JNR1444Y "Fundamentals of Neuroscience: Cellular and Molecular"

Lecture 2 *Sep 20, 2016*

Glial Cell Physiology and Glial-Neuronal Interactions

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RECOMMENDED READING:

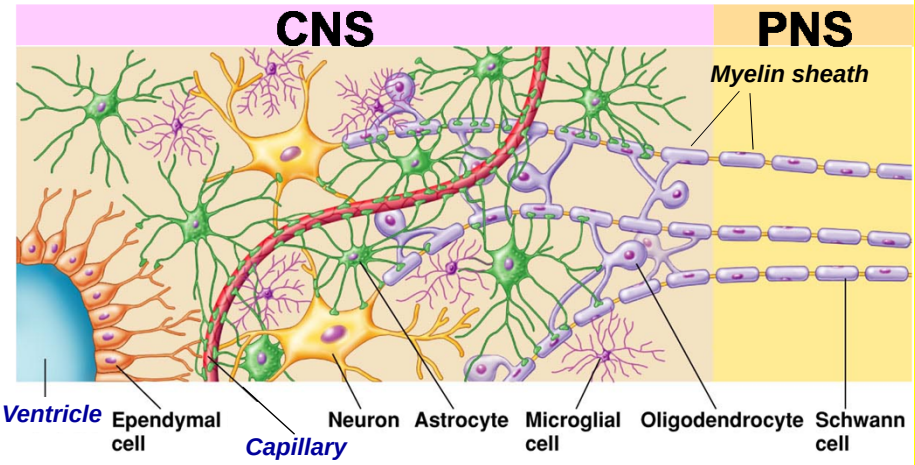
Kandel ER; Schwartz JH; Jessell TM; Siegelbaum SA & Hudspeth AJ
Principles of Neural Science, 5th edition (2013). ***Part II***

ADDITIONAL READING:

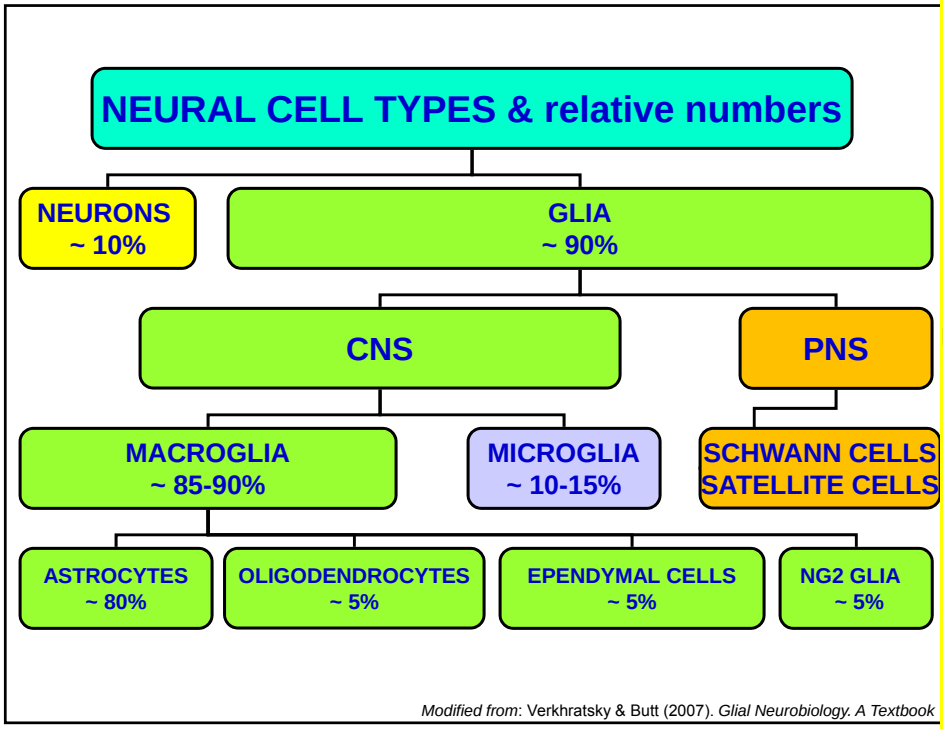
Verkhratsky A & Butt A (2007). **Glial Neurobiology. A textbook**. *John Wiley & Sons.*
Kettenmann H & Ransom BR, eds *(1995)* **Neuroglia**. *Oxford Univ. Press*
Kettenmann H & Ransom BR, eds *(2005)* **Neuroglia**, 2nd ed. *Oxford Univ. Press*
Kettenmann H & Ransom BR, eds *(2012)* **Neuroglia**, 3rd ed. *Oxford Univ. Press*

NEUROGLIA: Introductory Notes:

- **Neurons** (*excitable cells*)
- **Glial cells** (*"non-excitable" cells*)



the switch from oligodendrocyte



Modified from: Verkhratsky & Butt (2007). *Glial Neurobiology. A Textbook*

Dude used NeuN => saw how

General notes

ble":

rate action potentials.

s a wide variety of ion channels, receptors and transporters

connections:

not form or receive typical synaptic connections

eceive and send signals to each other and to neurons using ransmitters and gap junctions

cal diversity:

of glial cells have extensive processes

f them have true axons

diversity:

ous aspects of the development, functional activity, n, regeneration and remodelling of neurons and their ..

4

may have exceptional AP abilitiesdo respond to cha

glia = glue => hasn't been provenhousekeeping *=> clean up NT spillover => turnoverglymphatic system: espe

NEUROGLIA: Diverse roles in supporting neuronal function

- **In development**:
  - Scaffolding for neuronal migration (radial glia)
- **Mechanical support** of neurons in CNS
- **Housekeeping:**
  - Stabilizing the extracellular ionic media (*e.g.*, ***K^+ spatial buffering***) and participating in the ***turnover of neurotransmitters***
  - ***"Glymphatic" system*** *(brain drainage system)*
- **Scavenging**:
  - removing cell/tissue debris after injury or neuronal death
- **Formation and maintenance of myelin sheath** of axons
- Active role in **blood-brain barrier**
- Role in information handling and **memory storage**

5

NEUROGLIA: Underestimated before… …Overestimated now?

"To those who believed in glial cells during the long, dark period when the neuron dominated brain science."
(Kettenmann H & Ransom BR, (1995) dedication on First Edition of Neuroglia)

A revolution of views in the past two decades:
ASTROCYTES, FROM BRAIN GLUE TO COMMUNICATION ELEMENTS: THE REVOLUTION CONTINUES.
Volterra A & Meldolesi J (2005), Nat Rev Neurosci 6, 626-640

Glial cells … for many years have attracted little scientific attention. Neurophysiologists concentrated their research efforts instead on neurones and neuronal networks, because it was thought that they were the key elements responsible for higher brain function. Recent advances, however, indicate this isn't exactly the case....
From back cover of Verkhratsky A & Butt A. (2007) Glial Neurobiology. A textbook

6

Glia => major journal for glial researchThey were boringresponded to glutamineGlia began to be interesting in 60'sGlia can release gluta

MACROGLIA cell types

CNS

Astrocytes:

- Protoplasmic astrocytes
- Fibrous astrocytes
- Radial astrocytes
  - (e.g., Bergmann glia, Müller cells)
- Perivascular astrocytes
- Interlaminar astrocytes
- Immature astrocytes, precursors, glioblasts
- Marginal astrocytes
- Tanycytes
- Velate astrocytes

Oligodendrocytes:

- Myelinating oligodendrocytes
  - 4 types (I, II, III, IV)
- Oligodendrocyte precursor cells

Ependymal glia:

- Ependymocytes
- Choroid plexus cells

NG2 glia
("polydendrocytes"; oligodendrocyte precursors)

PNS

Schwann cells:

3 types:

- Myelinating
- Non-myelinating
- Perisynaptic

Satellite cells:

- **In peripheral ganglia**: cover the cell bodies of neurons

(**Microglia** not covered in this lecture: CNS resident macrophages; mesodermal origin)

7

ependymocytes line ventr

Electrophysiologically glia is studied much less than neurons

PubMed search: Publications with patch clamp studies

Year	Neurons	Astrocytes	Oligodendrocytes	N : A : O
2001	1327	48	8	100 : 3.6 : 0.6
2002	1258	32	13	100 : 2.5 : 1.0
2003	1628	37	11	100 : 2.3 : 0.6
2004	1406	43	5	100 : 3.1 : 0.3
2005	1558	43	9	100 : 2.7 : 0.5
2006	4898	200	56	100 : 4.1 : 1.1
2007	5091	152	33	100 : 2.9 : 0.6
2008	2404	66	20	100 : 2.7 : 0.8
2009	3905	109	37	100 : 2.8 : 0.9
2010	3521	116	29	100 : 3.3 : 0.8
2011	2836	111	20	100 : 3.9 : 0.7
2012	1151	46	8	100 : 4.0 : 0.7
2013	1100	37	8	100 : 3.4 : 0.7
2014	981	26	4	100 : 2.7 : 0.4
2015	1003	33	9	100 : 3.3 : 0.9

8

less confirmation in Glial researchNeurons have tons of confirmationmost of the studies are not electrophysiological

potassium is elevated extracellularly => affects the membrane potential of Glial cells => depol

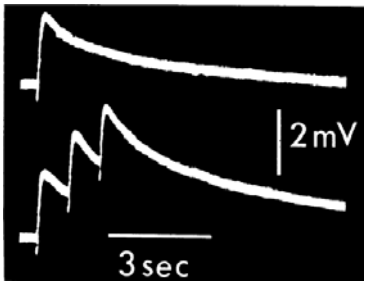
recorded memb pot of r

FIRST INSIGHTS INTO GLIAL-NEURONAL INTERACTIONS: Glial sensing the neuronal activity

Intracellular recording from glia in optic nerve:

Action potentials → depolarization of neighboring glial cells:

- **Single AP in axon →** long-lasting depolarization of glial cell
- **Repetitive APs → summation** of glial cell depolarizations**, and (!!!) faster decay**



J Neurophysiol 29:788-806 (1966)

EFFECT OF NERVE IMPULSES ON THE MEMBRANE POTENTIAL OF GLIAL CELLS IN THE CENTRAL NERVOUS SYSTEM OF AMPHIBIA[1]

R. K. ORKAND,[2] J. G. NICHOLLS,[3] AND S. W. KUFFLER
Neurophysiology Laboratory, Department of Pharmacology, Harvard Medical School, Boston, Massachusetts

(Received for publication February 7, 1966)

NEURONS AND GLIAL CELLS in most parts of the nervous system are intimately apposed, separated from each other by channels about 150 Å. wide. It is natural to wonder whether the two types of cell influence one another either "electrically" (i.e., by current flow from one cell to the other) or by the release of a substance. It has been shown in the leech nervous system that if

9

PART 1: Astrocytes

- Overview of astrocyte functions
- Types of astrocytes
- Astrocytic networks:
  - Ion channels in astrocytes: many K^+. few Na+
  - Gap junctional coupling
  - Spatial K^+ buffering
  - Ca^{2+} waves in astrocytic networks
  - Astrocyte microdomains
- Astrocytes and synaptic function
  - Gliotransmission and glial modulation of synaptic transmission
  - Astrocytic glutamate transport and turnover
  - Neurotransmitter receptors in astrocytes
- Blood-brain barrier (BBB)
  - astrocyte-vascular coupling
- Metabolic astrocyte-neuronal cooperation
- Reactive astrocytes

