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CSF formation and uptake: AQPs and solute coupled transport

the microcosmos

Aristotelis S. Filippidis M.D., Ph.D.
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Boston University, Boston Medical Center

ASNR 2013 meeting, IHIWG, San Diego, CA, USA, May 24th 2013

What is this and what is this not ?

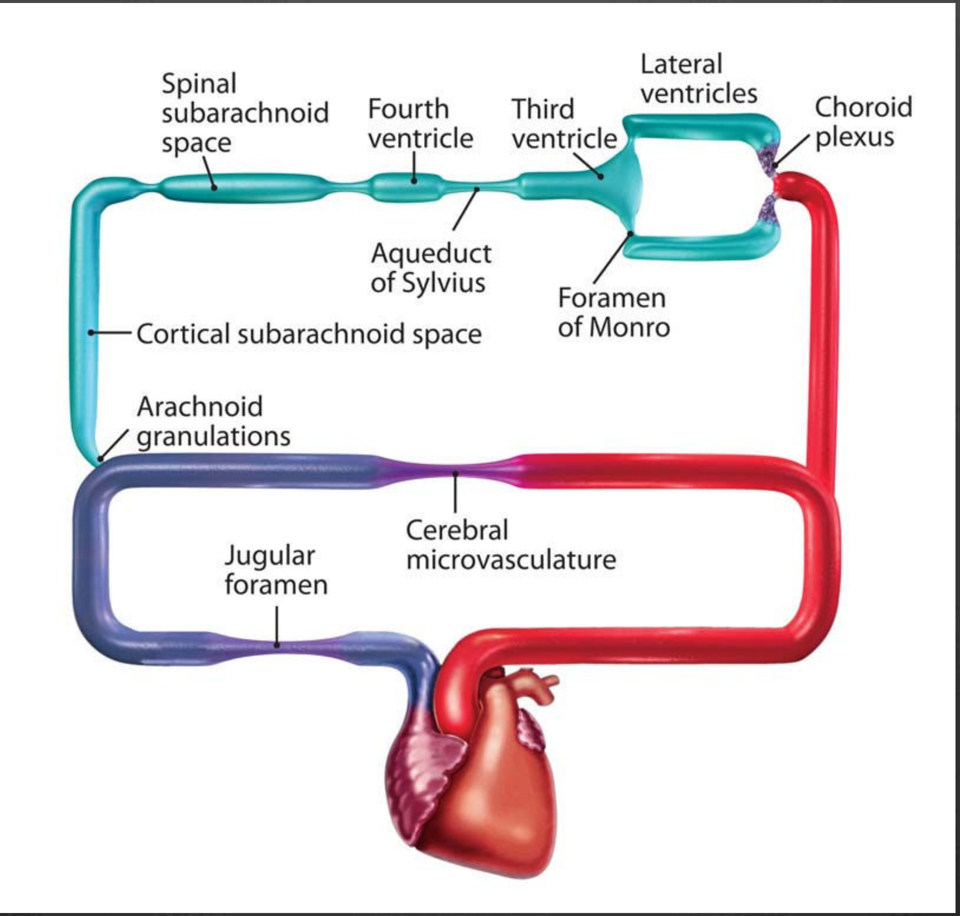
- It is about solute-coupled water transport and AQP4
- THIS IS about “Ion & Water mechanics”
- THIS IS about identifying analogies
- THIS IS about skimming for evidence
- THIS IS about teleology*

Plato and Aristotle, “The school of Athens”, Raphael 1509

*Τελεολογία = teleology:
a definition induced in philosophy by *Plato* and *Aristotle* meaning that *the evolution of structures happens always with a purpose*

"CSF formation and uptake"

- Redefine in CSF-ISF flow balance between compartments
- When this balance shifts reducing uptake we have cerebral edema formation



Rekate HL.The definition and classification of hydrocephalus: a personal recommendation to stimulate debate. Cerebrospinal Fluid Res. 2008 Jan 22;5:2. doi: 10.1186/1743-8454-5-2.

Brain edema types

(revised Klatzo classification)

