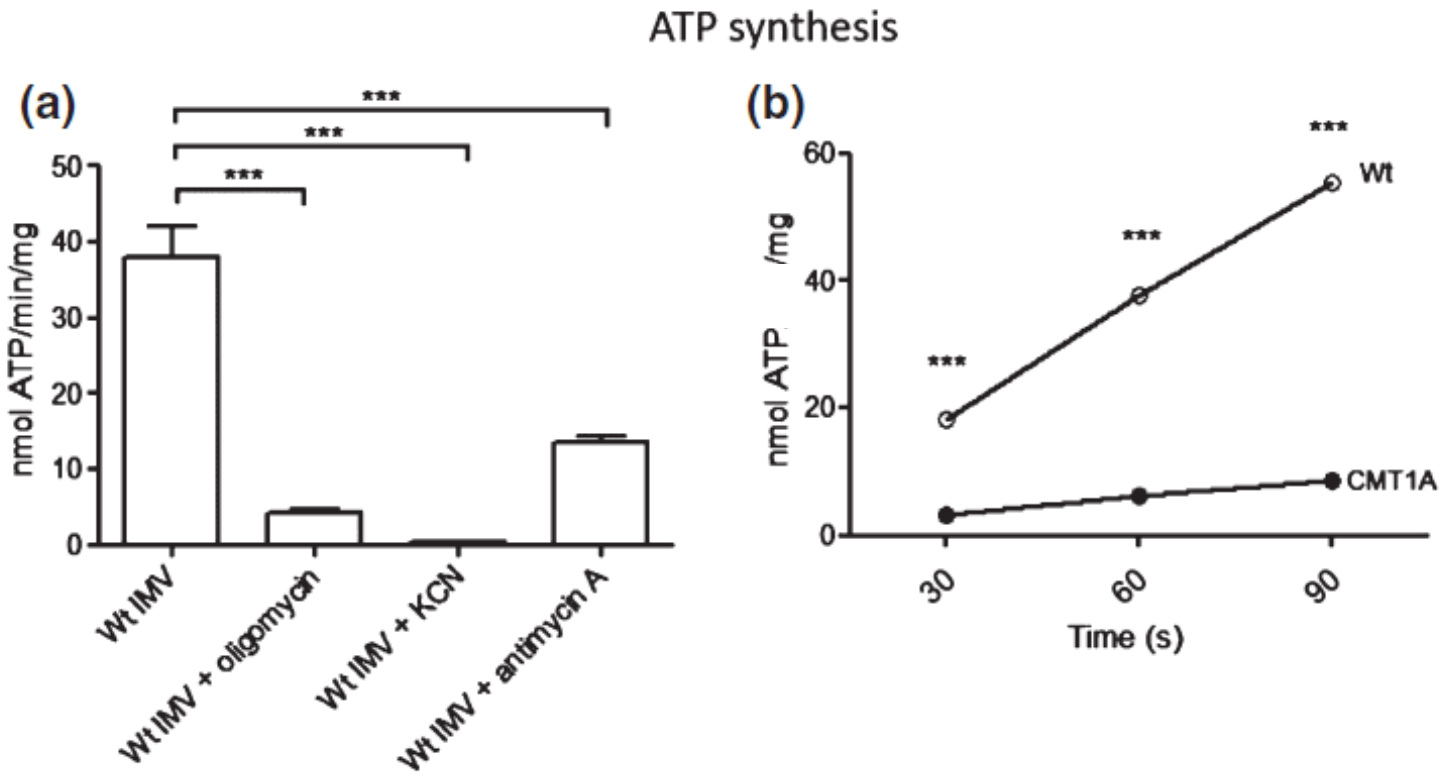
Oxydative phosphorylation in sciatic nerve myelin and its impairment in a model of dysmyelinating peripheral neuropathy

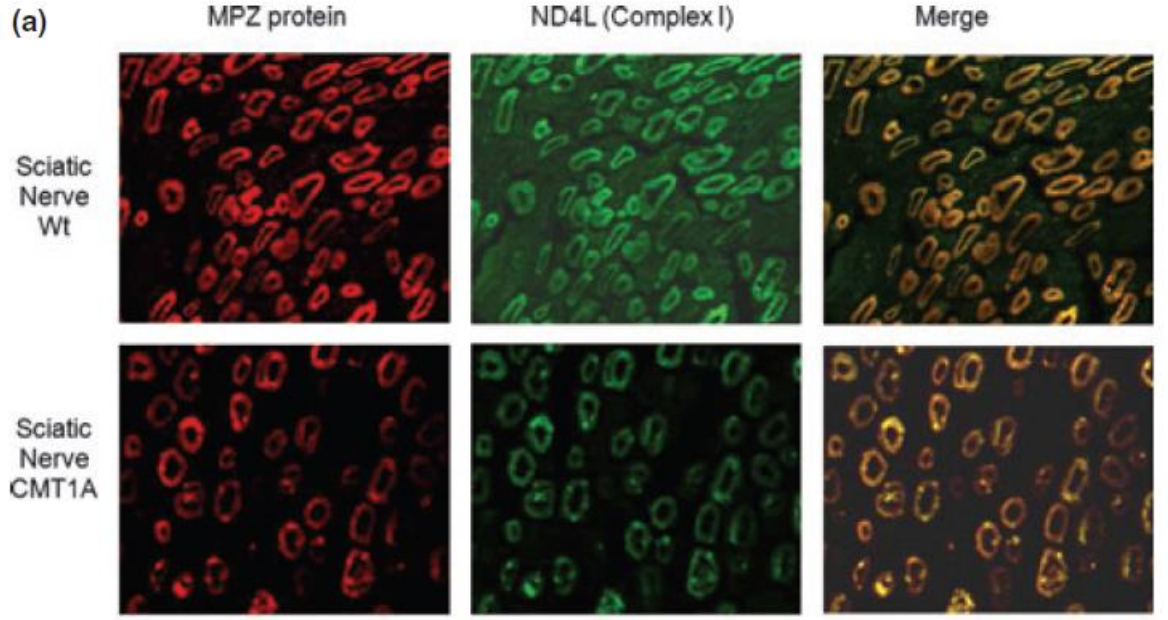
Silvia Ravera,*[,1] Lucilla Nobbio,†[,1] Davide Visigalli,† Martina Bartolucci,* Daniela Calzia,* Fulvia Fiorese,† Gianluigi Mancardi,† Angelo Schenone,† Alessandro Morelli* and Isabella Panfoli*

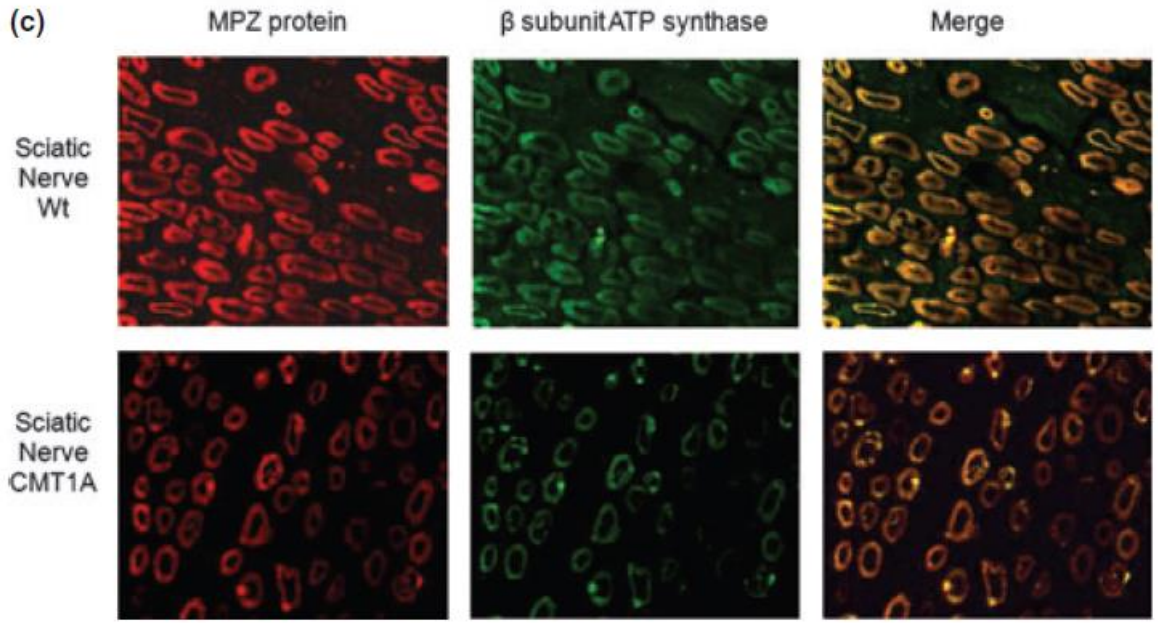
**DIFAR, University of Genoa, Italy*

†*DINOGMI, University of Genoa, Italy*

ATP Synthesis in Sciatic nerve-derived isolated myelin vesicles (IMV) from Wt and dysmyelinating peripheral neuropathy (CMT1A) rats




(a)
MPZ protein
ND4L (Complex I)
Merge
Sciatic Nerve Wt
Sciatic Nerve CMT1A

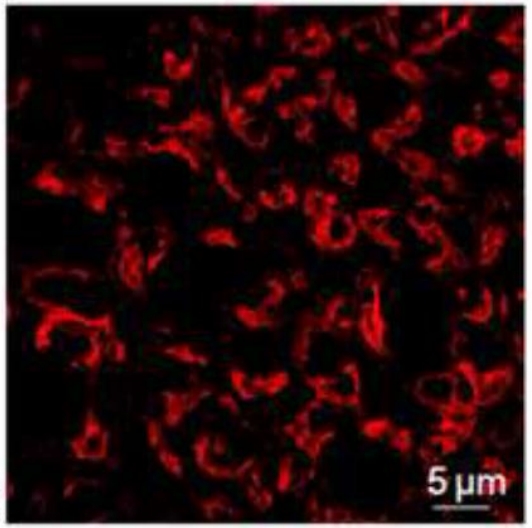

(c)
MPZ protein
β subunit ATP synthase
Merge
Sciatic Nerve Wt
Sciatic Nerve CMT1A