10

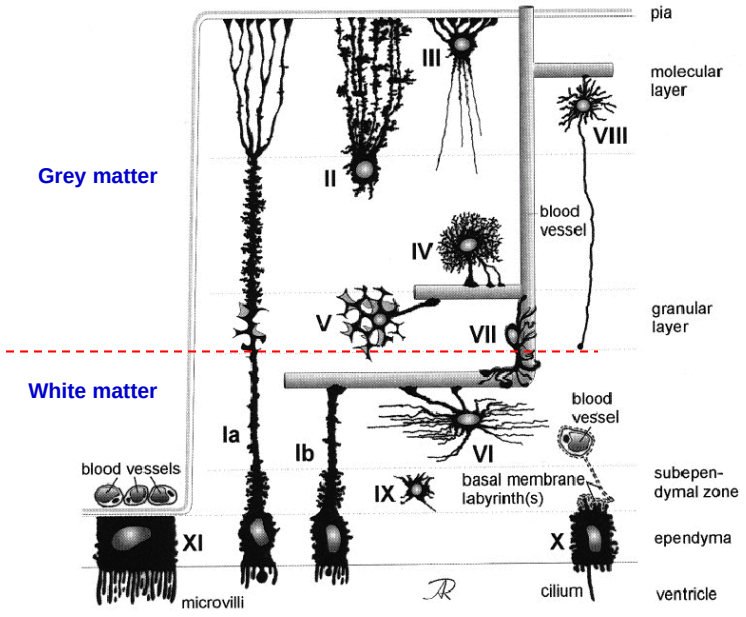
ASTROCYTE FUNCTIONS:

	Established	← Probable	← Emerging
Normal	❑ **Control of $[K^+]_o$** ❑ **Control of extracellular levels of neurotransmitters (NTs)** ➢ Synthesis of NT precursors (glutamate and GABA) ❑ **Neuronal pathfinding**	❑ **Brain energy metabolism** ❑ Role in **blood-brain barrier** ❑ Brain water homeostasis (AQP4) ❑ Regulation of extracellular pH	❑ **Modulation of excitatory and inhibitory synapses** ❑ Regulation of synaptogenesis ❑ Regulation of neurogenesis in adult brain (e.g., in hippocampus)
Pathological	❑ Alexander disease ❑ Cytotoxic brain edema ❑ Glioma formation	❑ Hepatic encephalopathy ❑ Stroke/injury: ➢ Free radical scavenging ➢ Glutamate homeostasis	❑ Trophic modulation of post-injury neural repair and axon regrowth ❑ Release of cytokines and chemokines ❑ Neuroinflammation

Modified from: Ransom, Behar & Nedergaard, 2003 (TINS)

11

Which of these cells is astrocyte?

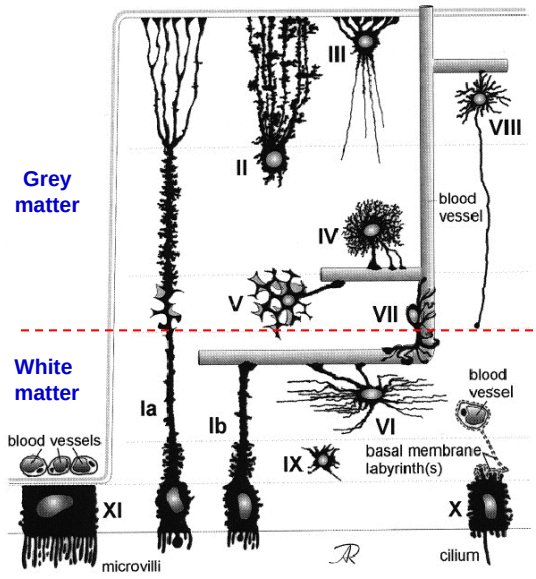


Modified from: Reichenbach & Wolburg 2005

12

6: fibrous astrocyte => protoplasmic astrocytesall are astrocytesall express protein typical for glial cells GFAP

ASTROCYTES AND EPENDYMOGLIAL CELLS IN BRAIN TISSUE:



Modified from: Reichenbach & Wolburg 2005

***I:* tanycyte** (*Greek: tanyos = to stretch)* periventricular glial cells with cytoplasmic processes reaching pial (Ia) or vascular (Ib) surfaces

***II:* radial astrocyte** (e.g., Bergmann glial cell)

***III:* marginal astrocyte**

***IV:* protoplasmic astrocyte**

***V:* velate astrocyte**

***VI:* fibrous astrocyte**

***VII:* perivascular astrocyte**

***VIII:* interlaminar astrocyte**

***IX:* immature astrocyte/glioblast**

X: ependymocyte

***XI:* choroid plexus cell** *(choroid plexus – a structure that secretes cerebrospinal fluid*

13

tube is blood vesselnumbe

Astrocyte marker GFAP

Glial **F**ibrillary **A**cidic **P**rotein - **the most popular marker for astrocytes.**

- major component in astrocytic intermediate filaments (~10 nm diam) – visualization of the stem processes (*~15% of astrocyte volume*)
- NOT present in neurons
- Present in all types of astrocytes
  - but in some types, only at early developmental stages, e.g. in ependymal cells and some tanycytes
  - **Bergmann glia, and Müller cells**: detectable GFAP only in adult tissue

The major reason for classifying a cell as astrocytic in nature is their expression of GFAP during at least one point in their life span.

14

intermediate filamentsin main processes=> not body or fine processeshow good we see depends on resolutionGFAP level may differ

ASTROCYTES: *protoplasmic vs. fibrous*

Protoplasmic Astrocytes:

- **In the gray matter**
  - **Numerous processes, spread more or less radially from the soma**, and many extend fine, complex lamellar side branches.
  - At least one of the cell processes bears one or several perivascular end-feet on blood vessels.
  - Every protoplasmic astrocyte establishes its own primarily exclusive territory .

processes branch extensively

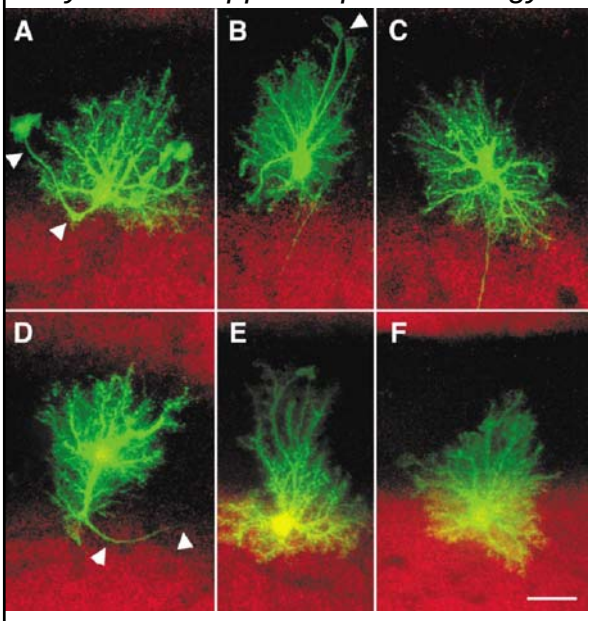
Fibrous Astrocytes:

- **In white matter tracts**
  - Somata: often arranged in rows between the axon bundles.
  - Processes: comparatively smooth, frequently oriented in parallel to the axons, with multiple finger-like outgrowths towards nodes of Ranvier.
  - Every fibrous astrocyte possesses several perivascular and/or subpial end-feet (e.g., in optic nerve).
  - The processes are generally longer (up to 300 μm) than those of protoplasmic astrocytes (50 μm).

rarely branch

Example: Protoplasmic astrocytes

Grey matter. Hippocampal dentate gyrus



The cells were filled with a fluorescent dye Lucifer Yellow.

Microinjection in fixed tissue

Most processes are densely ramified, similar to other types of protoplasmic astrocytes:

water solubleenargement

Interlaminar astrocytes

a ***long siphon process*** (indicated by small arrowheads) that frequently reach well beyond the extent of other processes of the same cell.

little long foot => transport something little ar

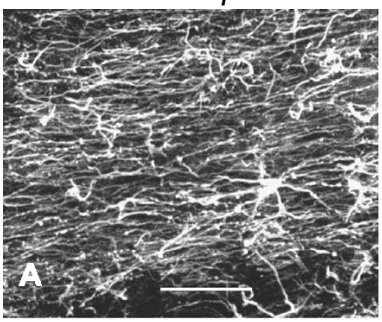
Scale bar: 20 μ.....

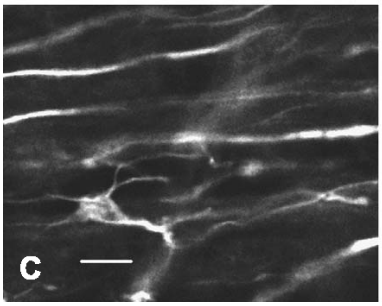
16

Bushong EA, Martone ME, and Ellisman MH (2003) J Comp Neurol 462:241-251

Example: Fibrous astrocytes

White matter. Spinal cord





GFAP

GFAP labelled astrocytes in **white matter** of mature rat spinal cord.

Long astrocytic processes run between the axons parallel to rostro-caudal axis.

Scale bars: A, 50 μm, C, 10 μm.

bodies may not be seen => express less grape fap

Modified from:
Mills, Velumian, Agrawal, Theriault, Fehlings (2004) Confocal imaging of changes in glial calcium dynamics and homeostasis after mechanical injury in rat spinal cord white matter. *NeuroImage* 21:1070-1083.

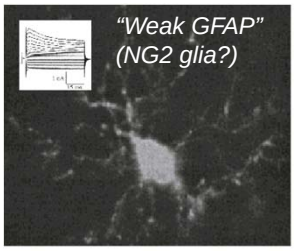
17

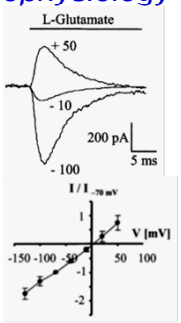
Astrocytes: "Glutamate receptor"and "transporter" types

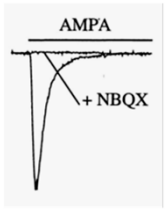
Hippocampus of transgenic (GFAP/EGFP) mice:

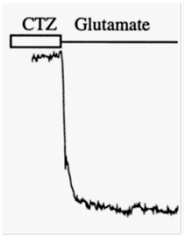
Morphology & Electrophysiology *Pharmacology*

GluR type

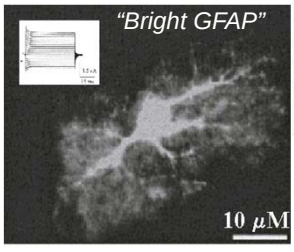


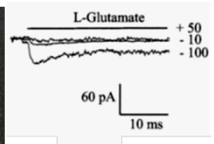


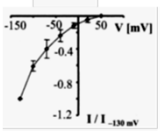


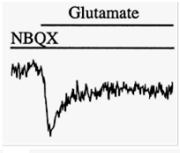


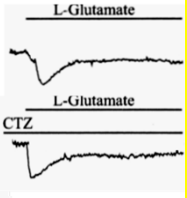
GluT type

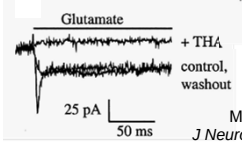












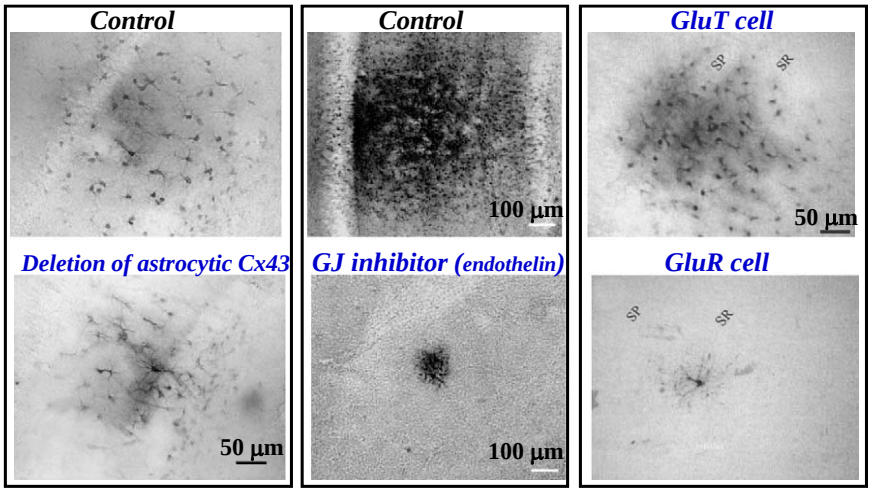
Matthias et al (2003) *J Neurosci* 23:1750-1758.