HYDROCEPHALIC
or
INTERSTITIAL

CYTOTOXIC
or
CELLULAR

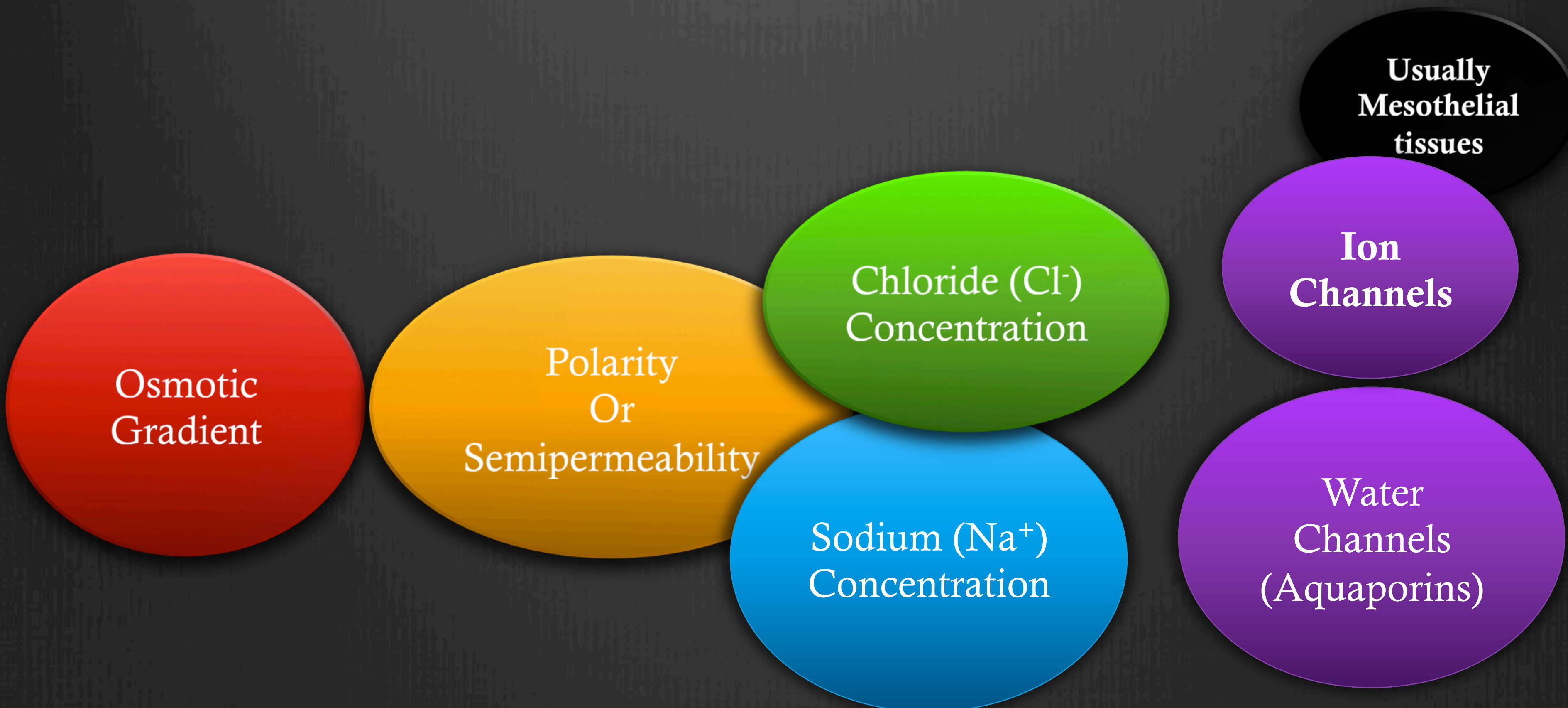
VASOGENIC

Klatzo.
Evolution of brain edema concepts.
Acta neurochirurgica Supplementum (1994)
vol. 60 pp. 3-6

What is
solute coupled transport?

Thinking beyond structures ➔ "Ion & Water mechanics"

The Critical Mixture for solute-coupled transport



Solute-coupled water transport: *the analogy*

Fluid movement, CFD

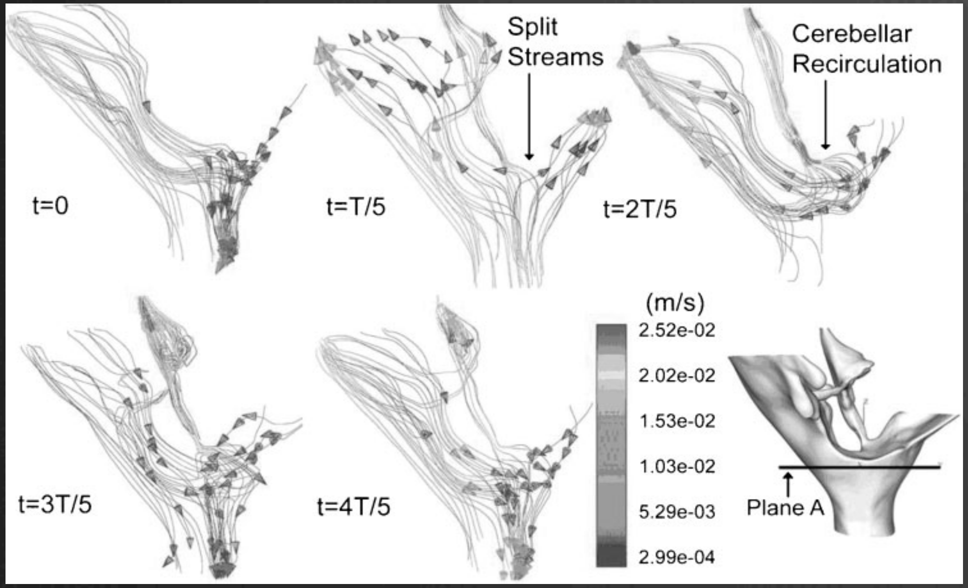
In Macro scale

Pressure gradients & pulse amplitude important

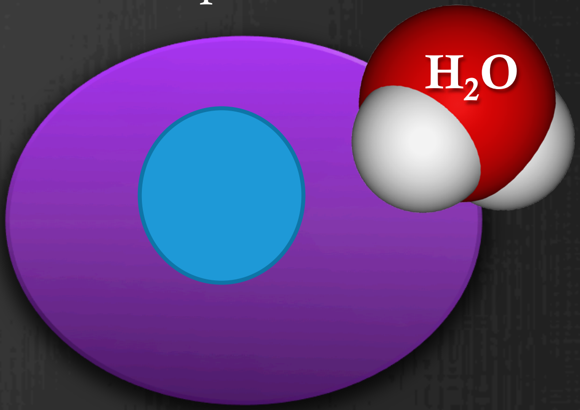
Water movement

In Cellular scale

Osmotic gradient & cellular permeability important



Schaffer N., Martin B., Loth F.
Cerebrospinal fluid hydrodynamics in type I Chiari malformation
Neurological Research (2011), 33:3, 247-260

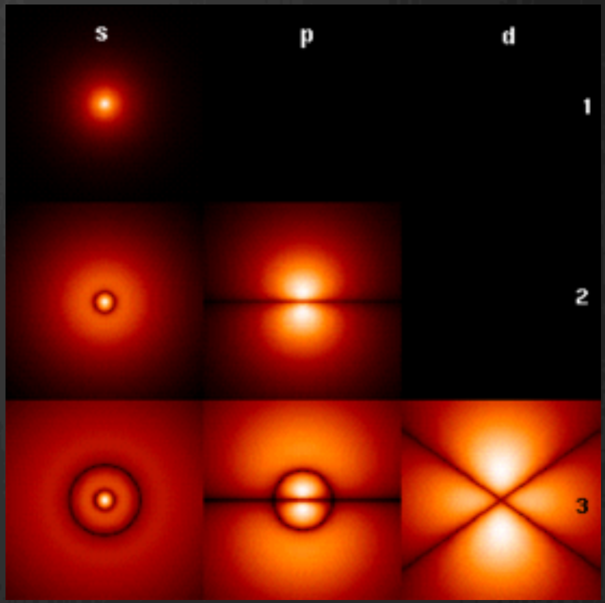


"Ion & Water Mechanics"