Gap-junction in myelin

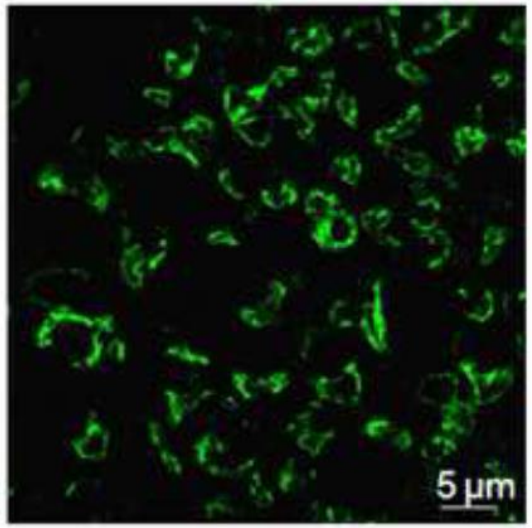
A

B

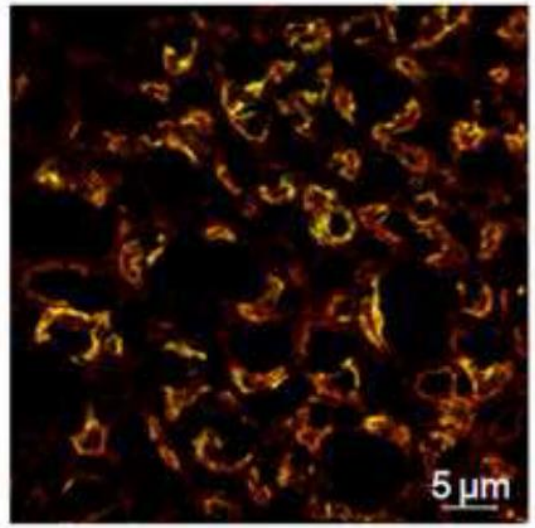
	Forebrain homogenate	Crude myelin	Isolated myelin
Cx32 (32 kDa)	5.2 ± 0.8	5.8 ± 0.6	4.7 ± 0.5



C



D



E

immunohystochemically stained with Ab against MBP

immunohystochemically stained with Ab against Cx32

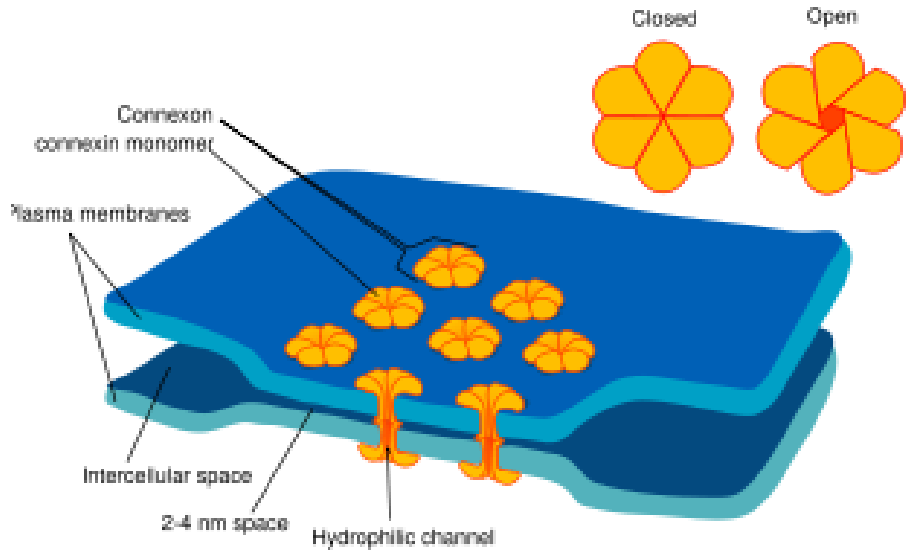
Merge

(3)

Hypothesis of molecular mechanism in respiring/energizing myelin sheath

GAP JUNCTIONS :

Structures that allow the transport of molecules throught two cells, by mass action. Considering that ATP is very concentrated in the cell it is possible hypothesize that connexins could transport ATP.



Gap Junctions between Cells Expressing Connexin 43 or 32 Show Inverse Permselectivity to Adenosine and ATP

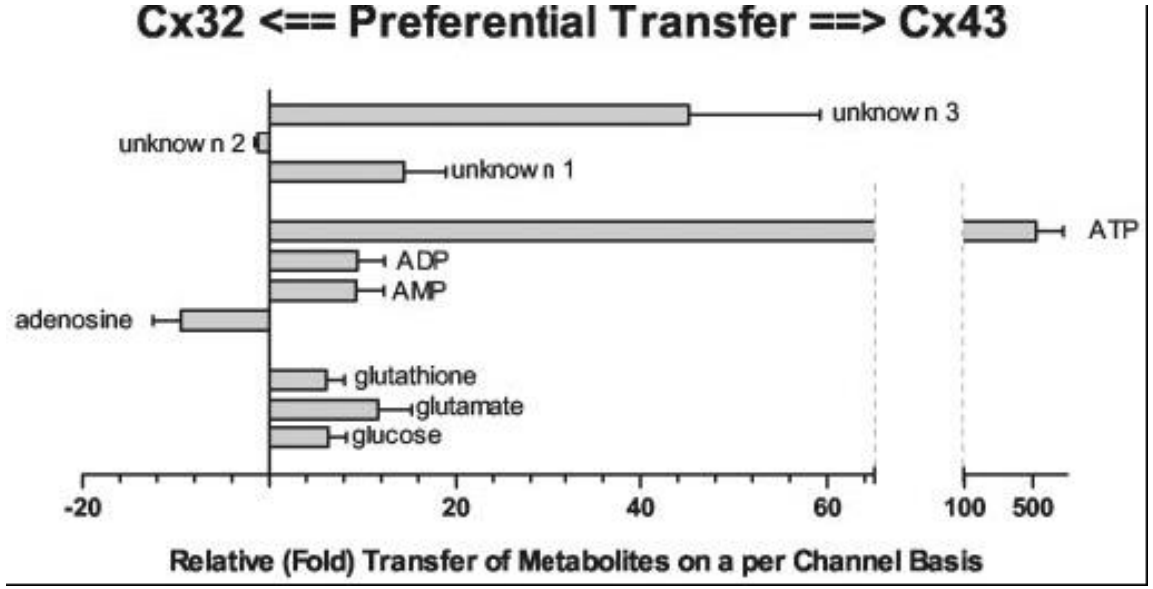
Gary S. Goldberg‡§, Alonso P. Moreno¶, and Paul D. Lampe

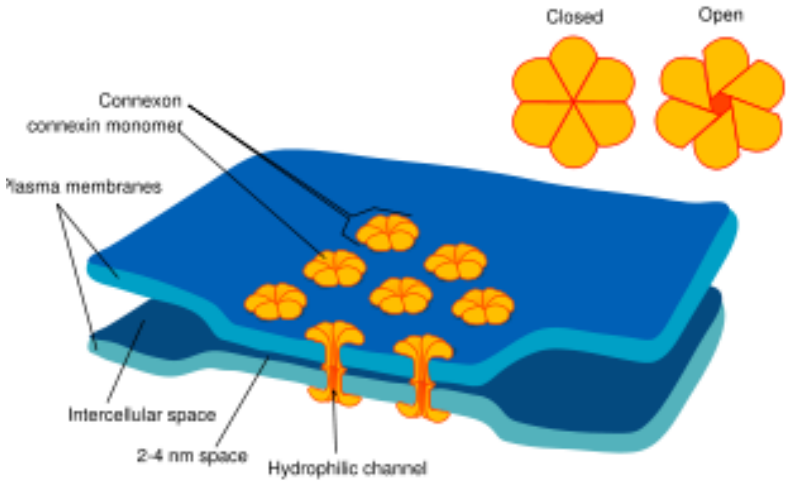
From the ‡Department of Physiology and Biophysics, State University of New York, Stony Brook, New York 11794-8661,

the ¶Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, Indiana 46202, and

Fred Hutchinson Cancer Research Center, Seattle, Washington 98109

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 39, Issue of September 27, pp. 36725–36730, **2002.**