18

GluT=> glutamine transporte

ASTROCYTIC SYNCYTIUM: *Example of gap junctional coupling:*

A dye injected into a single astrocyte labels large groups of astrocytes



Cx43 inactivation (*left*) decreases dye transfer by ~50% compared to littermate controls **Gap junction inhibitor endothelin (*middle*)** has a much stronger impact than deletion o Cx43 alone. **Astrocytes expressing GluT (*top right*)** show extensive coupling while **GluR cells (*bottom right*)** are not coupled. The injected cells are in the center of each panel.

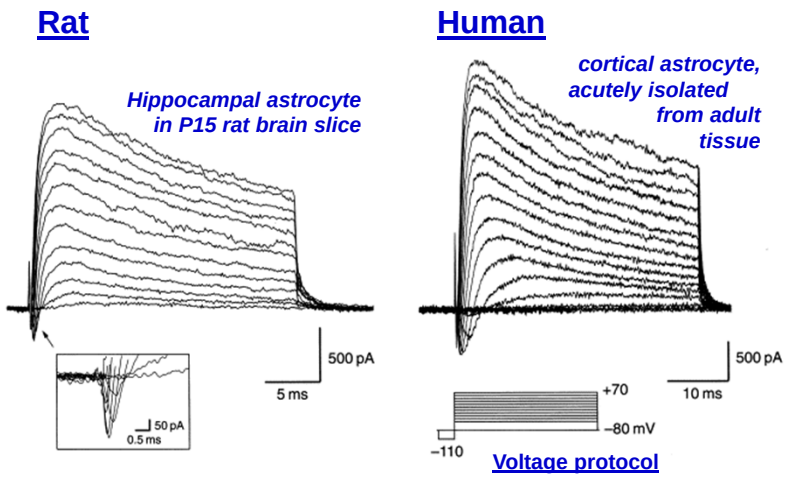
Mouse hippocampal slices.

Theis et al (2005) Trends Neurosci 28:188-195.

19

hit one astrocytes with injectio

Ion channels is astrocytes: few Na^+, many K^+:



The relative proportion of Na^+ to K^+ currents shows a dominance of K^+ currents.

I_{Na} is too small to generate action potentials

Whole-cell voltage clamp recording

Sontheimer H, Black JA, and Waxman SG (1996) Trends Neurosci 19:325-331.

20

low expression of Na+ channels => therfore astrocytes no APlots of K+ channels

ASTROCYTIC SYNCYTIUM:
Role in spatial buffering of $[K^+]_o$

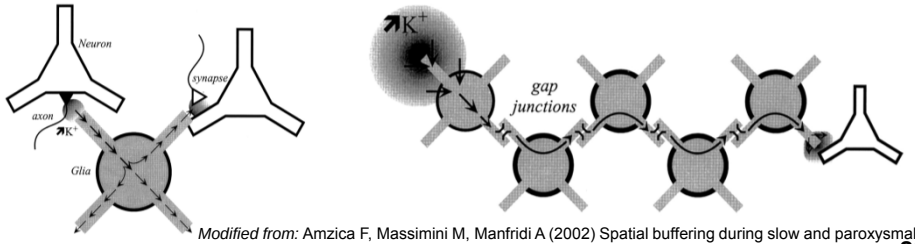
Spatial buffering of K^+_o:
Stabilizing extracellular K^+ levels and reducing local accumulations of K^+_o by redistributing them to other parts of the extracellular space.

all measures indirectno direct

Astrocytes are more efficient than neurons in spatial buffering due to:

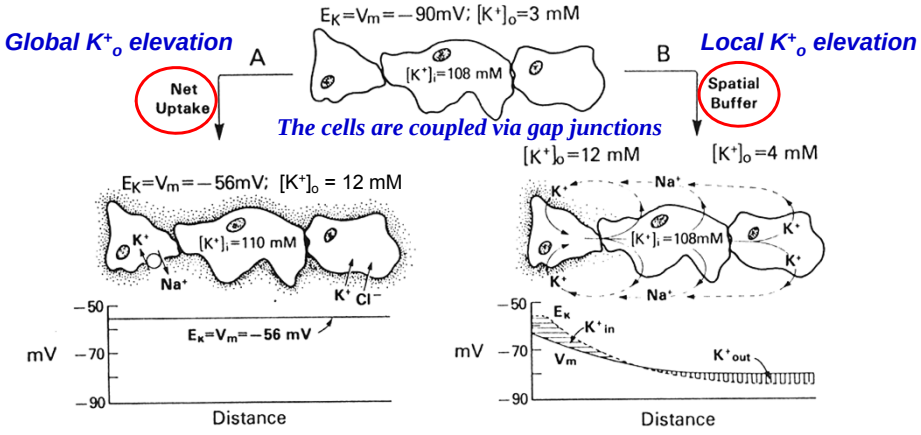
- Gap junctional communication with each other
- Spatial networks allowing to dump K^+ well away from the source

Buffering by one astrocyte *Buffering by a network of coupled astrocytes*



Modified from: Amzica F, Massimini M, Manfridi A (2002) Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. J Neurosci 22:1042-1053

Spatial buffering of $[K^+_o]$: mechanism



A: Global K^+ elevation: Glial cells accumulate K^+ either by the activity of a Na/K–ATPase or by a pathway in which K^+ is co-transported with Cl^-.

In this mechanism of $[K^+]_o$ regulation, the V_m in the glial syncytium is spatially uniform at −56 mV.

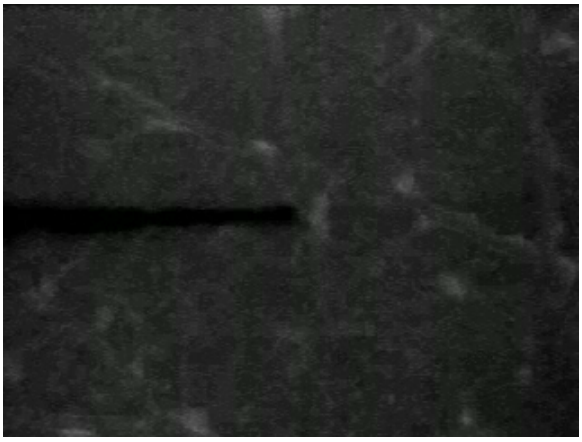
B: Local K^+ elevation: Local increases of $[K^+]_o$ → glial depolarization that spreads through the glial syncytium.

The local difference between V_m and E_K drives the K^+ uptake in regions of elevated $[K^+]_o$ and K^+ outflow at distant regions.

R.K. Orkand, Glial-interstitial fluid exchange, *Ann NY Acad Sci* **481** (1986), pp. 269–272

if affects all cells with K+ extracell increase => all depolarize together => change potassium membrane potential => steady membran

CALCIUM WAVES in astrocytic networks



Mechanical stimulation of a single astrocyte initiates a Ca^{2+} wave that propagates in all directions from the point of origin.

Retina, viewed from the vitreal surface.
The wave propagates through both astrocytes (large polymorphic cells) and Müller cell end-feet (smaller, round profiles).

25 μm

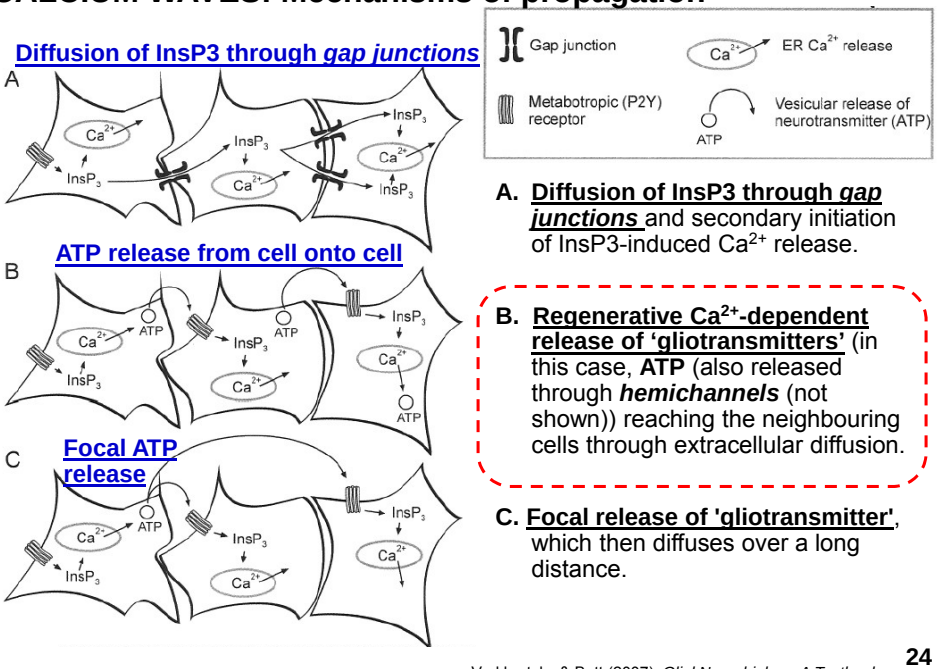
Glial cells were labeled with a **Ca^{2+} indicator dye** (Fluo-4 AM or Calcium Green-1 AM) and were imaged with a video-rate confocal microscope.

The video is shown at **0.75 times normal speed**.

Eric Newman http://www2.neuroscience.umn.edu/eanwebsite/index.htm **23**

stimulate one cell => spre

CALCIUM WAVES: Mechanisms of propagation



A. **Diffusion of InsP3 through *gap junctions*** and secondary initiation of InsP3-induced Ca^{2+} release.

B. **Regenerative Ca^{2+}-dependent release of 'gliotransmitters'** (in this case, **ATP** (also released through ***hemichannels*** (not shown)) reaching the neighbouring cells through extracellular diffusion.

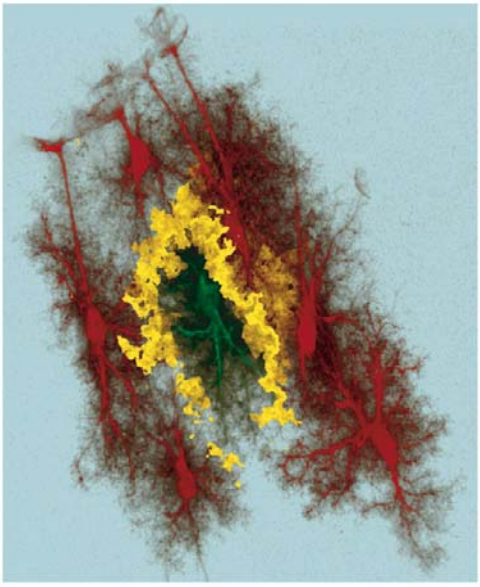
C. **Focal release of 'gliotransmitter'**, which then diffuses over a long distance.

Verkhratsky & Butt (2007). *Glial Neurobiology. A Textbook* **24**

most likely ATP => receptors for ATP ionotropic (P2x5 receptor) ATP released acts on pyrinergic receptor in next cell => InsP3 goes in =

ASTROCYTES: territories: Non-overlapping microdomains

Demonstrated with injection of fluorescent tracers



Individual astrocytes in the hippocampal CA1 molecular layer were filled with different coloured fluorescent dyes, Alexa 468 (**green*: the central cell***) and Alexa 488 (**red*: 6 surrounding cells***).

The discrete region where the fine terminal processes of the adjoining astrocytes are closest to one another (*although **not actually overlapping***) is revealed as **yellow**

Note that the 'boundary' of each astrocyte has a distinct surface that abuts neighboring astrocytes.

The long thin processes that extend from each cell shown in this figure end in sheet-like surfaces that line the adjacent blood vessel.

E. Bushong and M. Ellisman, The Natl Center for Microscopy and Imaging Research, University of California, San Diego, USA.

Volterra A, Meldolesi J. (2005) Astrocytes, from brain glue to communication elements: the revolution continues. Nature Reviews Neuroscience 6:626-640.