Solute-coupled water transport: *analogy*

Newtonian Physics
Macro scale

Quantum physics
Subatomic scale

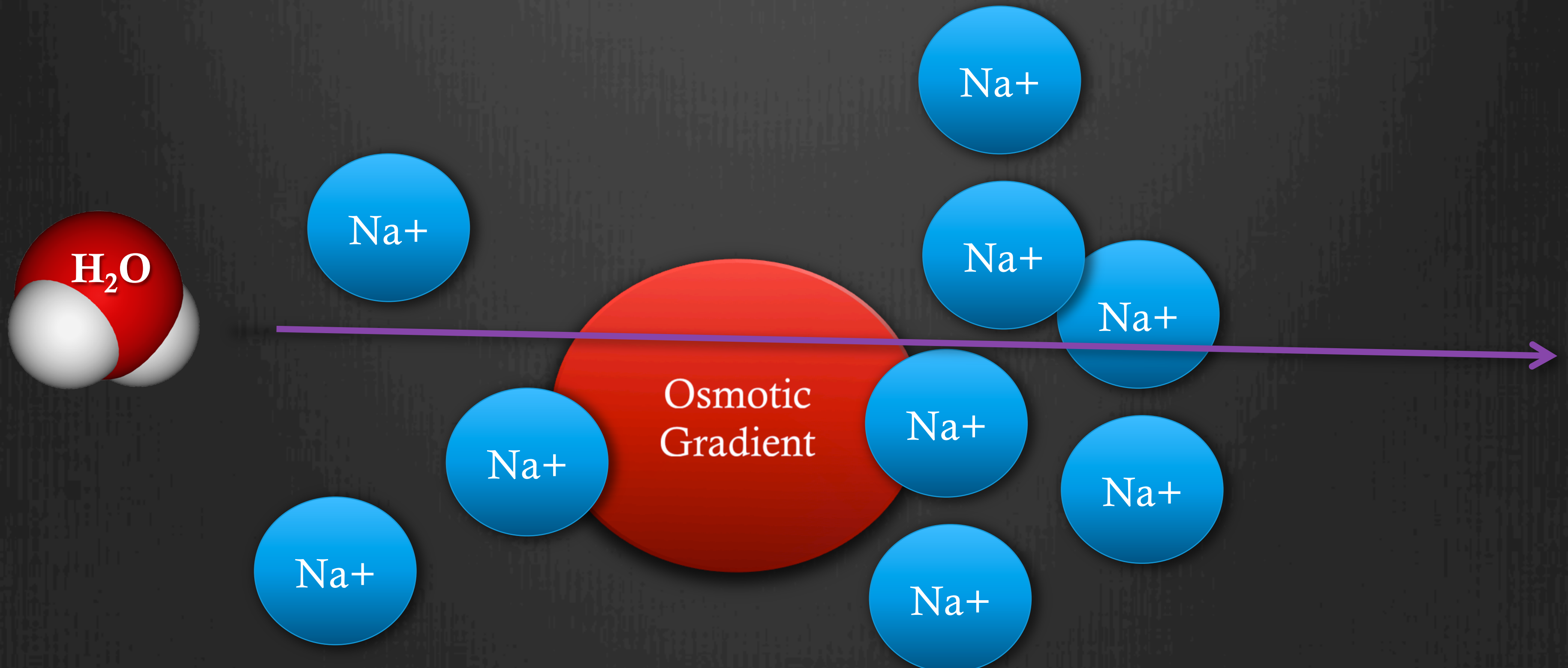


Thinking beyond structures ➔ "Ion & Water mechanics"

Water FOLLOWS Sodium in polarized epithelia

$\sigma = 1$

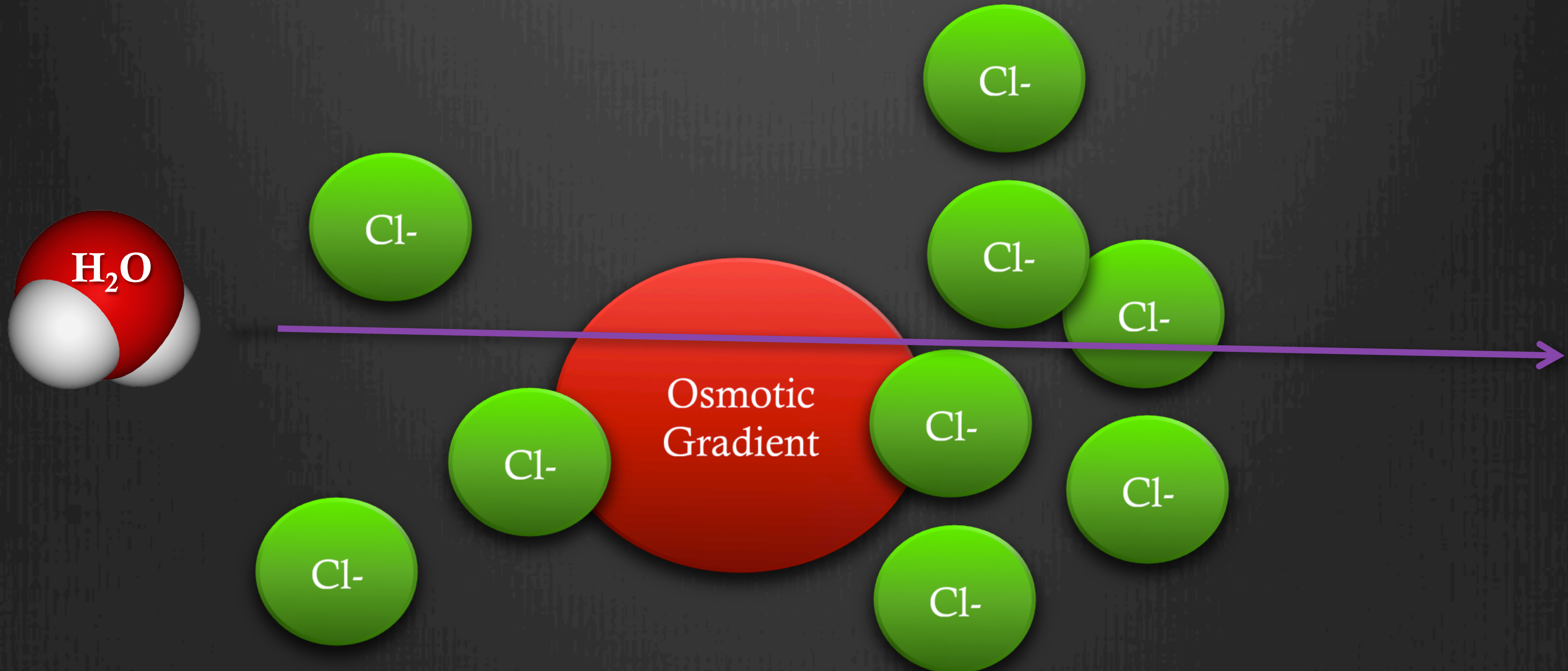
(e.g. choroid plexus, pleura, pericardium, omentum, nephron)



Thinking beyond structures ➔ "Ion & Water mechanics"