Myelin is rich in Gap-Junctions

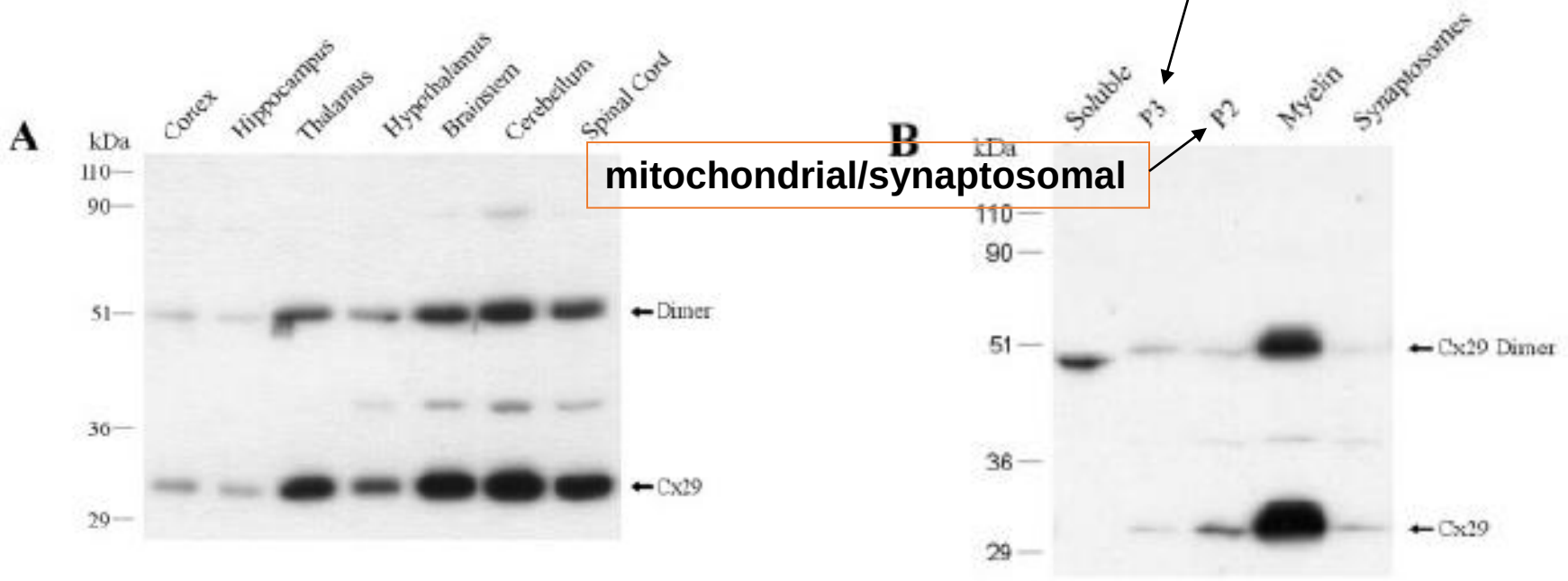
THE JOURNAL OF COMPARATIVE NEUROLOGY 464:356–370 (2003)

Connexin29 and Connexin32 at Oligodendrocyte and Astrocyte Gap Junctions and in Myelin of the Mouse Central Nervous System

JAMES I. NAGY,[1]* ANDREI V. IONESCU,[1] BRUCE D. LYNN,[1] AND JOHN E. RASH[2]
[1]Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba R3E 3J7, Canada

Microsomal membrane

mitochondrial/synaptosomal

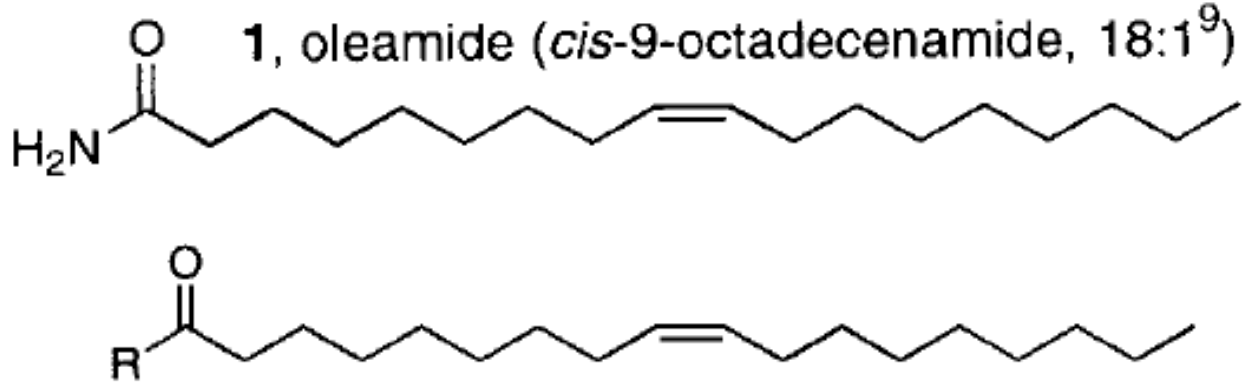


Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 4810–4815, April 1998
Chemistry

Chemical requirements for inhibition of gap junction communication by the biologically active lipid oleamide

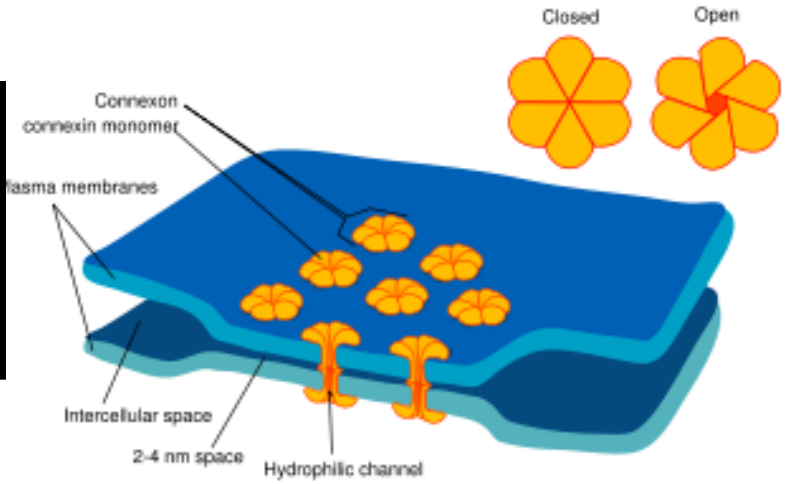
DALE L. BOGER*†, JEAN E. PATTERSON*, XIAOJUN GUAN‡, BENJAMIN F. CRAVATT*, RICHARD A. LERNER*, AND NORTON B. GILULA‡

Departments of *Chemistry and ‡Cell Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