25

each astrocyte has spatial

Astrocyte and the synapse: Glutamate removal

critical for maintenance of low extracellular concentrations of glutamate, protects neurons from excitotoxic injury

I. GLT (EAAT2) and GLAST (EAAT1) and some EAAC

II. EAAT4 (only in cerebellar Purkinje cells) **and EAAC** (throughout the brain).

III. GLT (EAAT2) in glutamatergic nerve terminal.

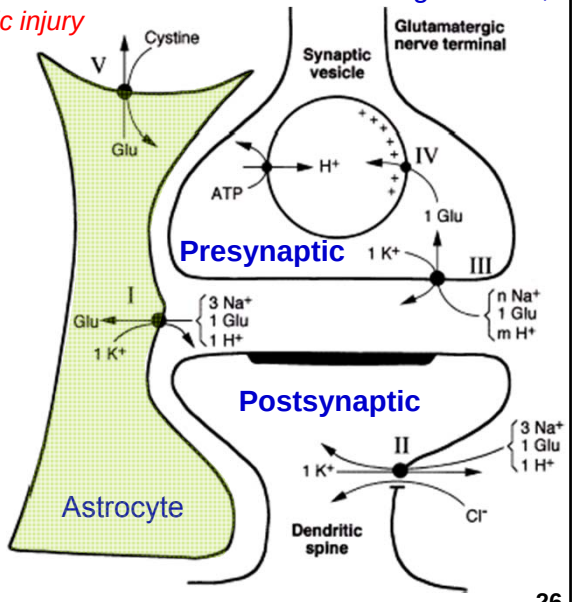
IV. vGLUT – on synaptic vesicles.

V. Glutamate-cystine exchanger.

Stoichiometry of glutamate uptake via EAAC and GLT:

1 Glu taken up together with n Na^+ & m H^+ ↔1 K^+:

=>Electrogenic!

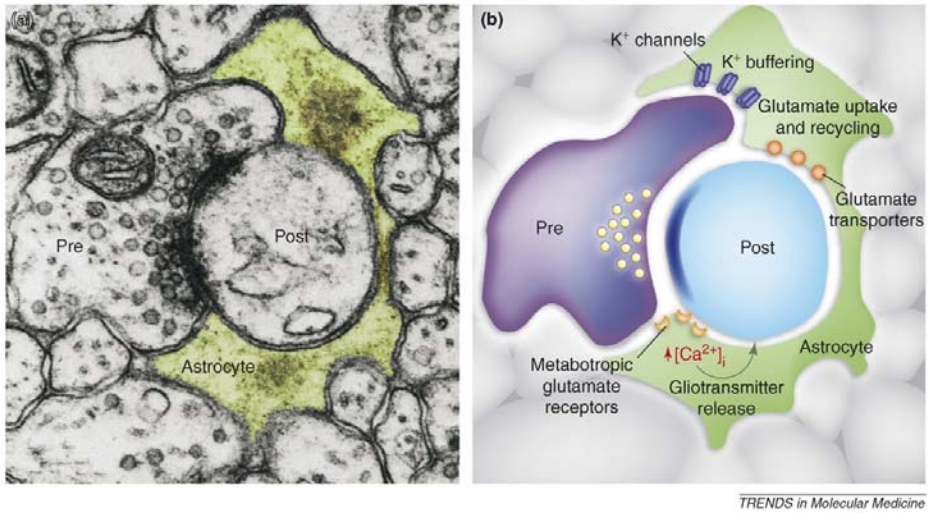


Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1-105.

26

different transporters

Astrocyte and the synapse: the “tripartite synapse”



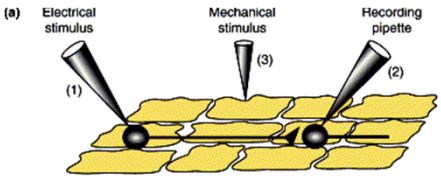
Halassa, M. M., Fellin, T. & Haydon, P. G. The tripartite synapse: roles for gliotransmission in health and disease. *Trends in Molecular Medicine* **13**, 54-63 (2007)

27

structures aren't as clear

GLIOTRANSMISSION: Astrocytic modulation of synaptic transmission between neurons

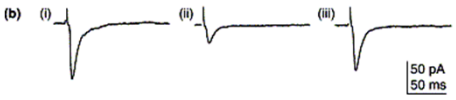
Co-culture of astrocytes and neurons



(1) A presynaptic neuron is stimulated with electrical pulses

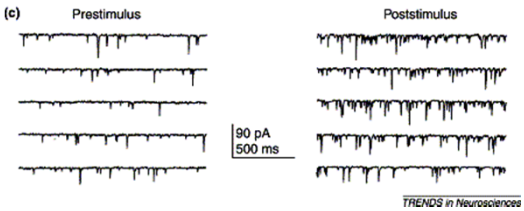
(2) synaptic currents (EPSCs) are recorded from the postsynaptic neuron.

(3) Astrocytes beneath the neurons (yellow) are activated mechanically.



(b) Stimulation of an astrocyte reduces the amplitude of the EPSC (ii).

This effect can be blocked by mGluR antagonists. (*Araque et al., 1998*).



(c) The frequency of mEPSCs increases after stimulation of a neighbouring astrocyte. (*Araque et al., 1998*).

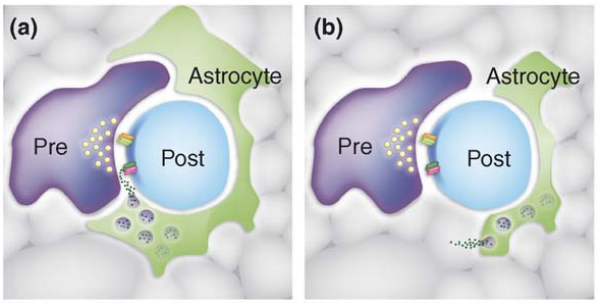
From: Eric A. Newman (2003) *New roles for astrocytes*: Regulation of synaptic transmission. Trends Neurosci., 26 (10), 536-542

28

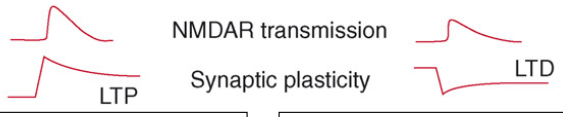
astrocytes in culture don't

GLIOTRANSMISSION: Astrocytic D-serine and synaptic plasticity

astrocytic process close to synapse



astrocytic process retracted from synapse



(a) D-serine released from astrocyte → postsynaptic NMDARs → enhanced NMDAR-current and LTP (red traces).

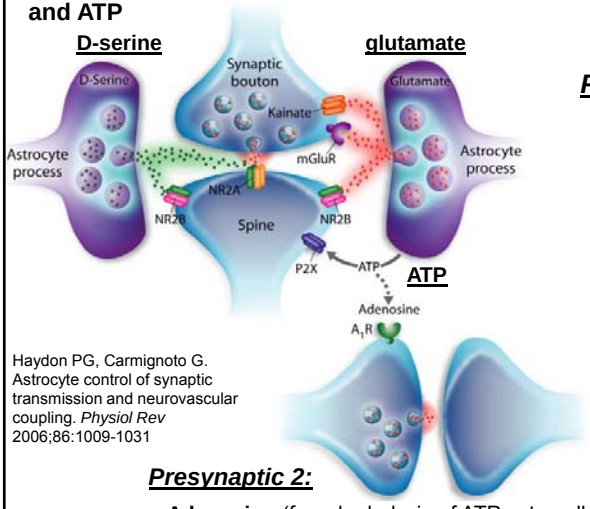
(b) D-serine released from astrocyte dissipates → less activation of postsynaptic NMDARs → reduced NMDAR-mediated synaptic transmission and prevalense of LTD.

Halassa, Fellin & Haydon (2007)

29

GLIOTRANSMISSION: Astrocyte-derived signals act both pre- and postsynaptically to regulate synaptic transmission

Astrocytic release of glutamate, D-serine, and ATP



Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* 2006;86:1009-1031

Presynaptic 1:

Glutamate → mGluRs and kainate receptors to enhance synaptic transmission.

Postsynaptic:

Glutamate → extrasynaptic NMDARs → depolarize the neuronal membrane → neuronal synchrony

D-serine → glycine-binding site of NMDARs and can regulate synaptic plasticity.

ATP → postsynaptic P2X receptors → depolarize the neuronal membrane and regulate the insertion of postsynaptic AMPA receptors.

Presynaptic 2:

Adenosine (from hydrolysis of ATP extracellularly by ectonucleotidases) → **presynaptic A1 receptors → heterosynaptic depression of excitatory synaptic transmission.**

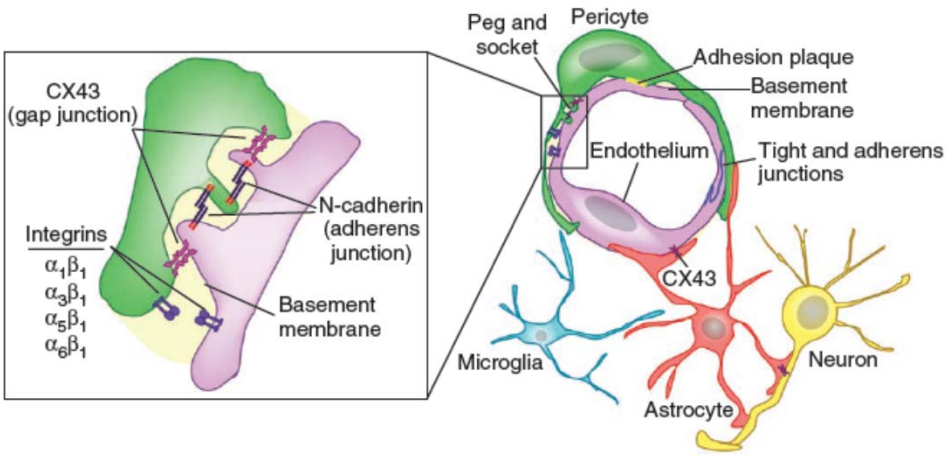
30

NMDA => glycine site to

tripartide synapseATP=>A

possible exam question => difference between protoplasmic vs fibrous cellsSchwan

BLOOD-BRAIN BARRIER (BBB): ASTROCYTES AND PERICYTES



Winkler, Bell & Zlokovic (2011). Central nervous system pericytes in health and disease. Nat Neurosci; 14:1398-1405

31

end feet touch endothelium

Metabolic astrocyte-neuronal cooperation

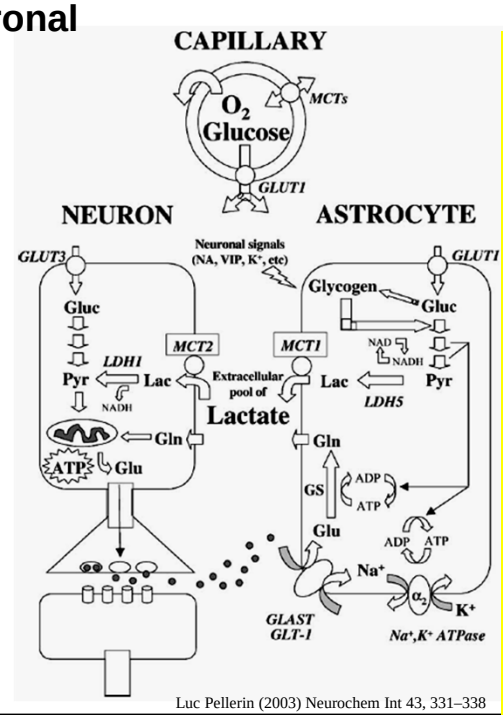
IN ASTROCYTES:

- **Glycogenolysis** → glucose → lactate
- **Glucose → lactate** by astrocyte-specific **LDH5.**
- ***Glutamate → glutamine*** (using glucose as energy substrate).
- **Lactate** → into the extracellular space via MCT1→ extracellular lactate pool.
- **Lactate released from astrocytes →** into neurons via their own specific MCT2.

IN NEURONS:

- **Lactate → pyruvate** by **LDH1**. Pyruvate then enters the tricarboxylic acid cycle.
- **Glutamine → glutamate**

GLUT: glucose transporter; **LDH**: lactate dehydrogenase; **MCT**: monocarboxylate transporter; **GS**: glutamine synthetase; **Gluc**: glucose; **Pyr**: pyruvate; **Lac**: lactate; **Glu**: glutamate; **Gln**: glutamine.