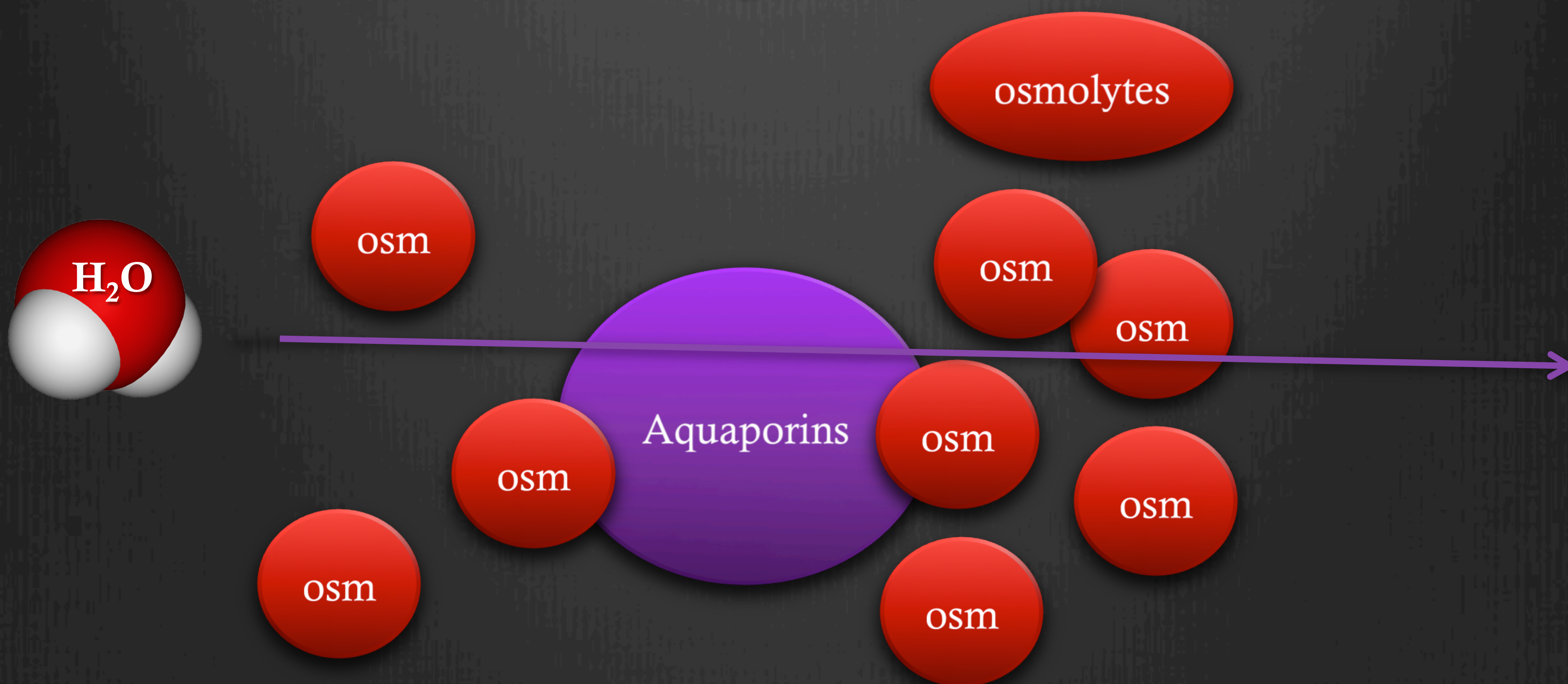
Water FOLLOWS Chloride in polarized epithelia

(e.g. sweat glands, salivary glands, bronchi)



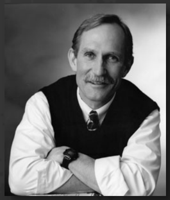
Thinking beyond structures → "Ion & Water mechanics"

Water FOLLOWS osmotic gradient of osmolytes through AQPs



AQUAPORINS

- Family of more than 13 water channel proteins
- First described in 1991 as aquaporin -1 (AQP1)
- Nobel prize in Chemistry 2003 *(Peter Agre)*



- Aquaporin-4 (AQP4) is the dominant form in the brain, with AQP1 being dominant at the choroid plexus

Agre et al.
Towards a molecular understanding of water homeostasis in the brain.
Neuroscience (2004) vol. 129 (4) pp. 849-50

AQP1 LOCALIZATION

- **Mostly at the apical surface of choroid plexus**
- Some presence at the basolateral surface of the choroid plexus
- Abundant in red blood cells