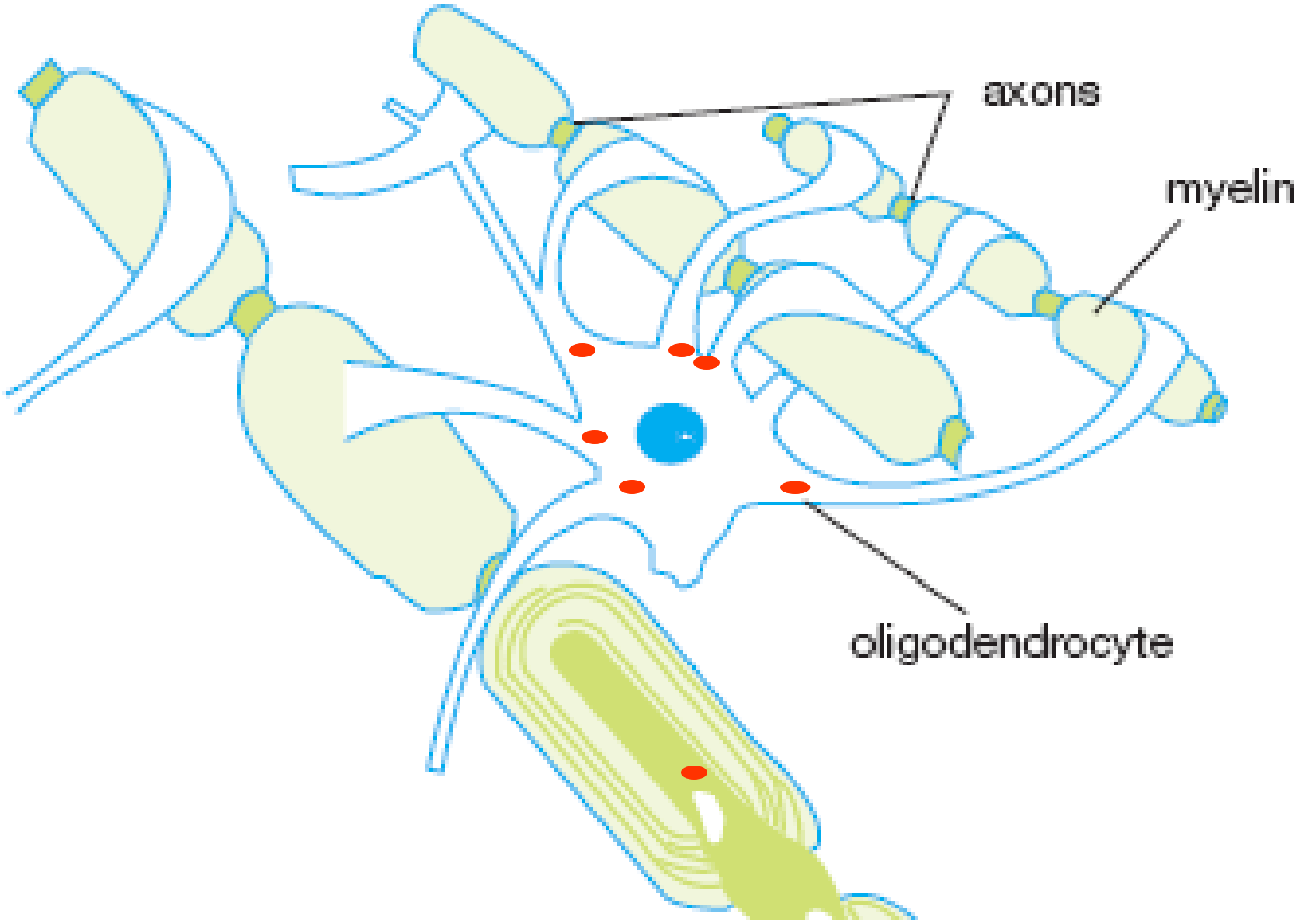


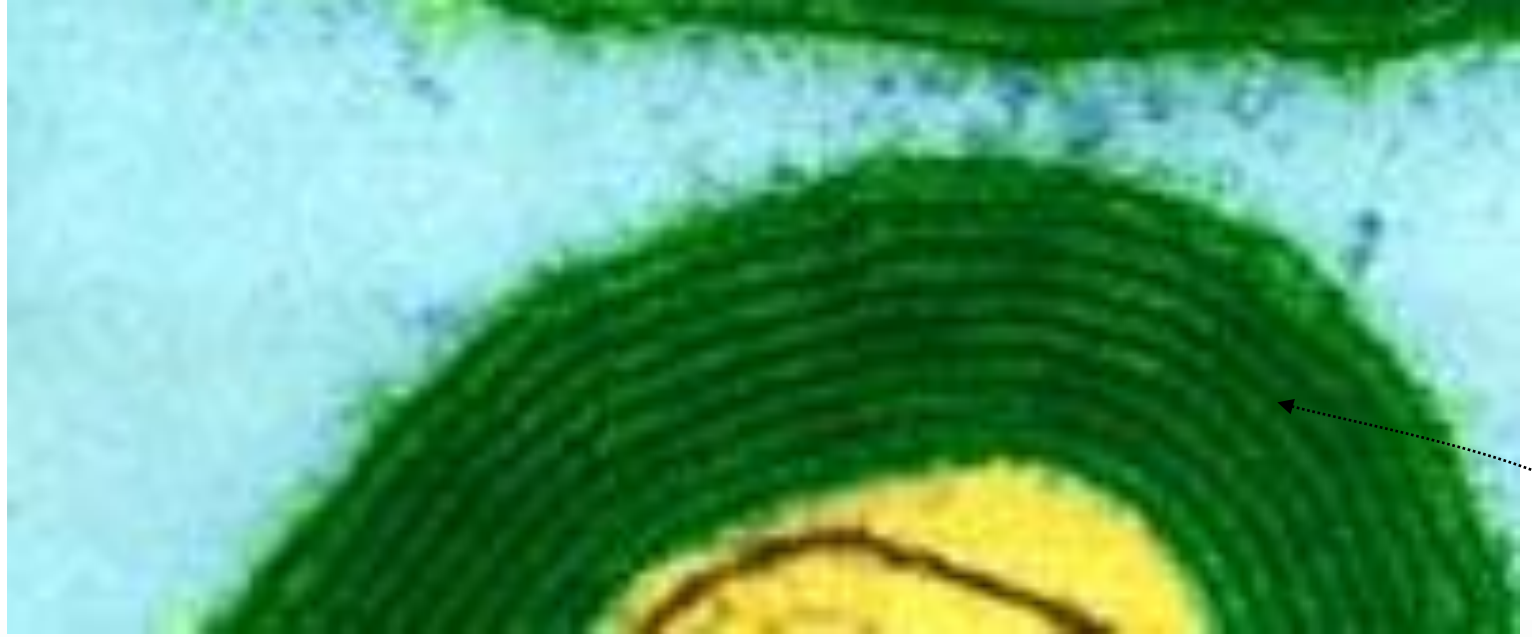
Agent (R)	% inhibition†, μM		
	100	50	20
NH_2 (oleamide)	100	100	100
OH (oleic acid)	0	0	0

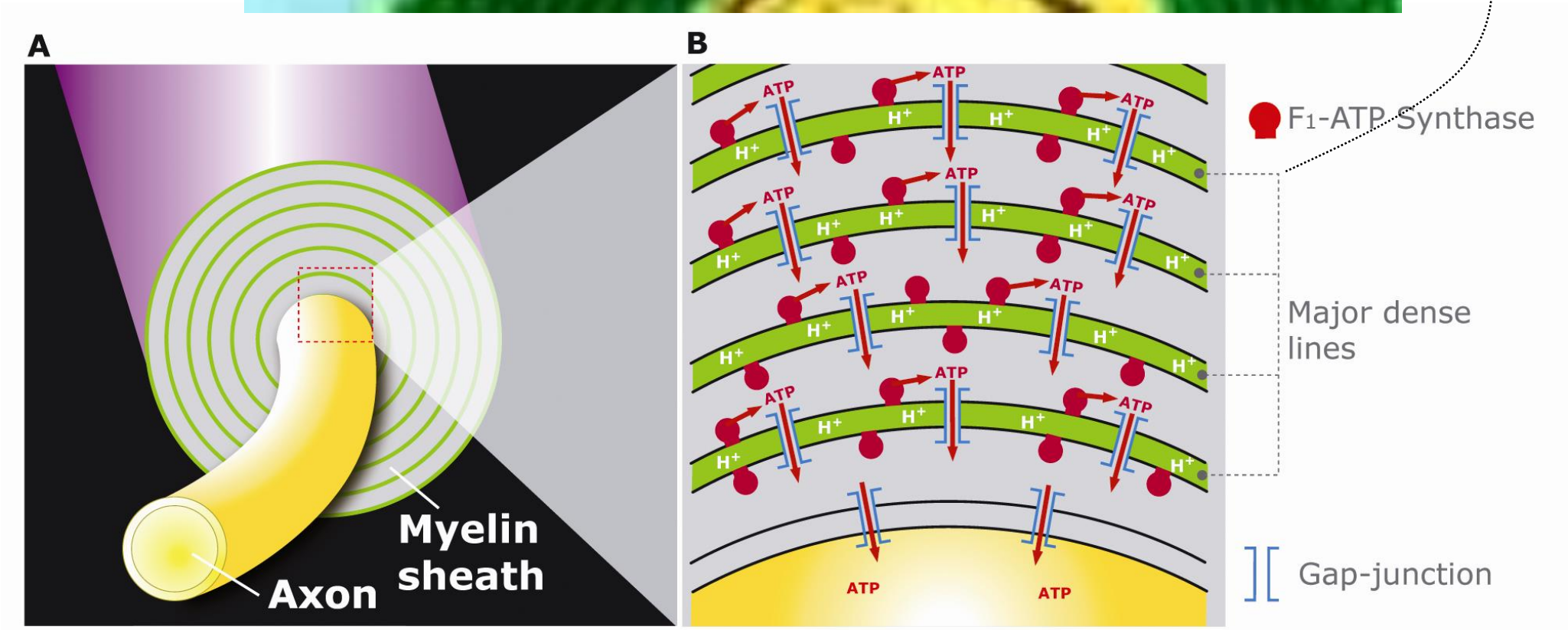
Test of ATP production with Oleamide, an inhibitor of Gap-Junctions



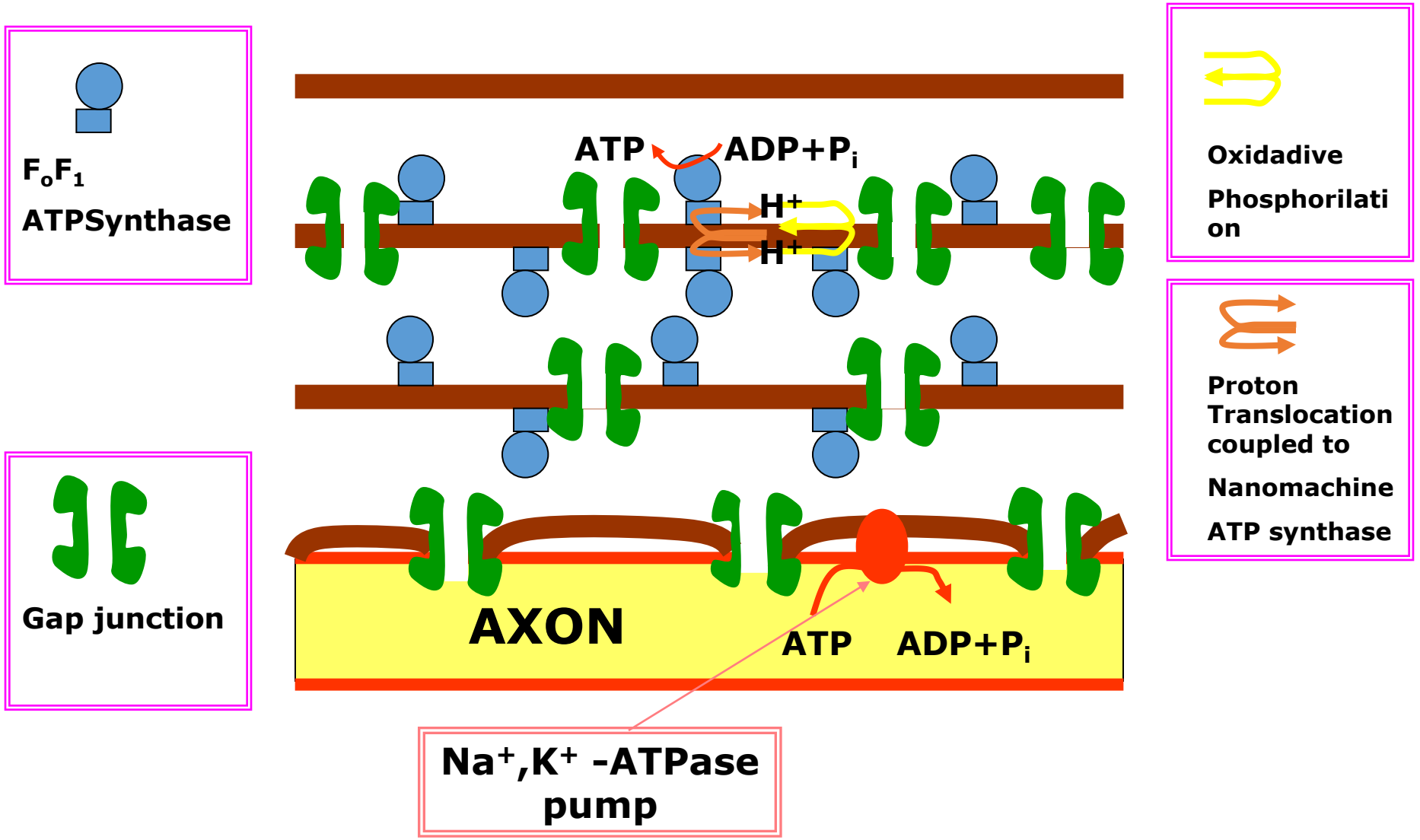
Sample	ATP produced (nmol/min/mg)
Myelin vesicles (+ DMSO)	35 ± 3.0
Myelin vesicles + Oleamide 50 μM (in DMSO)	5 ± 0,4
Myelin vesicles + Oleic Acid 50 μM (in DMSO)	35 ± 3.0


axons
myelin
oligodendrocyte




A
Myelin sheath
Axon
B
ATP
H+
F1-ATP Synthase
Major dense lines
Gap-junction

Hypothesis of Energized Myelin Sheath



(4)