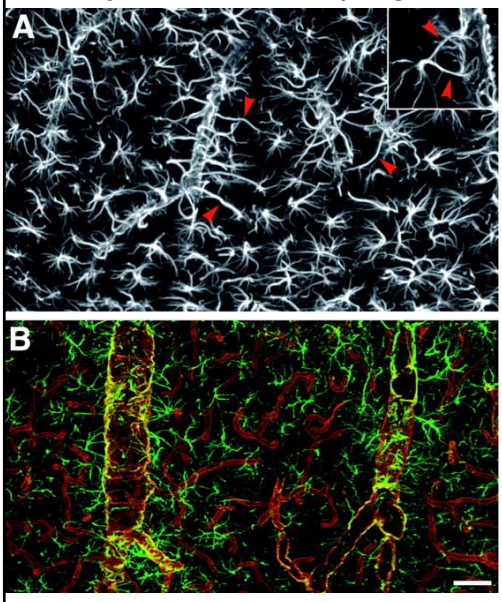


Luc Pellerin (2003) Neurochem Int 43, 331–338

glycogen only in astrocytes =

ASTROCYTIC NETWORKS: Perivascular astrocytes

Astrocyte-vascular coupling in brain cortex



A: GFAP immunolabeling: Astrocytes distributed symmetrically around blood vessels. Their vascular processes: straight, unbranched, and of larger diameter compared to other processes (▲). The surfaces of large to medium-size vessels were densely covered by GFAP+ astrocytic end-feet.

Inset: An astrocyte with two vascular processes.

B: **GFAP (green)/AQP-4 (red) double immunolabeling**: AQP-4 immunolabeling reveals that the entire network of vessels, including capillaries, is covered by astrocytic processes (some are GFAP negative). The AQP-4 labeling reveals continuous coverage of smaller vessels by astrocytic end feet.

Scale bar: inset, 40 µm; A, 10 µm; B, 60 µm.

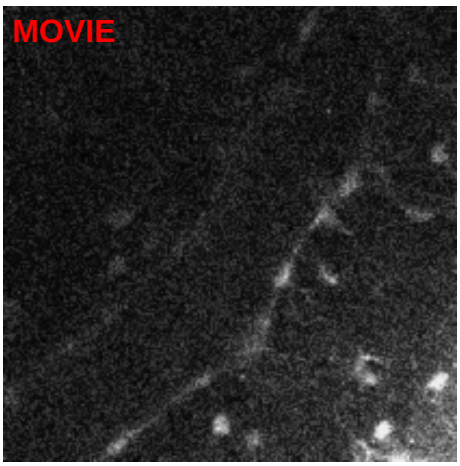
Simard et al J. Neurosci. 2003;23 9254-9262 (Nedergaard group)

33

stemmed processes always

ASTROCYTIC NETWORKS: Microvascular effects

Initiation of vasoconstriction



Two-photon photolysis of **DMNP-EDTA** in an astrocyte initiates a Ca^{2+} wave propagating into the astrocytes and astrocyte end-feet along the vessel wall. *Acquisition rate = 3.9 sec/frame (66 frames):*

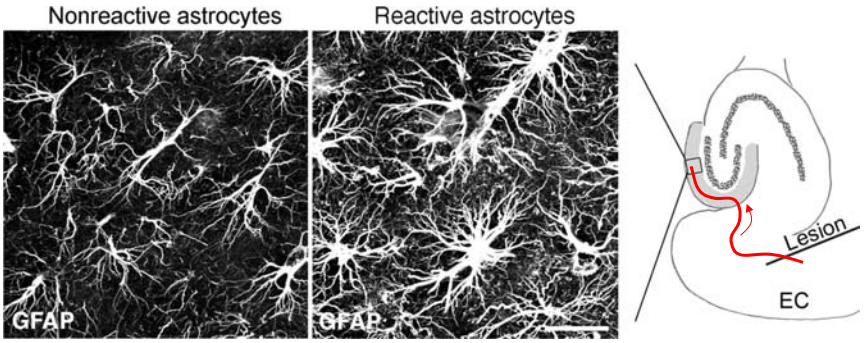
Two-photon transmitted and Ca^{2+} indicator fluorescence overlay showing arteriole constriction resulting from Ca^{2+} wave that propagates along the vessel wall *(same experiment as in the left frame)*

Mulligan, S. J. & MacVicar, B. A. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature 431, 195-199 (2004).

3

loaded astrocyte with cased ca

Reactive astrocytes and reactive gliosis



Injury → activated astrocytes, also called **reactive astrocytes**

Reactive gliosis:

- hypertrophy of astrocytes
- proliferation of microglial cells and astrocytes
- migration of activated astrocytes (*and microglia*) to injured site
- formation of glial scar

35

if you lesion => astrocytes be

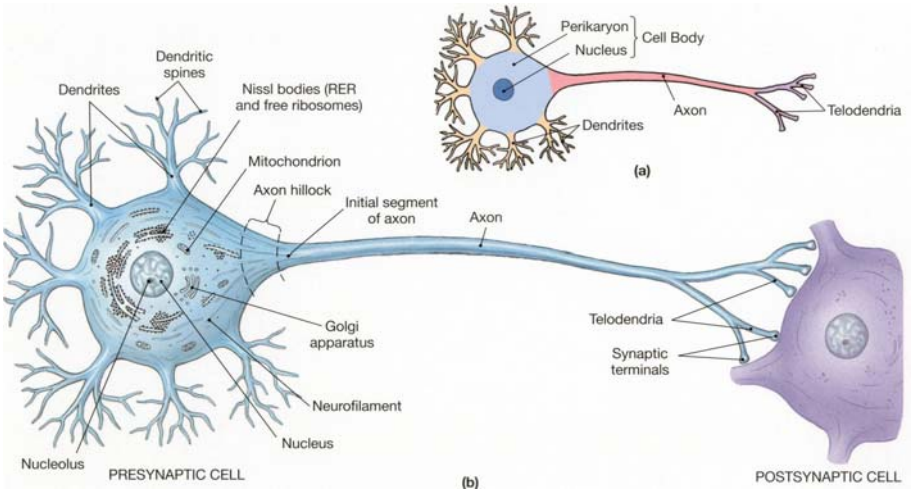
PART 2: Glia and axonal function

Myelin and myelin-producing cells

- ❑ The initial segment of the axon
- ❑ Myelin and saltatory conduction
- ❑ Safety factor of action potential propagation
- ❑ Myelin-forming cells:
  - ❑ Schwann cells (PNS)
  - ❑ Oligodendrocytes (CNS)
- ❑ Compactness of the myelin sheath
- ❑ Myelin proteins
- ❑ Paranodal and internodal myelin
- ❑ Functional architecture of the node of Ranvier and paranodal myelin specializations: spatial segregation of Na^+ and K^+ channels
- ❑ Intra-myelin cytoplasm
- ❑ Gap junctions of the myelin sheath: interactions within and beyond the myelin segment
- ❑ Neurotransmitter receptors expressed in oligodendrocytes and myelin
- ❑ Role of glial GluRs in pathophysiology of myelinated axons

36

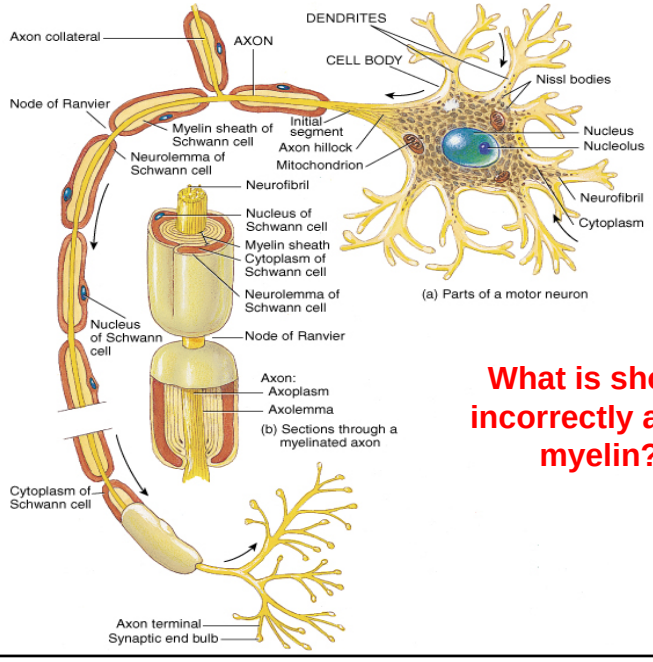
A NEURON WITH NON-MYELINATED AXON



What exactly is the initial segment of the axon?

37

A NEURON WITH MYELINATED AXON:



What is sho
incorrectly ab
myelin?

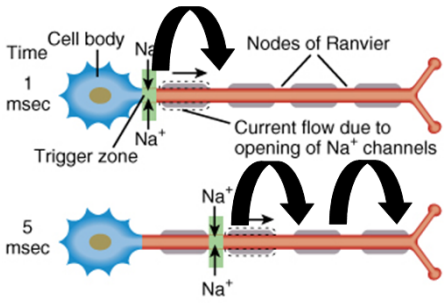
38

myelin determines bord

neuron from CNS => yet myelin is scl

The myelin sheath and saltatory conduction

The node of Ranvier: key element responsible for saltatory propagation of action potentials in myelinated axons.



The action potential "jumps" from one node of Ranvier to another

Q1: What conduction velocity would you expect for the 4 ms time interval shown here between the upper and lower parts?

Q2: Will this conduction velocity be acceptable for saltatory conduction (AP jumping from node 1 to node 2)?

use numbers from the table below

Classification of axons:

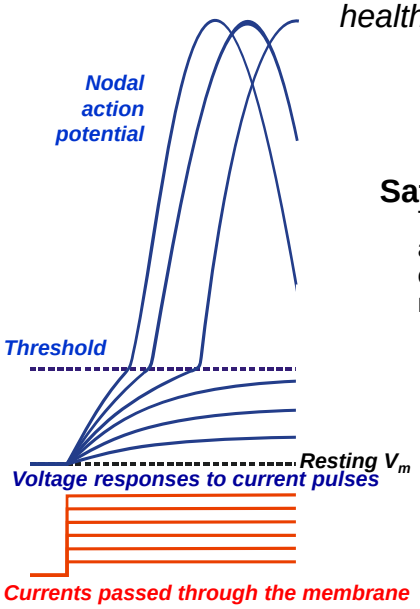
Axon group	A	B	C
Myelin sheath	present	present	absent
Axon diameter, μm	1-20	<4	0.3-1.5
Conduction velocity, m/s	5-120	3-5	0.5-2
Internodal length, μm	100-2000	<400	-

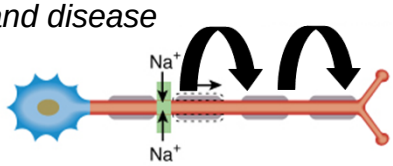
39

one myelin segment => 10

Safety factor of action potential propagation

importance for understanding the function of myelinated axons in health and disease





Safety factor (SF) of AP propagation:

The current generated by an action potential at the active node of Ranvier normally exceeds the current needed to bring the next node of Ranvier to threshold:

$$SF = I_{actual} / I_{sufficient}$$

Bigger currents will initiate an action potential **faster** (*see figure on the left for explanation*).

What will happen if the safety factor decreases?