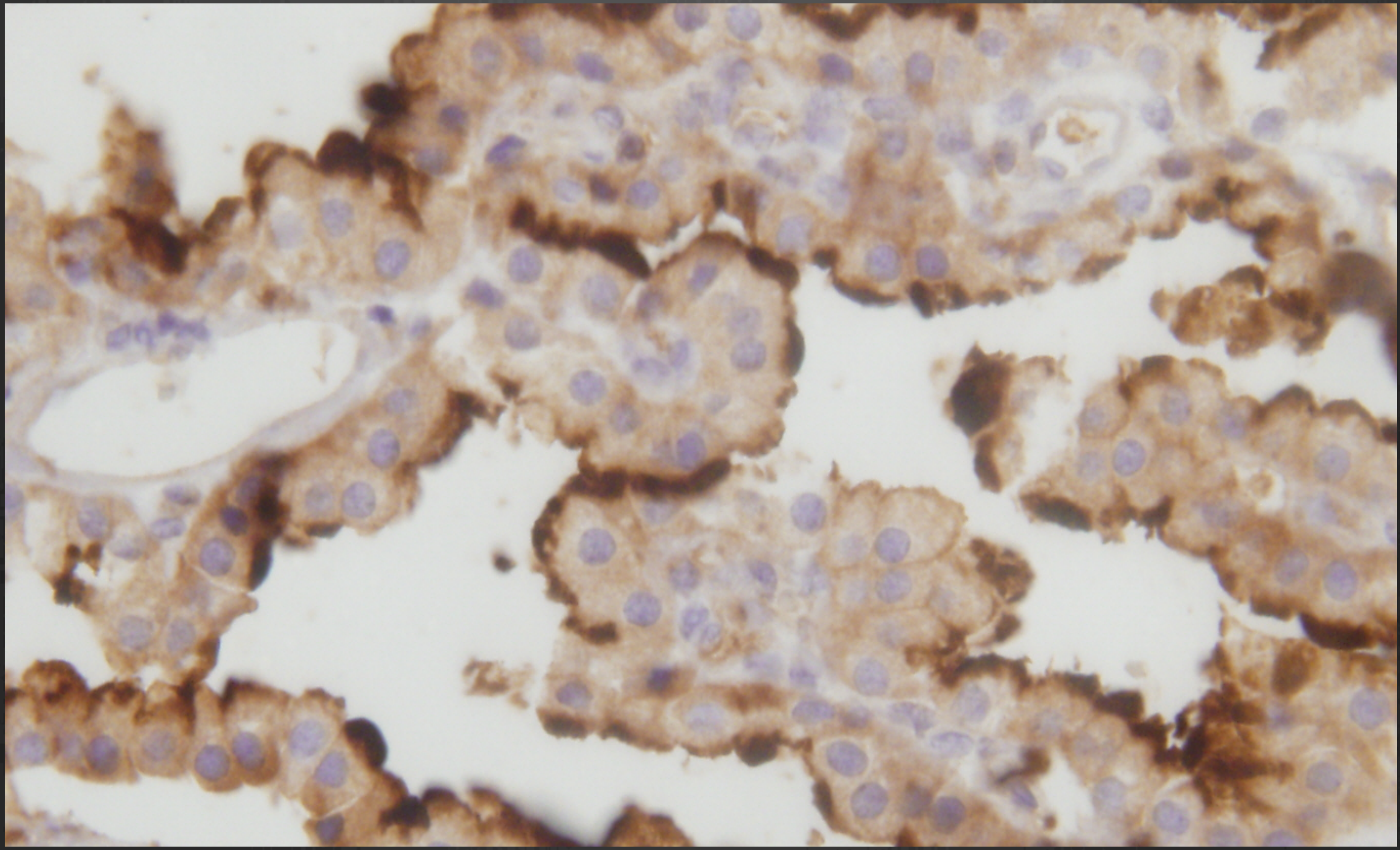
THEY NEED !!! an Osmotic Gradient

They are important for Hydrocephalus

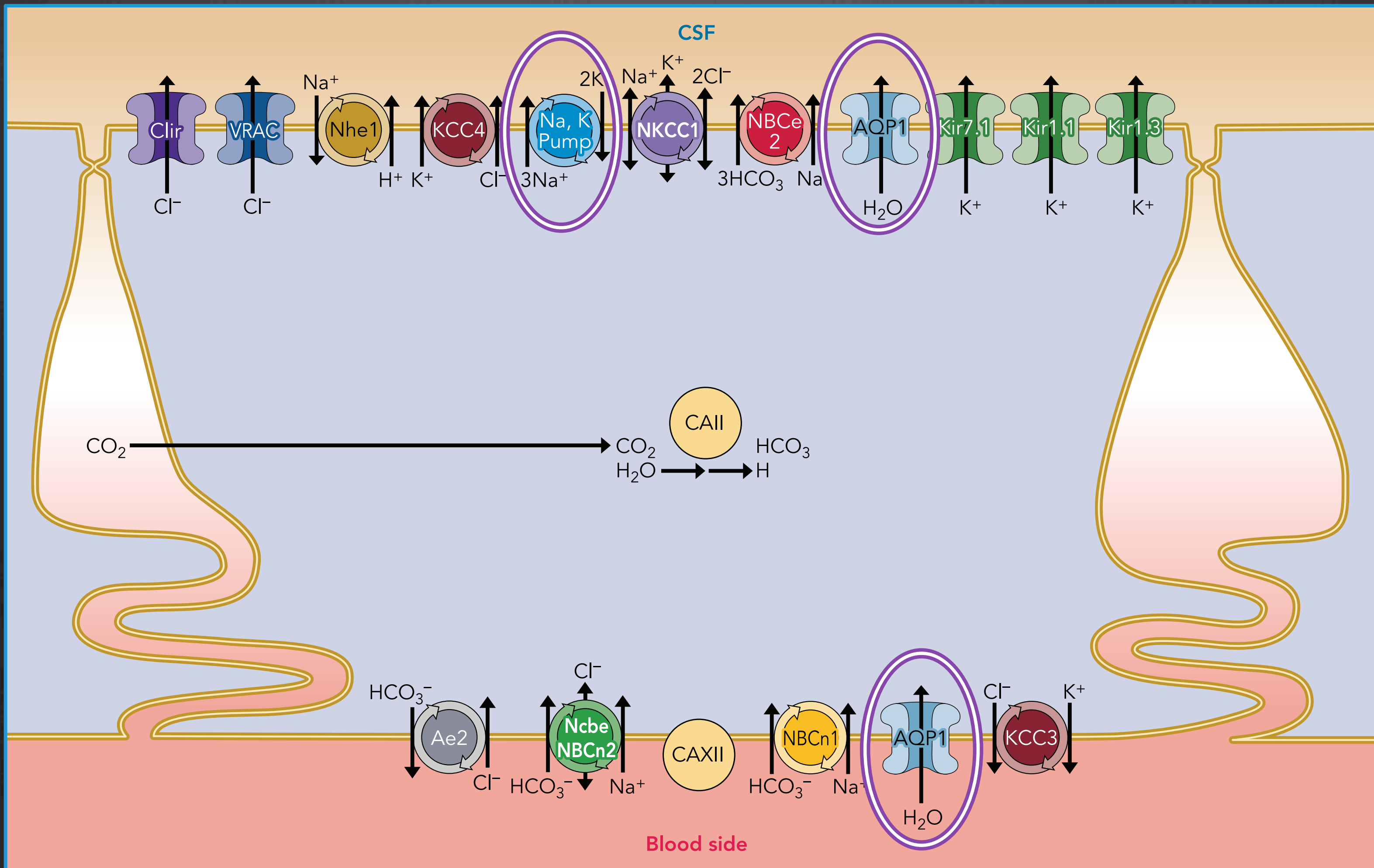
Badaut et al.
Aquaporins in brain: distribution, physiology, and pathophysiology.
J Cereb Blood Flow Metab (2002) vol. 22 (4) pp. 367-78

Filippidis et al.
Hydrocephalus and aquaporins: lessons learned from the bench.
Childs Nerv Syst. 2011 Jan;27(1):27-33. Epub 2010 Jul 13.

AQP1 on the choroid plexus



choroid plexus: ion channels



Damkier et al.
Epithelial Pathways in Choroid Plexus Electrolyte Transport.
Physiology (2010), 25, p 239-249

AQP4 LOCALIZATION

- **Glia Limitans**
- **Astrocyte foot processes around capillaries that form the Blood-Brain-Barrier (BBB)**
- Ependymal cells
- Supraoptic and suprachiasmatic nuclei of hypothalamus
- Cerebellum
- Hippocampal dentate gyrus,
- Hippocampal areas CA1-CA2
- Neocortex
- Nucleus of stria terminalis
- Medial habenular nucleus