Hypotheses on Proton movementation in biological membrane

Journal of Cerebral Blood Flow & Metabolism
12 sept 2013

Journal of Cerebral Blood Flow & Metabolism (2013), 1–5

www.jcbfm.com

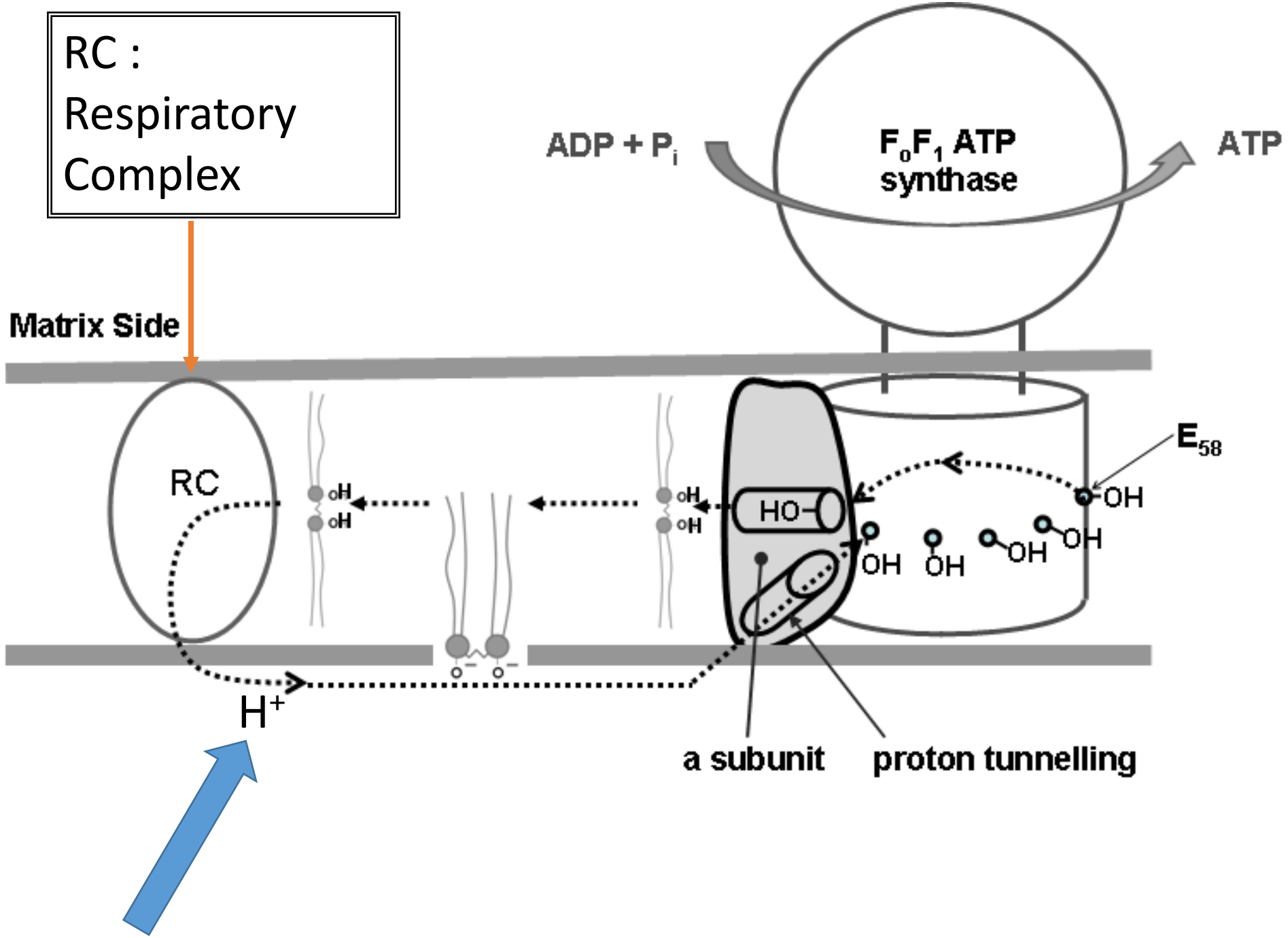
OPINION ARTICLE

Hypothesis of lipid-phase-continuity proton transfer for aerobic ATP synthesis

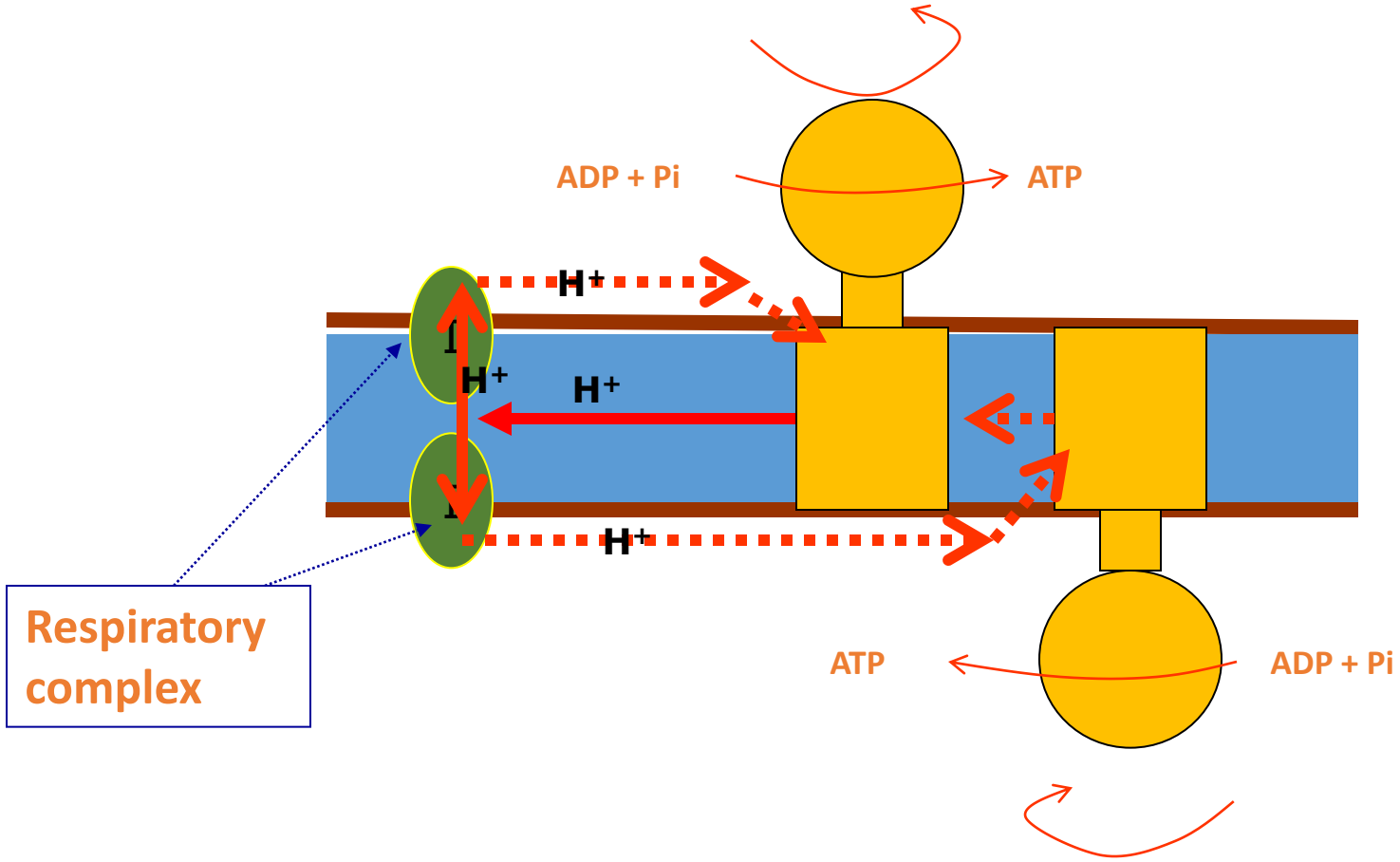
Alessandro M Morelli, Silvia Ravera, Daniela Calzia and Isabella Panfoli

The basic processes harvesting chemical energy for life are driven by proton (H^+) movements. These are accomplished by the mitochondrial redox complex V, integral membrane supramolecular aggregates, whose structure has recently been described by advanced studies. These did not identify classical aqueous pores. It was proposed that H^+ transfer for oxidative phosphorylation (OXPHOS) does not occur between aqueous sources and sinks, where an energy barrier would be insurmountable. This suggests a novel hypothesis for the proton transfer. A lipid-phase-continuity H^+ transfer is proposed in which H^+ are always bound to phospholipid heads and cardiolipin, according to Mitchell's hypothesis of asymmetric vectorial H^+ diffusion. A phase separation is proposed among the proton flow, following an intramembrane pathway, and the ATP synthesis, occurring in the aqueous phase. This view reminiscent of Grotthus mechanism would better account for the distance among the F_o and F_1 moieties of F_oF_1–ATP synthase, for its mechanical coupling, as well as the necessity of a lipid membrane. A unique active role for lipids in the evolution of life can be envisaged. Interestingly, this view would also be consistent with the evidence of an OXPHOS outside mitochondria also found in non-vesicular membranes, housing the redox complexes.