SV-60208

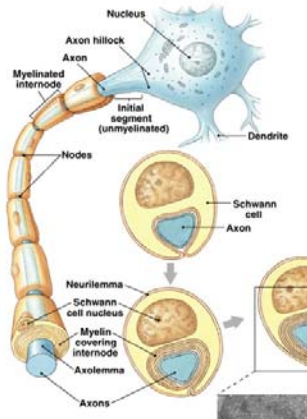
40

oligodendrocytes can't stre

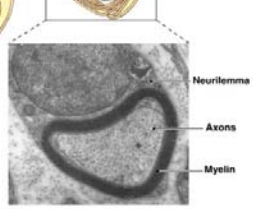
V does not respond immediately => exponential response to larger/larger stimsafety factor => to bypass damagerange of a

Schwann cells: 3 types (*PNS: nerves and MNJ*)

1) **Myelinating**



IHC markers:
- P0
- Myelin basic protein (MBP)
- S-100



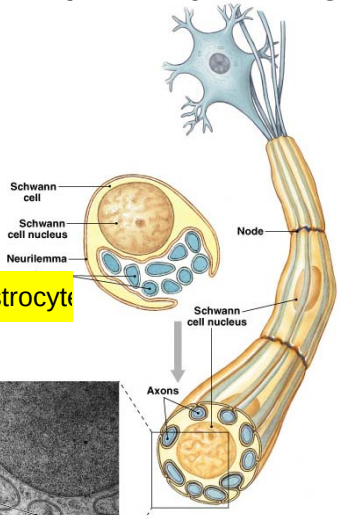
2) **Perisynaptic at NMJ** *(not shown):*

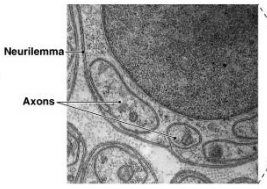
Functions: similar to perisynaptic astrocytes of the CNS;

IHC markers:
- GFAP
- S-100

works like endfoot/astrocyte

3) **Non-myelinating**

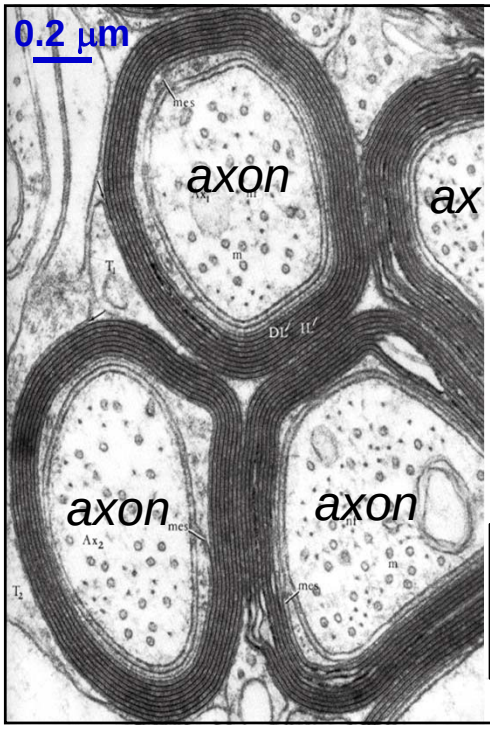




IHC markers:
- S-100
- GFAP

41

not elec insulation => create



The fine structure of myelin sheath:

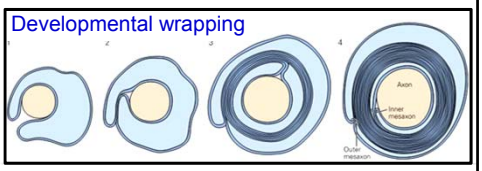
- Wrapped around the axon
- Compact
- Multilayer

Functional importance:
- Electrical insulator
- Mechanical protection of axon

Mature rat optic nerve

Image from:
Peters, Palay & Webster (1990)

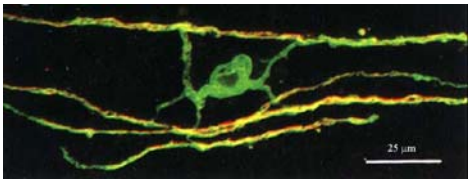
Developmental wrapping



42

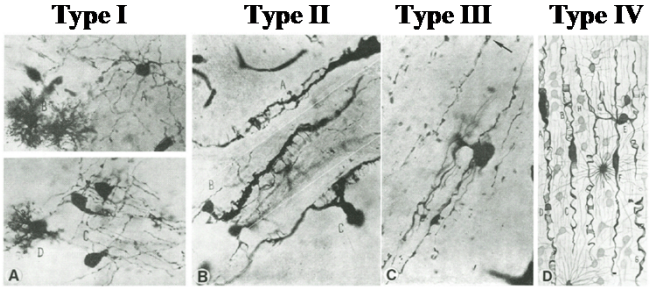
spiral structure => not con

Oligodendrocytes: can myelinate many axons



Double immunofluorescence labeling of a mature myelinating oligodendrocyte and its associated myelin sheath.
Rip (green) and **anti-MBP (myelin basic protein, red).**
Rat anterior medullary velum.
From: Butt, A. M. & Berry, M. Oligodendrocytes and the control of myelination in vivo: new insights from the rat anterior medullary velum. J Neurosci Res 59, 477-488 (2000).

Four morphological types of oligodendrocytes



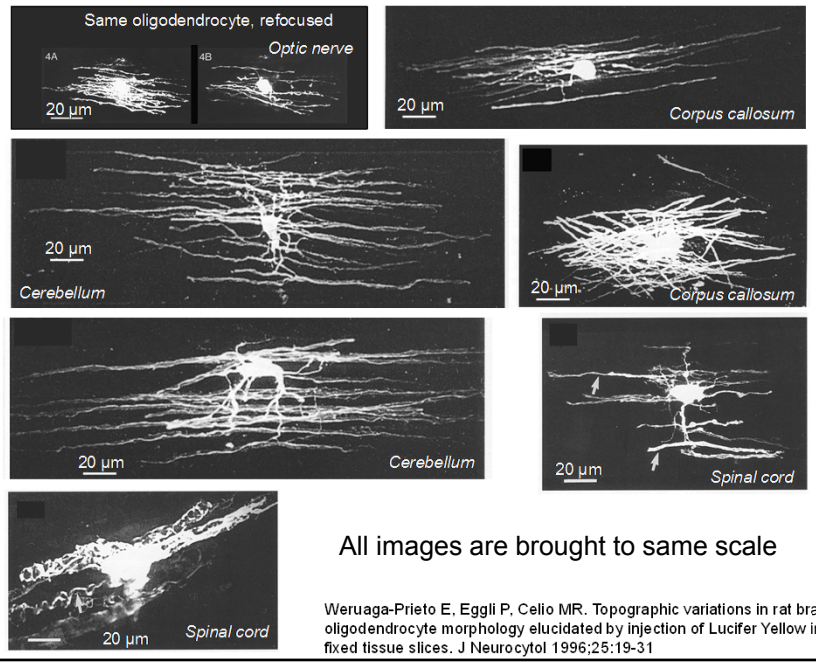
From:
Rio Hortega (1928)
Modified after
Szuchet (1995)

Smaller axons
Up to 60 axons per 1 OLG

Larger axons
Less axons per 1 OLG

43

Lucifer Yellow-injected oligodendrocytes in different parts of CNS



All images are brought to same scale

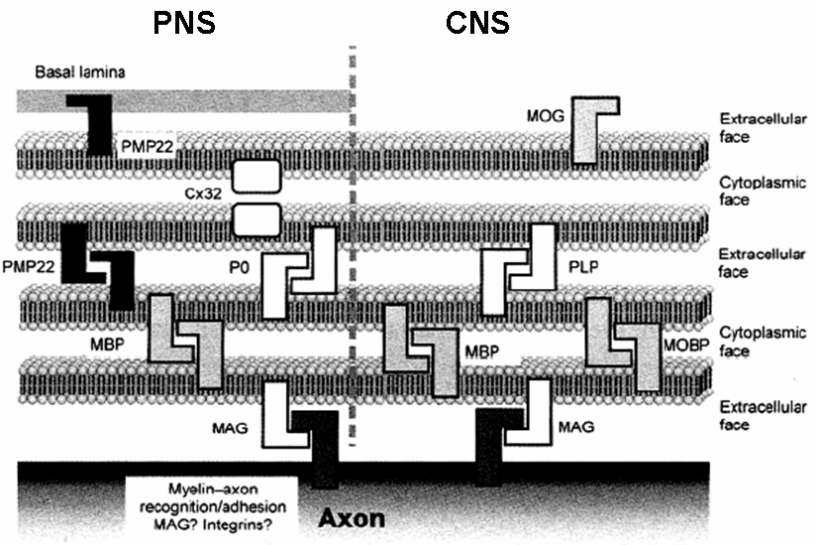
Weruaga-Prieto E, Eggli P, Celio MR. Topographic variations in rat brain oligodendrocyte morphology elucidated by injection of Lucifer Yellow in fixed tissue slices. J Neurocytol 1996;25:19-31

44

vedu medularian => th

oligodendrocytes => fixed =>

Keeping the myelin membranes together:
Major proteins of CNS and PNS myelin



Cx32 - connexin 32; **MAG** - myelin associated glycoprotein; **MOG** - myelin oligodendrocyte glycoprotein; **P0** - protein zero; **PMP22** - peripheral myelin protein 22

45

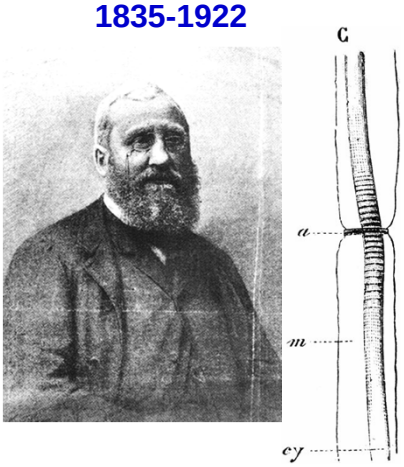
Modified from: Verkhratsky & Butt (2007). *Glial Neurobiology. A Textbook*

layers of myelin => differ

The node of Ranvier and paranodal myelin specializations:

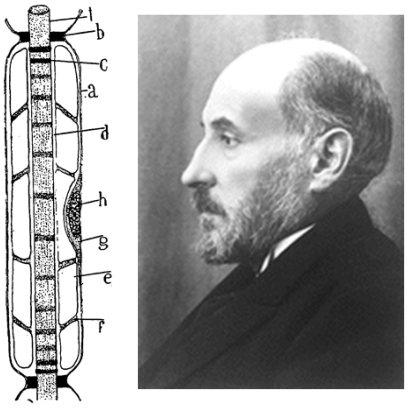
First descriptions of the node and paranodal myelin structures

Louis-Antoine Ranvier
1835-1922



Nodal and paranodal regions of a myelinated fiber as depicted by Ranvier (1878).

Santiago Ramón y Cajal
1852-1934

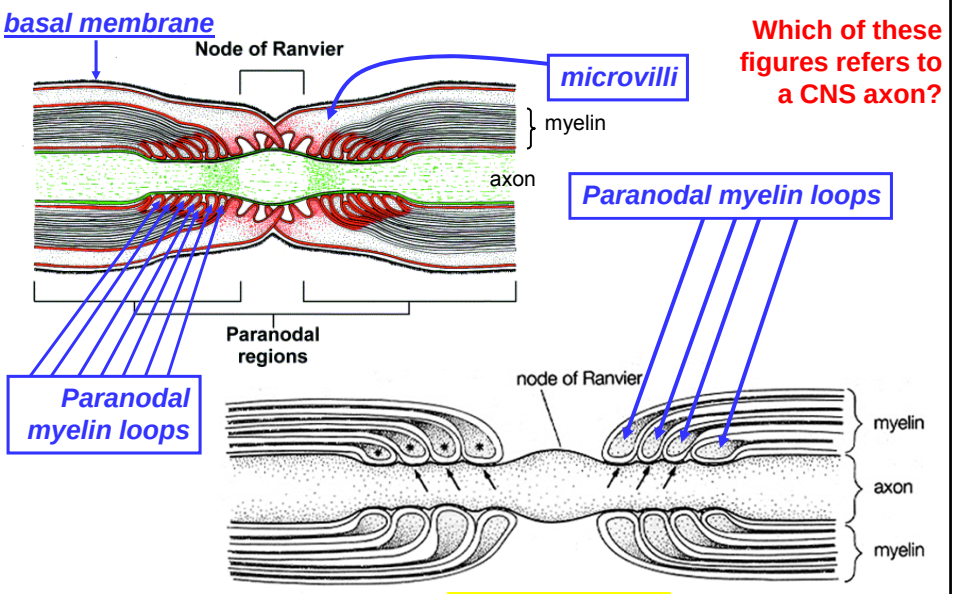


An internode as depicted by Cajal (1909) showing oblique Schmidt-Lanterman clefts.