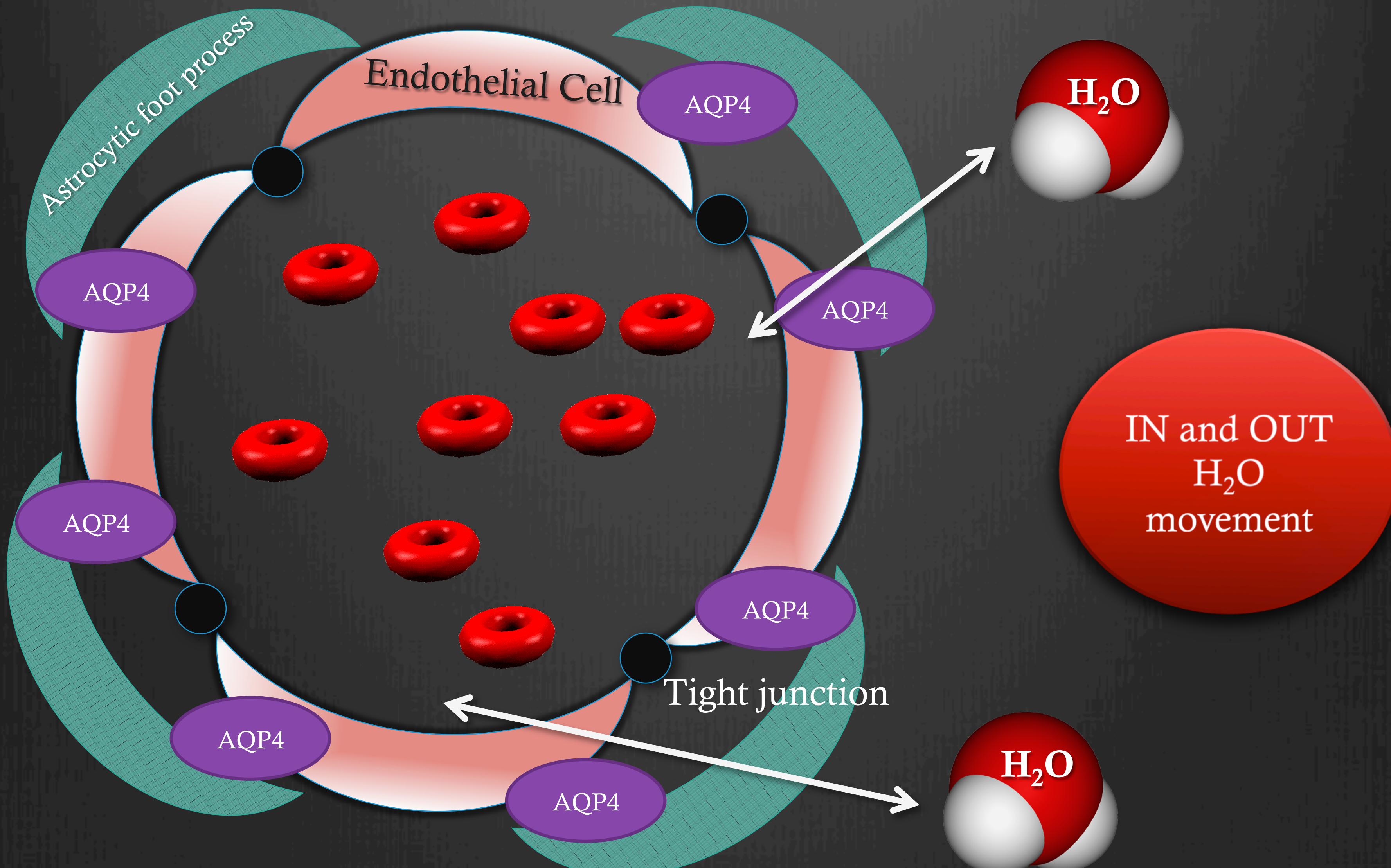
THEY NEED !!! an Osmotic Gradient

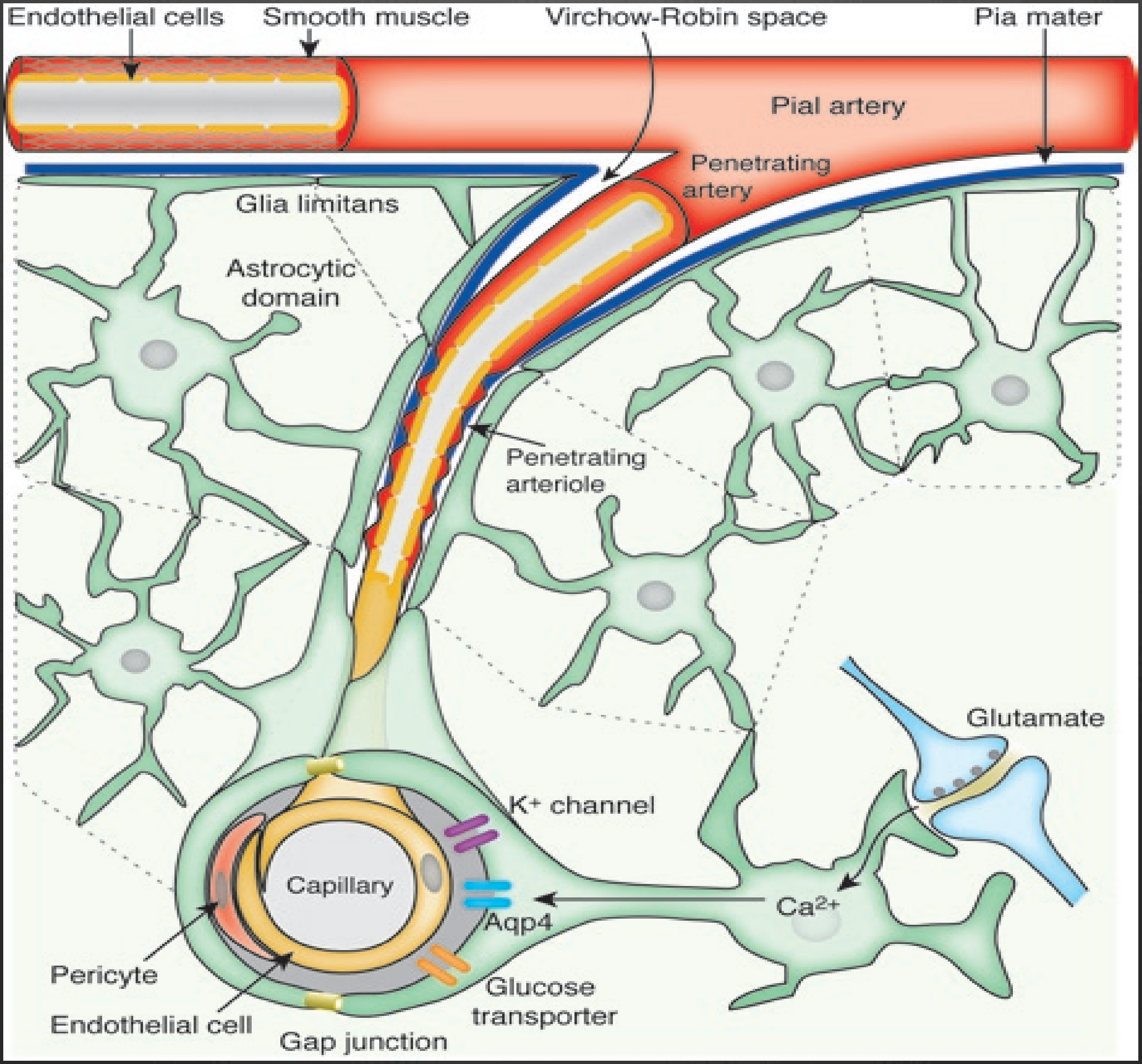
They are important for Hydrocephalus

Badaut et al.
Aquaporins in brain: distribution, physiology, and pathophysiology.
J Cereb Blood Flow Metab (2002) vol. 22 (4) pp. 367-78