RC :
Respiratory
Complex



This model is consistent with the observed be-face operativity of ATP-Synthase in myelin



(5)

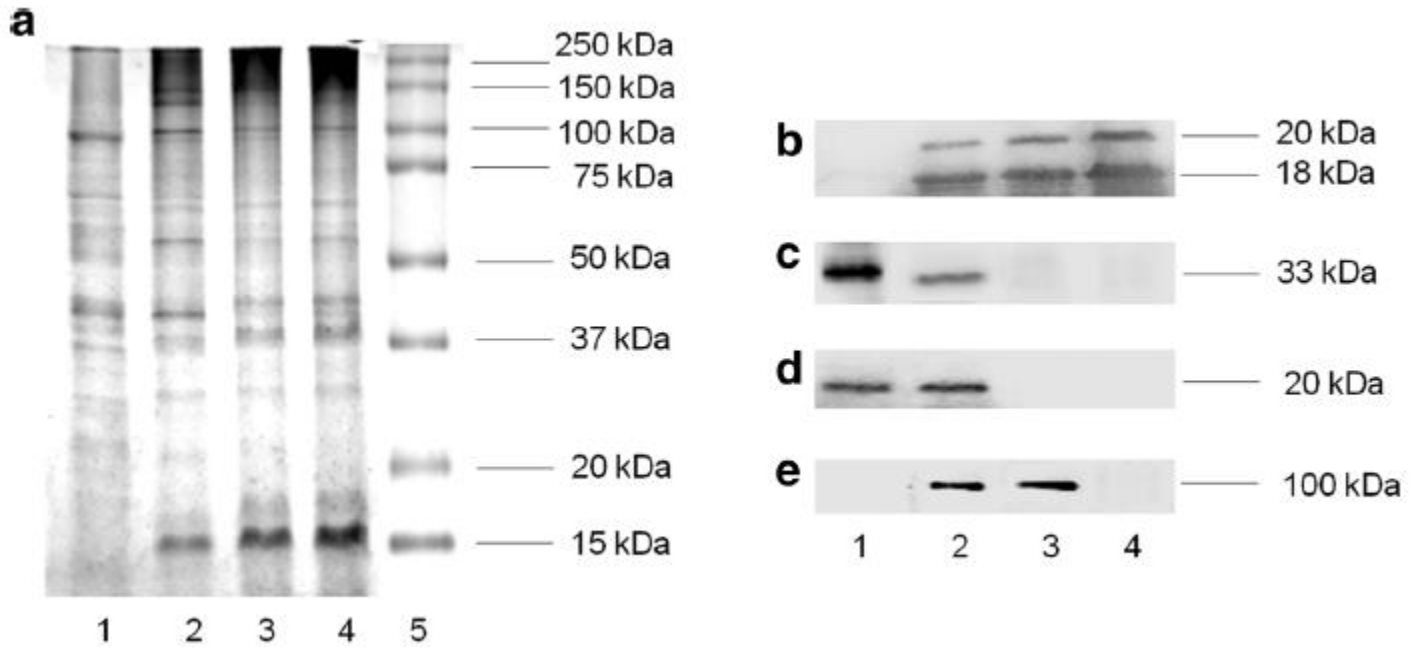
Support of Nerve Conduction By Respiring Myelin Sheath

Mol Neurobiol. 2015 Jun 2. [Epub ahead of print]

Support of Nerve Conduction by Respiring Myelin Sheath: Role of Connexons

Silvia Ravera[1] · Martina Bartolucci[1] · Enrico Adriano[2] · Patrizia Garbati[2] · Sara Ferrando[3] · Paola Ramoino[3] · Daniela Calzia[1] · Alessandro Morelli[1] · Maurizio Balestrino[2] · Isabella Panfoli[1]

1- Mitochondria
2 – Forebrain homogenate
3 – Crude myelin fraction.
4 – Isolated myelin



Western blot against:

← Myelin Basic Protein

← Adenine Nucleotide translocase

← Translocase Inner Membrane

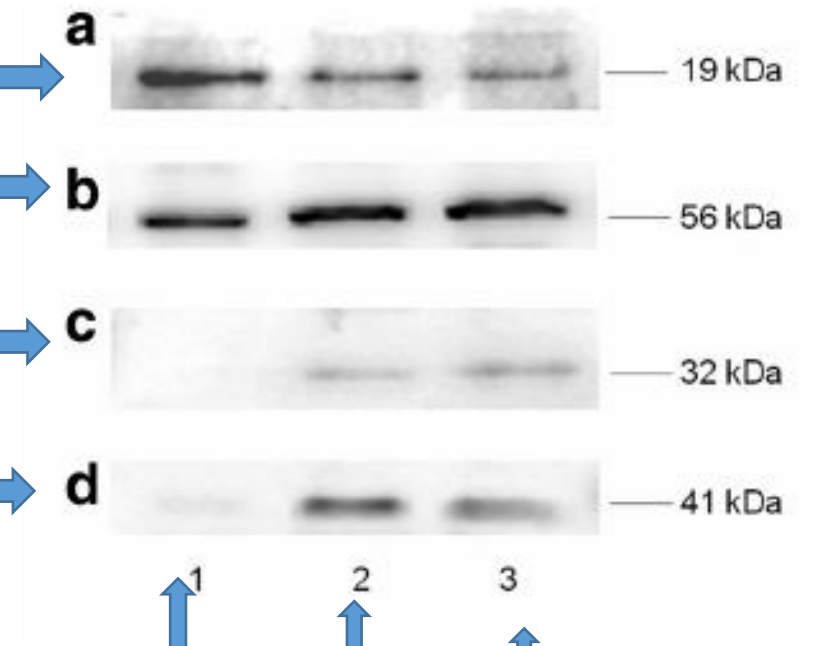
← Na/K ATP ase

Western Blot anti Cox IV

anti β subunit ATP synthase

anti Connexine 32

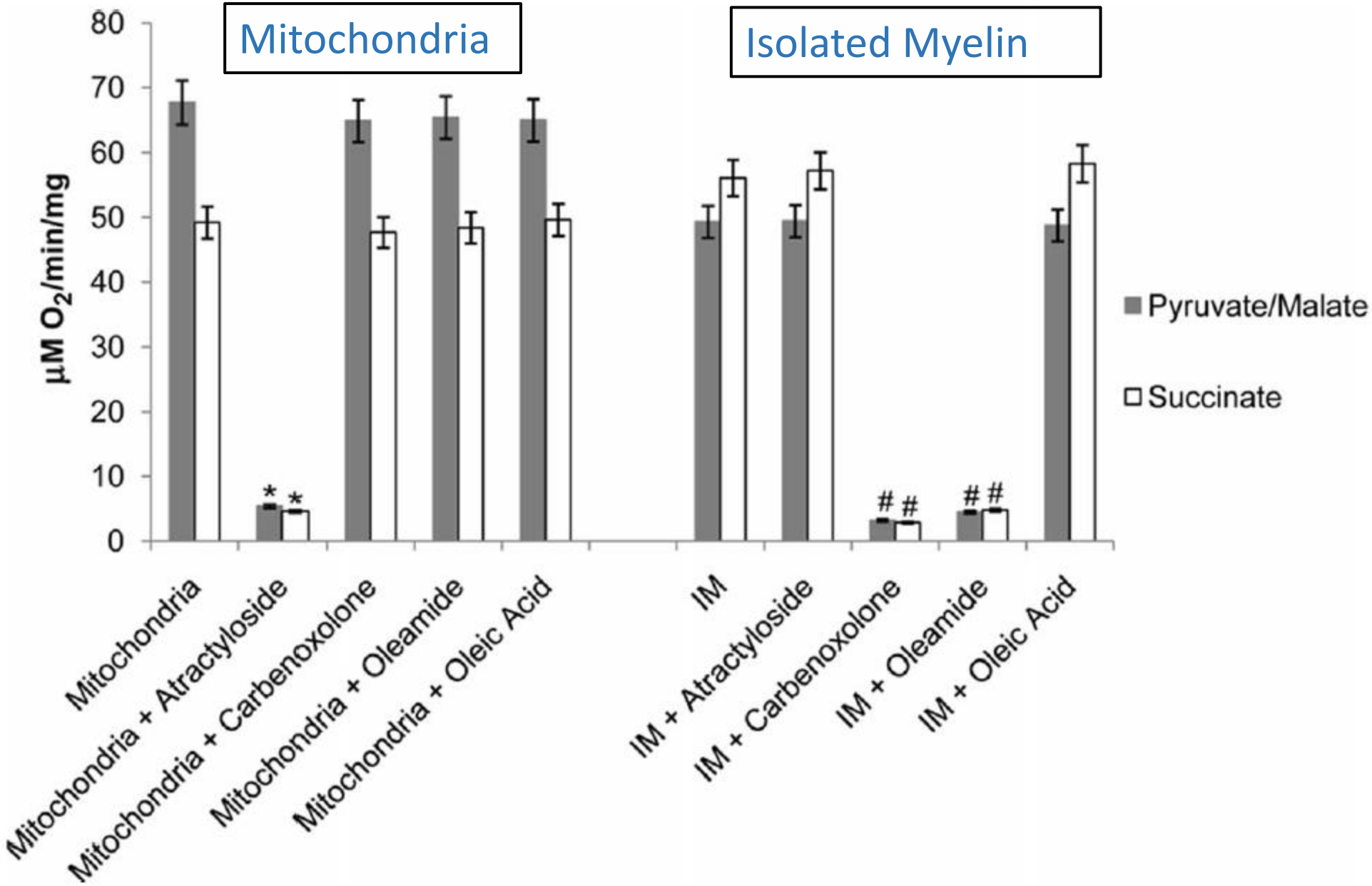
anti Connexine 43



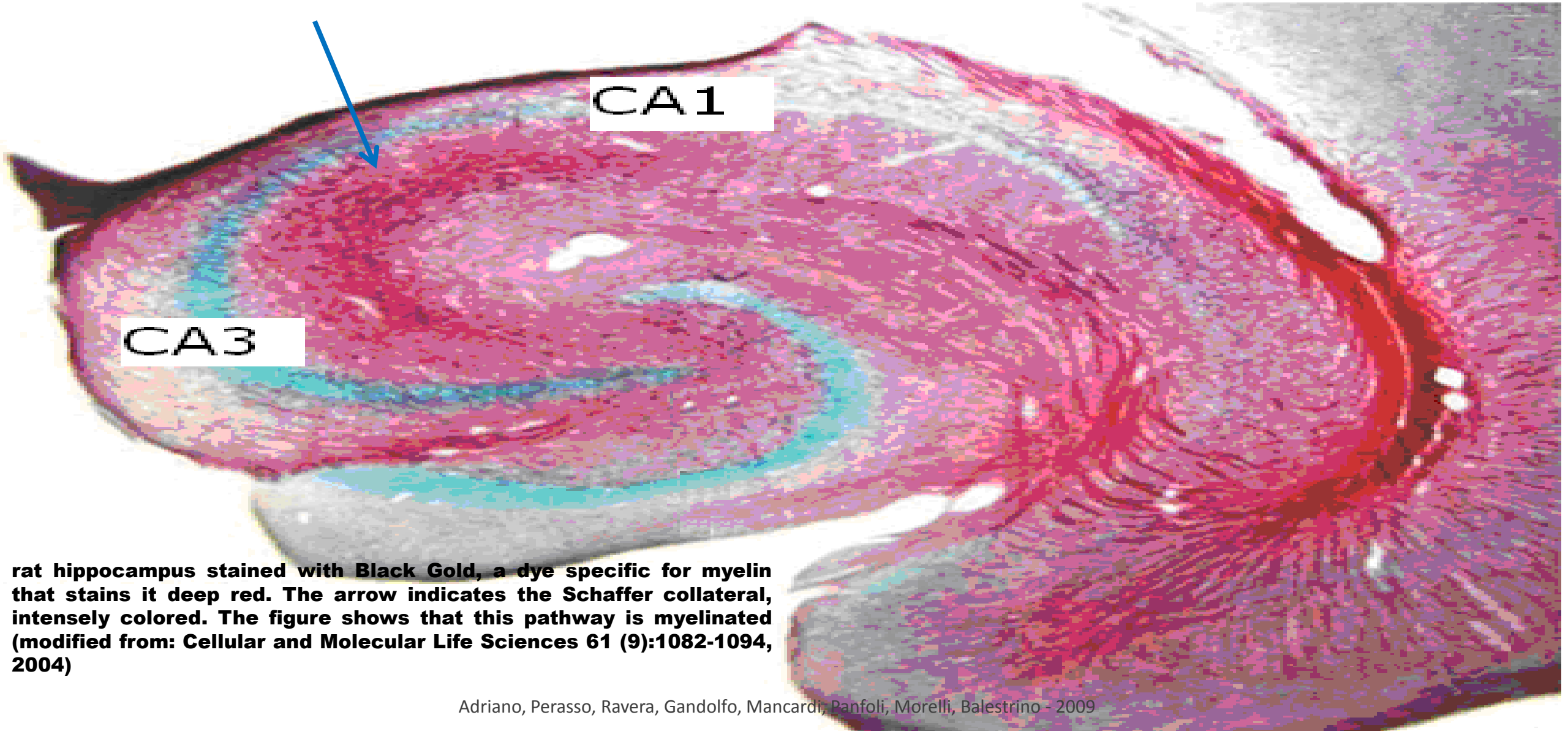
Mitochondria

Forebrain homog

Isolated Myelin

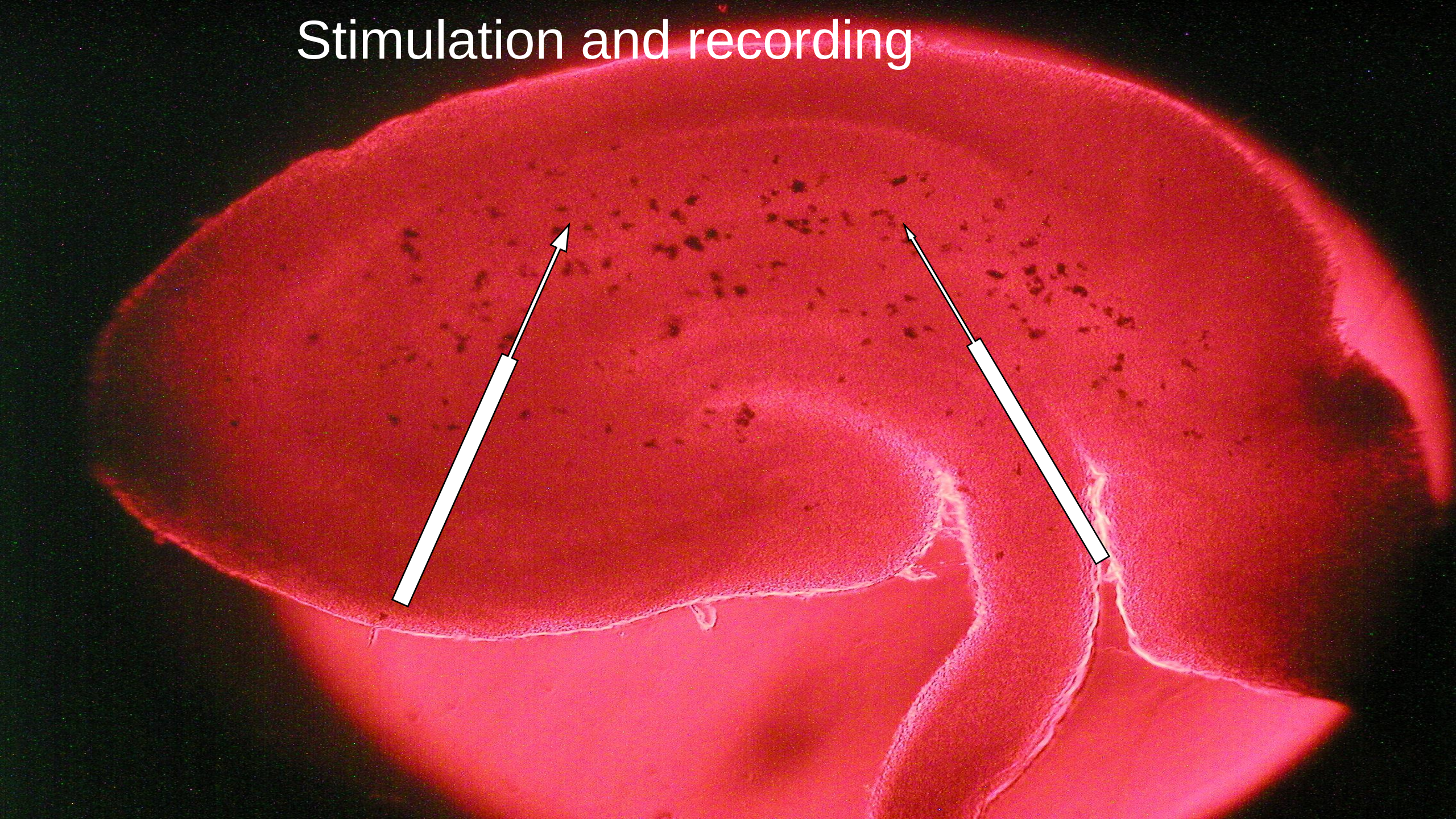

Mitochondria
Isolated Myelin
µM O2/min/mg
80
70
60
50
40
30
20
10
0
Pyruvate/Malate
Succinate
*
*
#
#
#
#
Mitochondria
Mitochondria + Atractyloside
Mitochondria + Carbenoxolone
Mitochondria + Oleamide
Mitochondria + Oleic Acid
IM
IM + Atractyloside
IM + Carbenoxolone
IM + Oleamide
IM + Oleic Acid

WE USED AS EXPERIMENTAL MODEL THE STIMULATION AND RECORDING OF 'NERVOUS IMPULSE AT THE SCHAFFER COLLATERAL , A CENTRAL NERVOUS MYELINATED PATHWAY