46

Reproduced from: Rosenbluth J (1999) Journal of Neurocytology 28:251-262

The node of Ranvier: *paranodal cytoplasmic loops*



Which of these figures refers to a CNS axon?

CNS

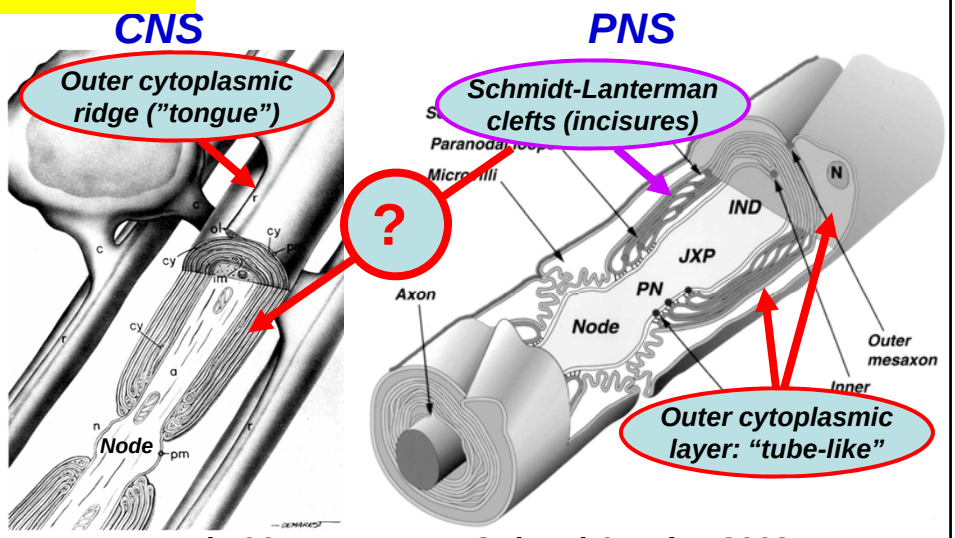
Alvarez-Leefmans FJ, Leon-Olea M, Mendoza-Sotelo J, A (2001) Neuroscience 104:569-582.

47

microvilli => PNS

Intra-myelin cytoplasm

longitudinal tongue



Bunge et al 1961

Spiegel & Peles 2002

48

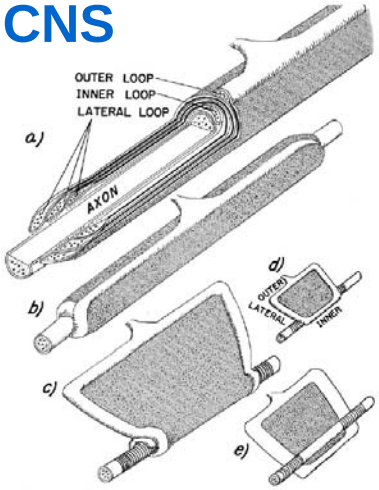
little holesare they pres

wtf is this shit?

Understanding the structure of paranodal myelin loops:

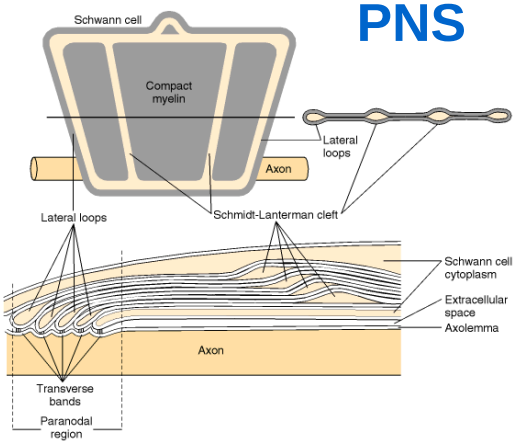
Hypothetical "unrolling" model (*based on EM studies)*

CNS



Hirano A, Dembitzer HM. A structural analysis of the myelin sheath in the central nervous system. *J Cell Biol 1967;34:555-567.*

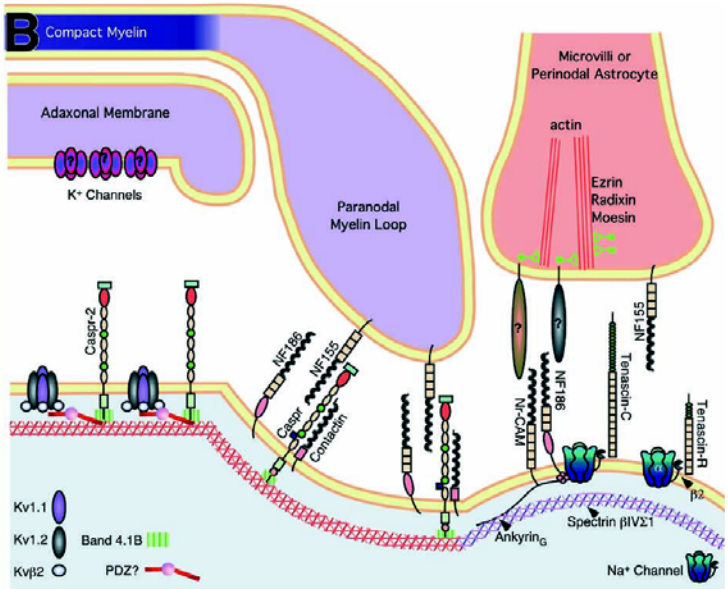
PNS



Morell & Quarles (1999). Myelin Formation, Structure and Biochemistry. In: **Basic Neurochemistry. Molecular, Cellular and Medical Aspects. Sixth Edition.** Part One. Cellular Neurochemistry and Neural Membranes. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=bnchm.figgrp.255

49

The node of Ranvier: *molecular architecture*



Not shown in this figure: molecular interactions between the paranodal loops

Scherer SS, Arroyo EJ (2002) Recent progress on the molecular organization of myelinated axons. J Peripher Nerv Syst 7:1-12.

50

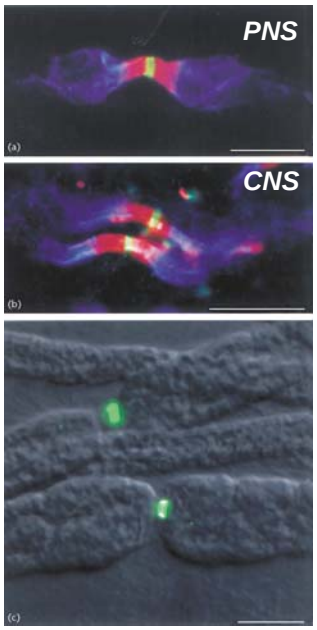
myelin doesn't hug di

Ion channels at the node of Ranvier:
Spatial segregation of Na^+ and K^+ channels

a

b

c



(a, b) Nodes of Ranvier in the peripheral and central nervous systems, labeled immunohistochemically for **Na^+ channels (green)**, **Caspr (red)*** and **Kv1.2 K^+ channel α subunits (blue)**.

Note the spatial segregation of Na^+ and K^+ channels, separated by Caspr in both PNS and CNS

(c) Four myelinated axons from the peripheral nervous system, visualized using Hoffman optics and immunofluorescence, two of which have **Na^+ channels clustered in the nodal gap (green)**.

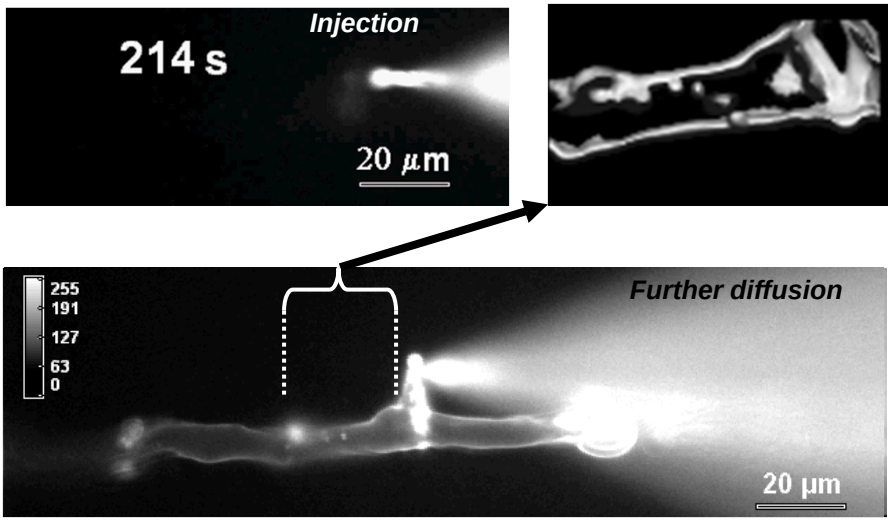
Bars: 10 μm

Caspr: *contactin-associated protein

Rasband MN (2000) Nature Encyclopedia of Life Sciences. Nature Publishing Group, http://www.els.net/, London

51

Cytoplasmic continuum revealed within a living CNS myelin sheath by injection of diffusible fluorescent dye



The isolated cytoplasmic "pockets" seen within myelin sheath in EM studies have been viewed earlier as "trapped" cytoplasm left after developmental compaction of myelin, or as "sheared" defects.

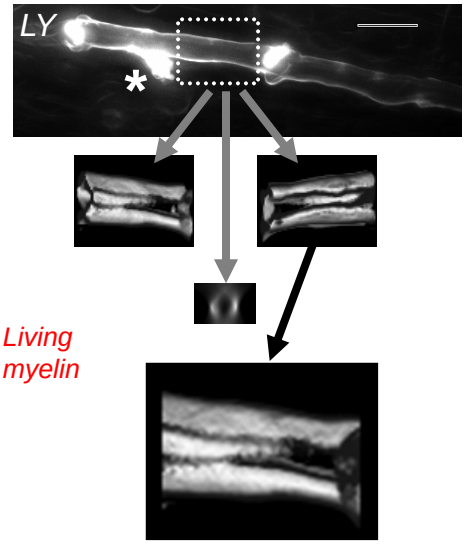
Velumian AA, Samoilova M, Fehlings MG. (2011) *NeuroImage* 56:27-34

52

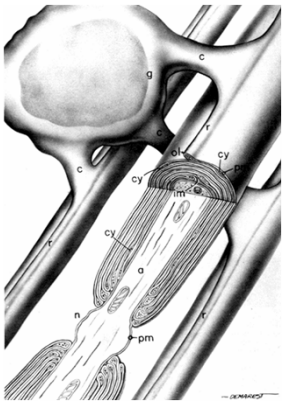
slightly sucked in T4 oligo

myelin has way more cytopasm => there for maintenance in living state

The outer cytoplasmic layer of CNS myelin:
Enveloping rather than assumed ridge-like shape



Living myelin



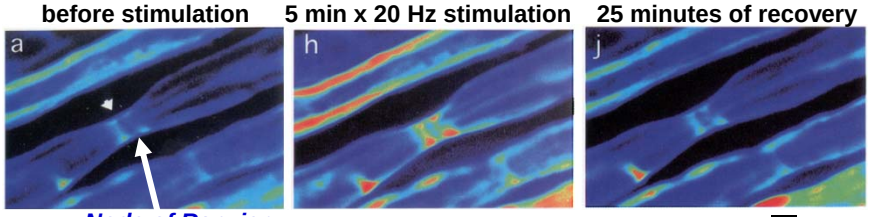
Histologically processed myelin:
collapsed cytoplasmic spaces due to **dehydration?**

Modified from:
Velumian, Samoilova & Fehlings (2010) *NeuroImage* online:

53

Intra-myelin cytoplasm actively responds to axonal activity by $[Ca^{2+}]_i$ transients

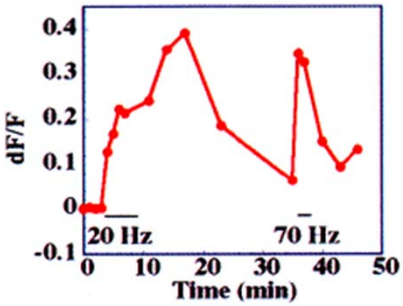
before stimulation **5 min x 20 Hz stimulation** **25 minutes of recovery**



Node of Ranvier

10 μm

Time course of Ca^{2+} transients at the node of Ranvier



The nerve activity evokes Ca^{2+} increase in paranodal area, along the myelin sheath, and at a restricted site of the myelin sheath (presumably, Schmidt-Lanterman cleft)

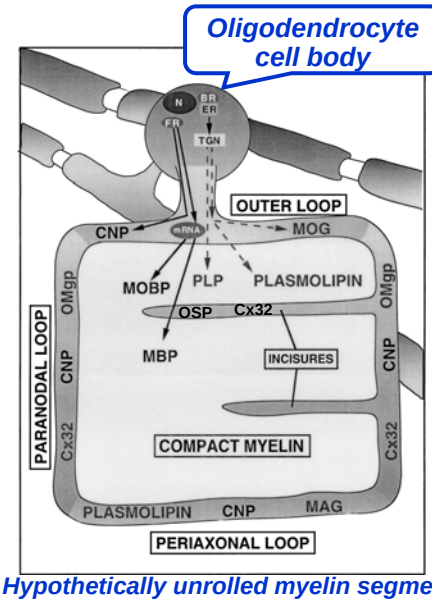
(PNS axons)

Modified from: Lev-Ram & Ellisman (1995) Axonal activation-induced calcium transients in myelinating Schwann cells, sources, and mechanisms. J Neurosci 15:2628-2637.