Filippidis et al.
Hydrocephalus and aquaporins: lessons learned from the bench.
Childs Nerv Syst. 2011 Jan;27(1):27-33. Epub 2010 Jul 13.

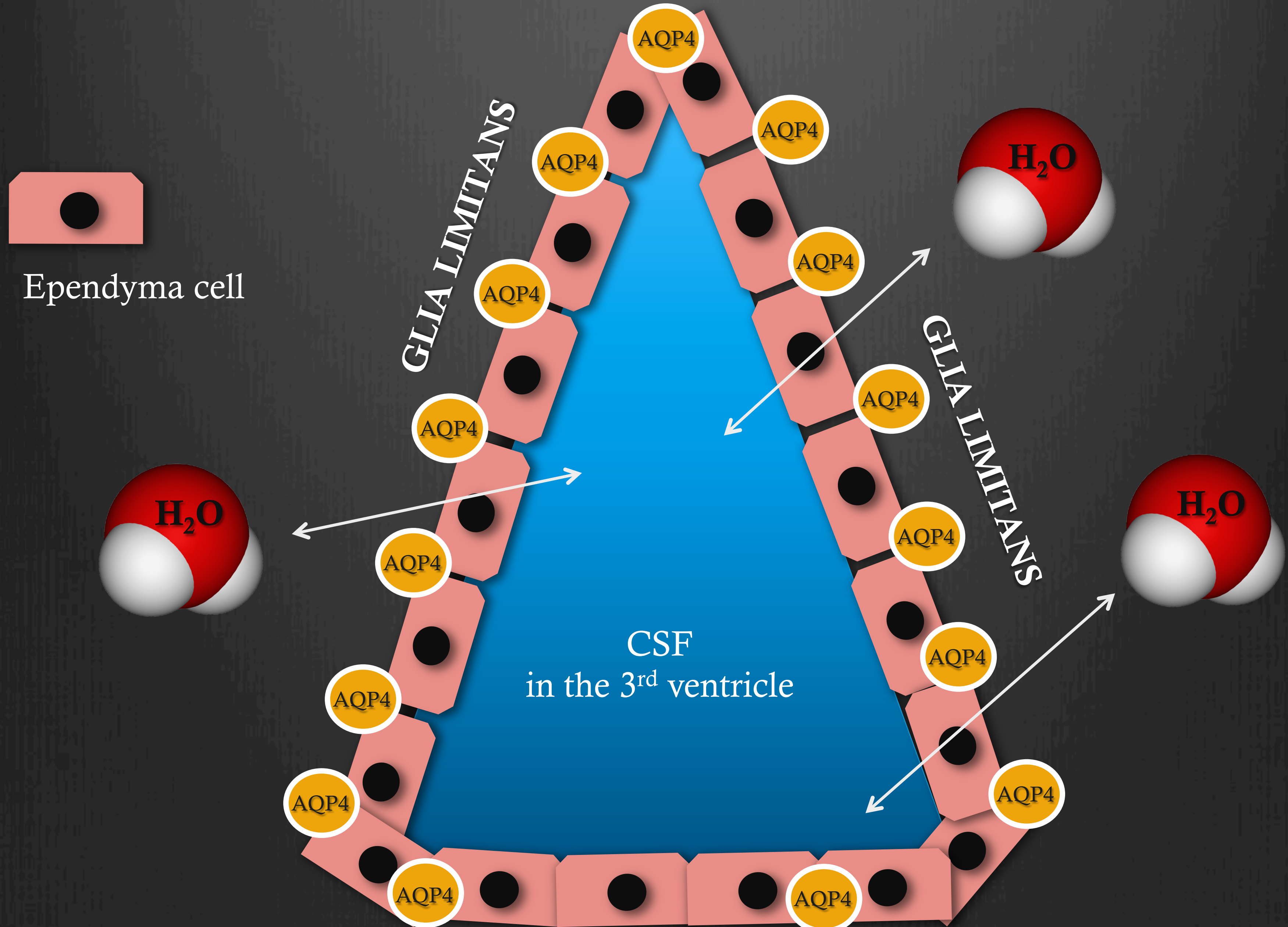
AQP4 and the Blood-Brain-Barrier and Cerebral vessels