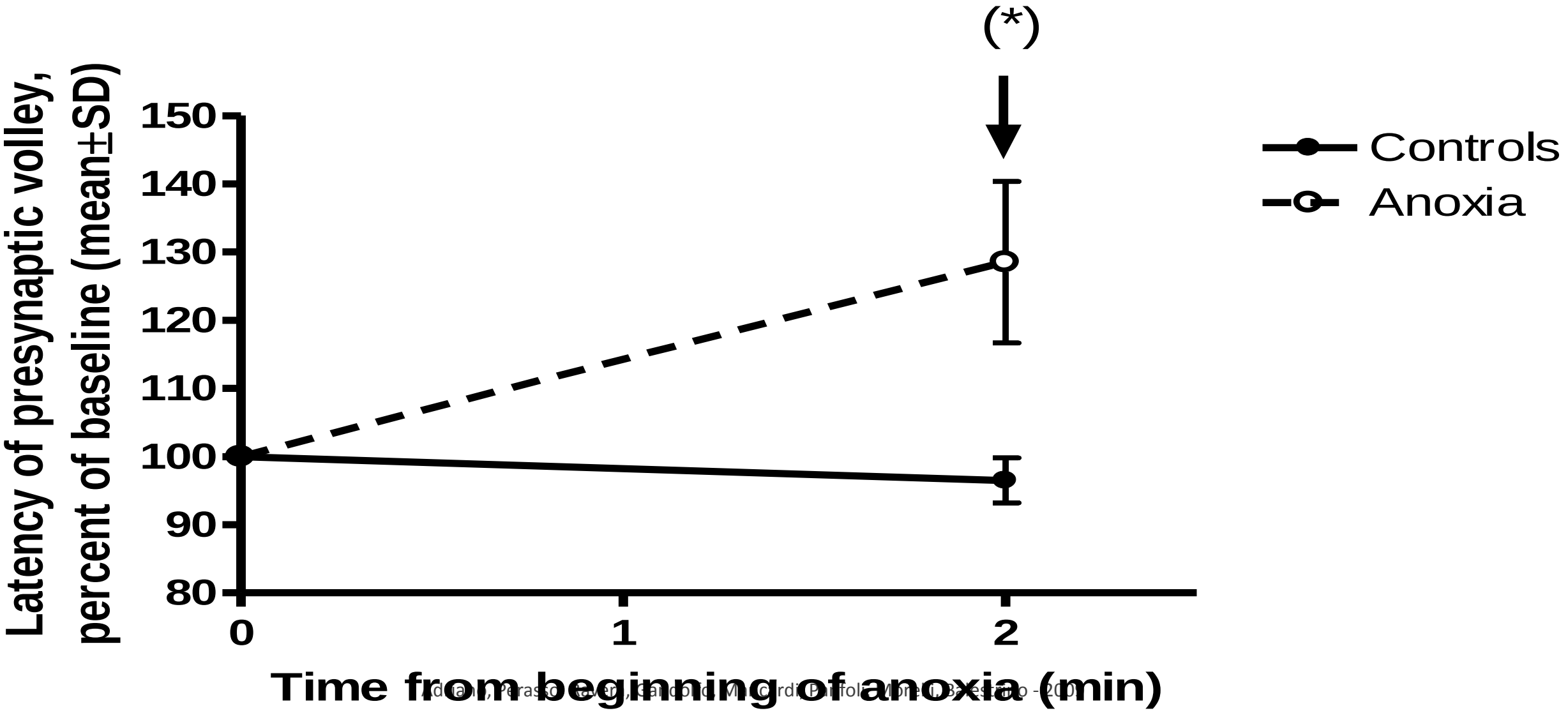


rat hippocampus stained with Black Gold, a dye specific for myelin that stains it deep red. The arrow indicates the Schaffer collateral, intensely colored. The figure shows that this pathway is myelinated (modified from: Cellular and Molecular Life Sciences 61 (9):1082-1094, 2004)

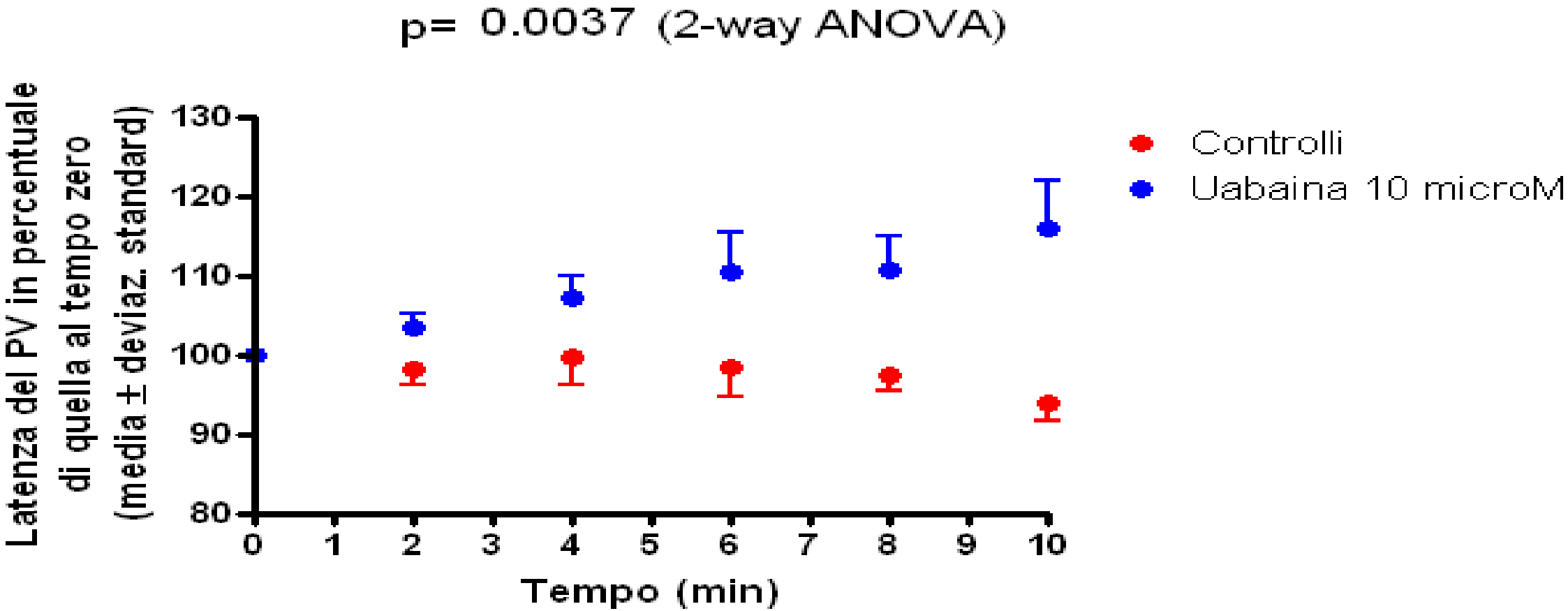
Stimulation and recording



Anoxia and velocity of conduction

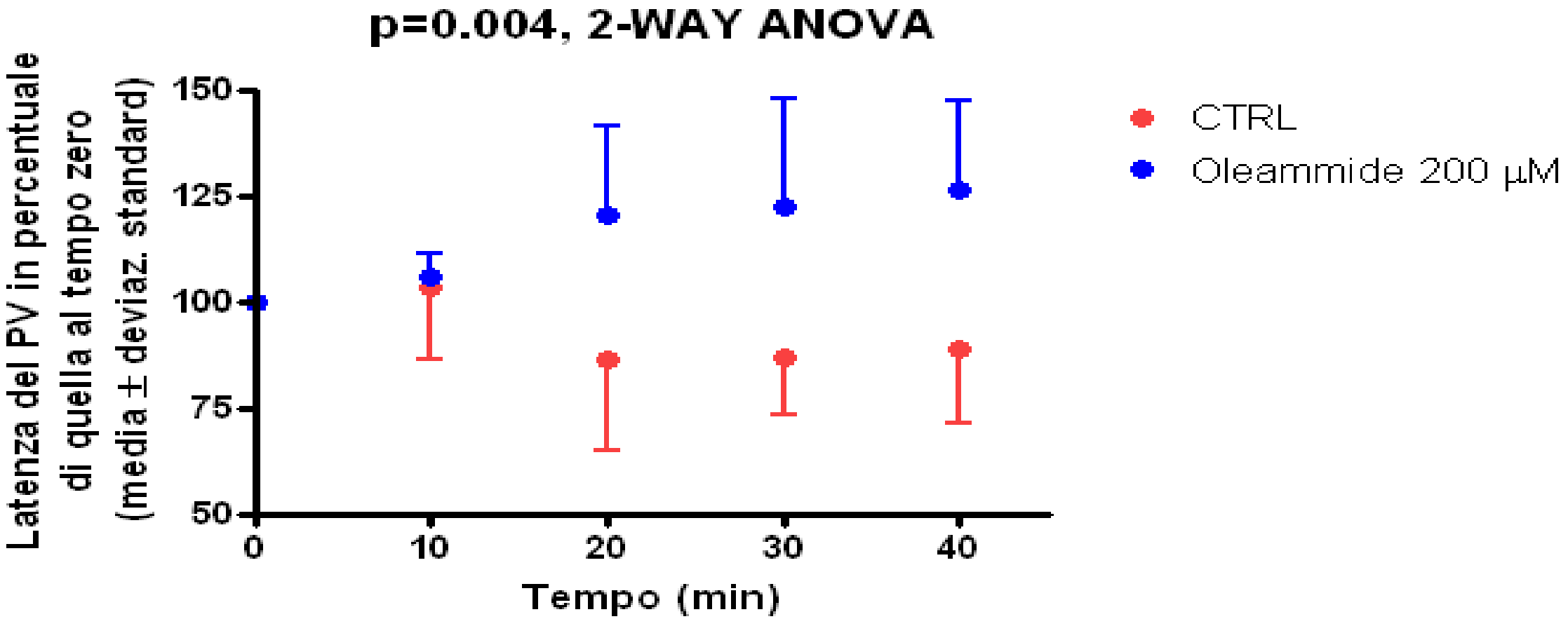


Inhibition of Na / K ATPase with uabaina reproduces the effects of ischemia and slows the conduction velocity in the Schaffer collateral



Adriano, Perasso, Ravera, Gandolfo, Mancardi, Panfoli, Morelli, Balestrino - 2009

The experiment mimics the effect of ouabain and 'ischemia: Blocking of Gap-Junctions between neurons and glia with Oleammide is compatible with a transfer of ATP from glia to axons.



Adriano, Perasso, Ravera, Gandolfo, Mancardi, Panfoli, Morelli, Balestrino - 2009


FIRST EYD
protein
purification
27/09/2010

Acknowledgements:

Isabella Panfoli, Silvia Ravera, Daniela Calzia, Martina Bartolucci

Dip.to di Farmacia, Università di Genova

Mario Pestarino, Simona Candiani

Dip.to di Scienze della Terra, dell'Ambiente e della Vita - Genova

Camillo Rosano

Centro di Biotecnologie Avanzate (CBA) - Genova

Gian Luigi Mancardi, Carlo Traverso, Angelo Schenone,

Maurizio Balestrino, Enrico Adriano

Dip.to di Neuroscience, Oftalmologia e Genetica, Università di Genova

Carlo Tacchetti, **Katia Cortese**

Dip.to di Medicina Sperimentale, Università di Genova

Vito Pistoia, Giovanni Candiano, Elisa Ferretti

Istituto Giannina Gaslini – Genova

Lucia Manni, Federico Caicci

Dipartimento di Biologia - Padova