54

calcium go upto go up need cy

Myelin proteins: *Cytoplasmic delivery and topographic distribution within the myelin sheath*



Hypothetically unrolled myelin segment

CNP - 2'3'-cyclic nucleotide 3'-phosphodiesterase
Cx32 - Connexin-32
MAG - myelin associated glycoprotein
MAL - myelin and lymphocyte protein
MOBP - myelin-associated/oligodendrocyte basic protein
MBP - myelin basic protein
MOG - myelin-oligodendrocyte glycoprotein
OMgp - oligodendrocyte/myelin glycoprotein
OSP - oligodendrocyte specific protein/claudin-11
PLP - proteolipid protein

MBP and MOBP are synthesized utilizing an mRNA transport mechanism. PLP, MOG, plasmolipin and other membrane proteins are synthesized and passed through the ER and Golgi pathway and targeted to the various domains.

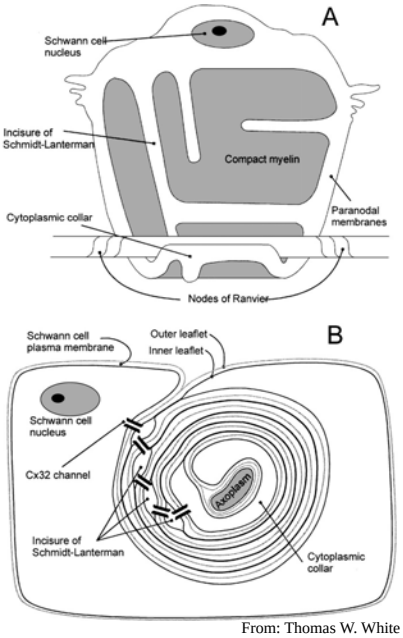
N: nucleus; **BR**: bound ribosomes; **FR**: free ribosomes; **mRNA**: messenger RNA; **TGN**: trans-Golgi network.

Combined from:
Kim & Pfeiffer (1999) and Kramer at al (2001)

55

Gap junctions within the myelin sheath

makes gap junctions with se



Connexin Cx32 forms **reflexive** (also called ***autologous***, i.e. interlamellar) gap junctions within, rather than between, Schwann cells.

(*A*) Diagram of unrolled Schwann cell showing that cytoplasm is retained at Schmidt-Lanterman clefts and paranodal loops, in continuity with the Schwann cell body and the cytoplasmic collar of the myelin sheath adjacent to the axon.

(*B*) Cross-sectional view of the connections formed by Cx32 at Schmidt-Lanterman clefts. Cx32 is specifically localized to incisures and paranodal membranes, where intracellular (as opposed to intercellular) channels form a shortcut between the Schwann cell body and periaxonal myelin cytoplasm.

From: Thomas W. White and David L. Paul. Annual Review of Physiology, Vol. 61: 283-310, 1999

56

Gap junctions of the myelin sheath:

Hypothetical roles of *heterologous* and *autologous* gap junctions

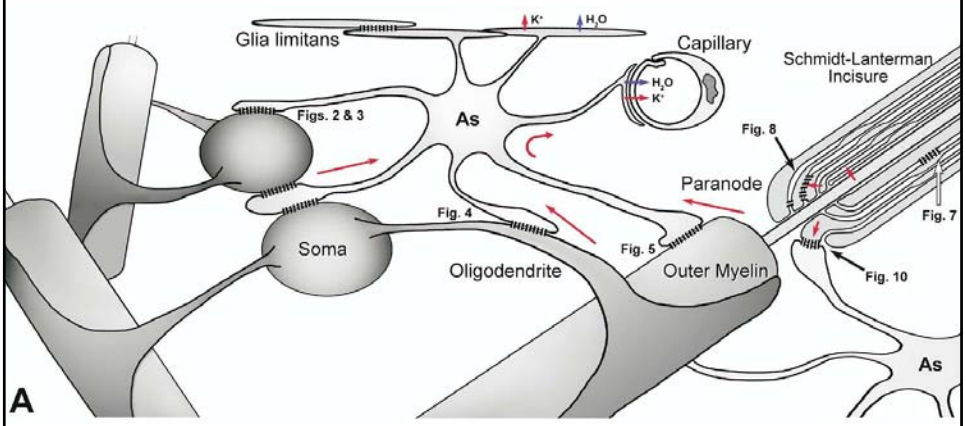


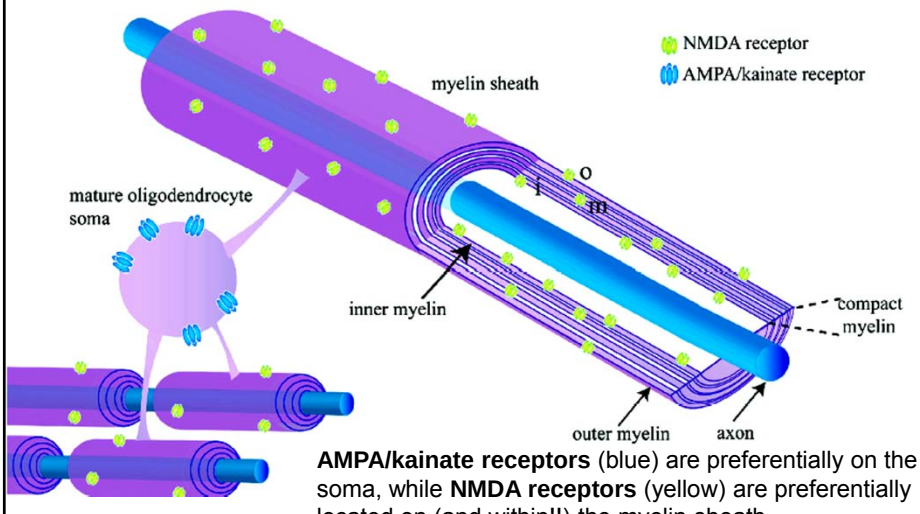
Diagram of locations of *heterologous* (between oligodendrocyte and astrocyte) gap junctions on oligodendrocyte cell body, dendrites, outer surface of myelin and everted paranodal loops, and *autologous* (i.e. interlamellar) gap junctions at Schmidt-Lanterman incisures and between paranodal loops.

Kamasawa, Sik, Morita, Yasumura, Davidson, Nagy & Rash (2005) Neuroscience 136:65-86.

57

astrocyte/ myelin cell are buddies => to siphon in potassium from under the myelin sheath to the astrocyte=>unstudied

Glutamate receptors are expressed in oligodendrocytes and the myelin sheath



AMPA/kainate receptors (blue) are preferentially on the soma, while **NMDA receptors** (yellow) are preferentially located on (and within!!) the myelin sheath.

This schematic diagram of the myelin ignores the fact that the oligodendrocyte cytoplasm is, in reality, thicker in the innermost and outermost turns of the myelin.

Karadottir R, Attwell D. Neurotransmitter receptors in the life and death of oligodendrocytes. *Neuroscience* 2007;145:1426-1438

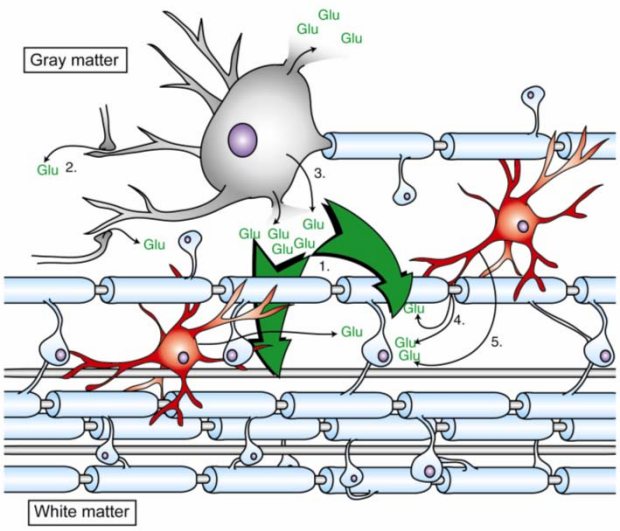
58

axomyelininc synapse?

Role of glutamate in pathophysiology of CNS white matter: *Sources of excitotoxic glutamate affecting axonal function in white matter*

Glutamate can be released to supraphysiological levels by:

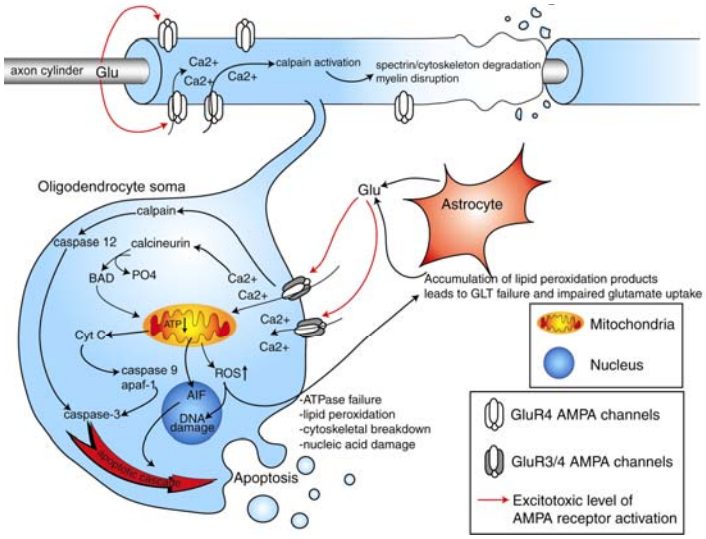
- Injured neurons in the neighboring grey matter
- Astrocytes in the white matter



Park, Velumian & Fehlings (2004) The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma 21:754-774.

59

Pathophysiology of myelinated axons:
Role of AMPA receptors in excitotoxicity and apoptosis of oligodendrocytes after spinal cord injury



Park E, Velumian AA, Fehlings MG (2004) The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma 21:754-774.

60

cytotoxic effect in oligode

Myelination of axons: important for fast transmission of signals along the axons…

BUT…

The price paid for myelination:

- The axons become dependent on the myelinating cells, which are highly susceptible to injury, particularly in CNS
- Most part of the axons becomes isolated from the environment: accumulation of ions / metabolites?
- No access for fast maintenance and repair due to the compactness of myelin membrane layers?
- Breakdown of the myelin after injury releases factors (e.g., myelin-associated inhibitors of axonal growth), which prevent regeneration

61