Costantino Iadecola & Maiken Nedergaard, *Nature Neuroscience*, 2007

AQP4 at Ependyma



"the short circuit" AQP4 at the BBB

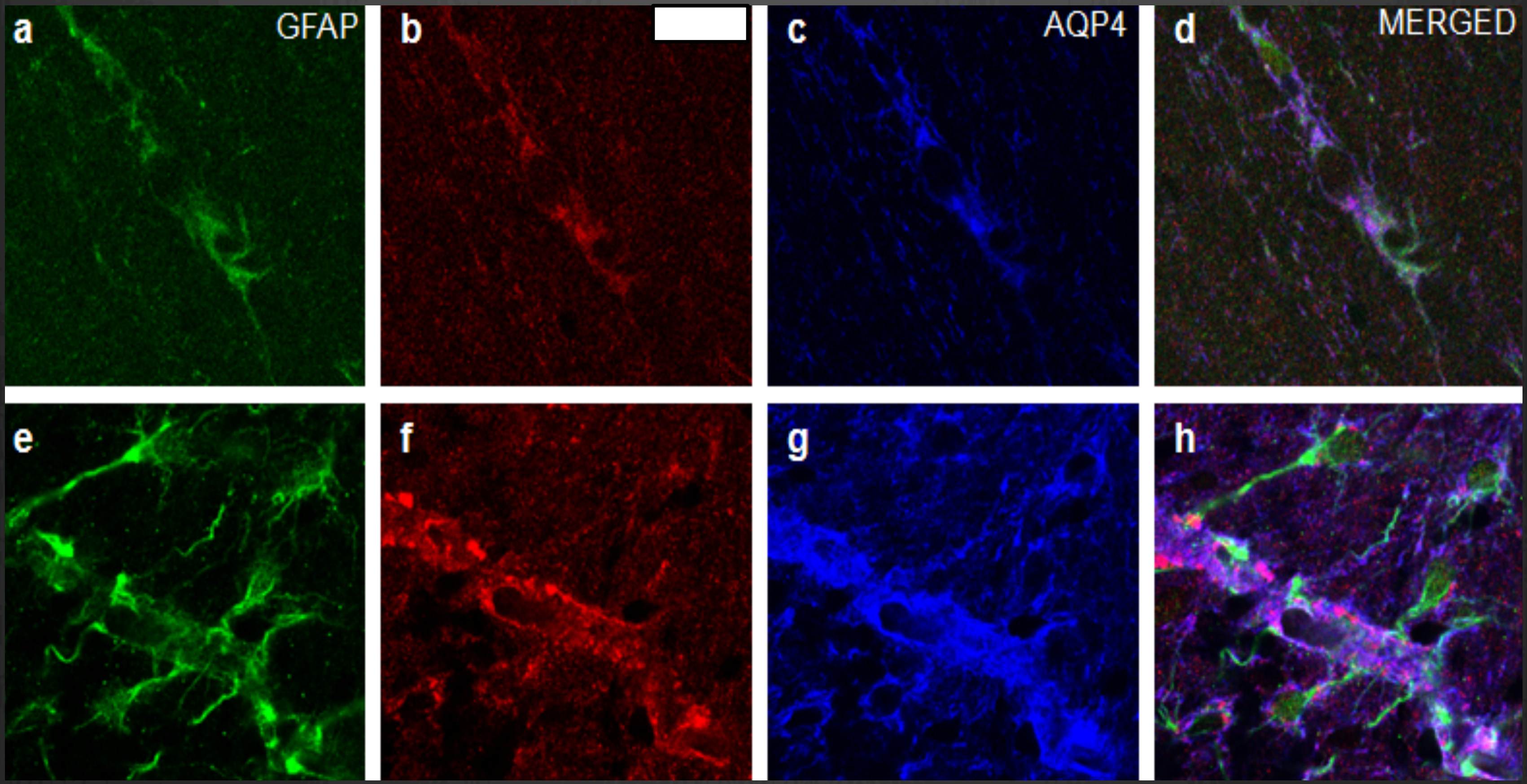


Image courtesy of Marmarou Lab

"Glymphatics"

RESEARCH ARTICLE

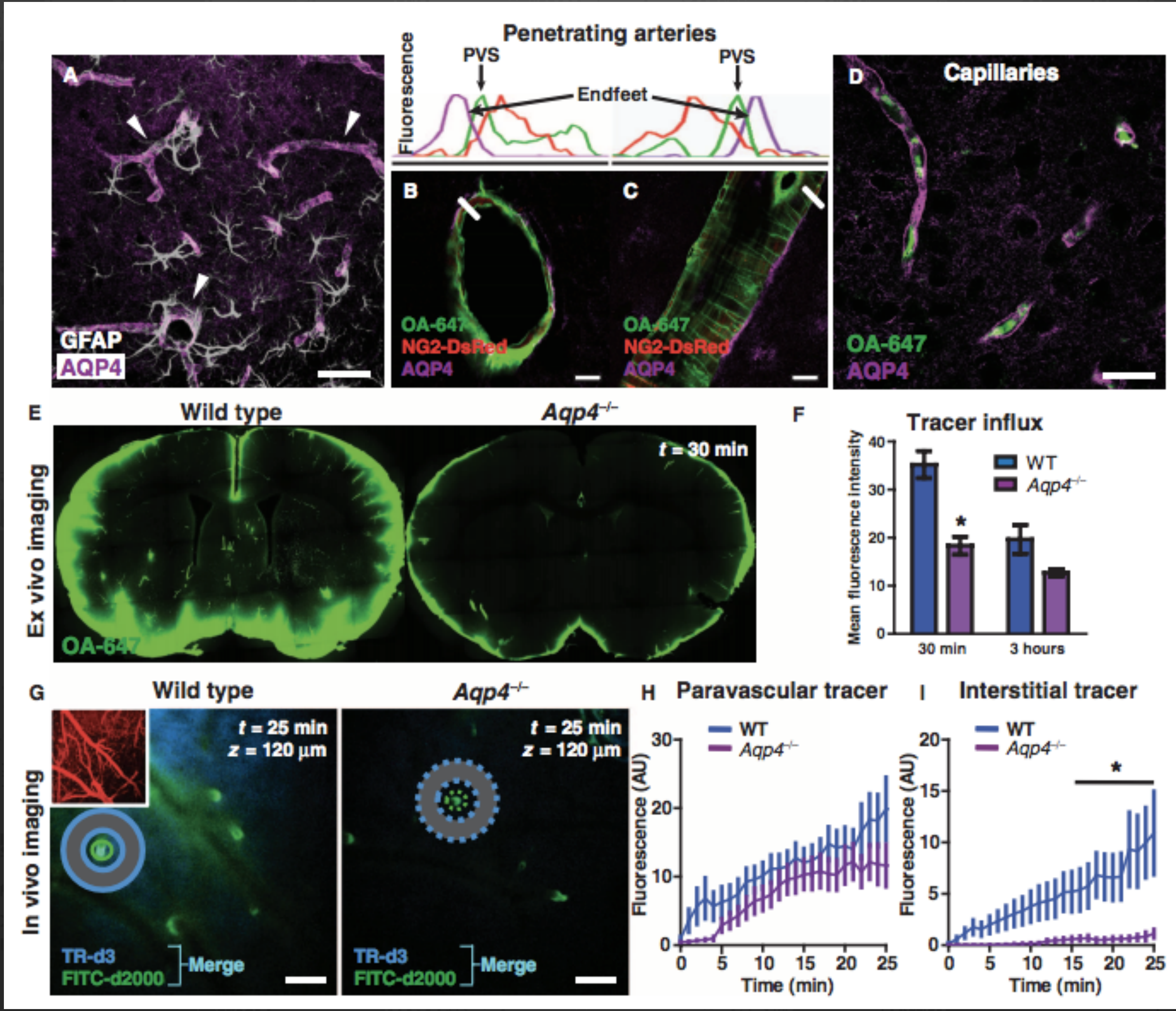
CEREBROSPINAL FLUID CIRCULATION

A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β

Jeffrey J. Iliff,[1]* Minghuan Wang,[1,2] Yonghong Liao,[1] Benjamin A. Plogg,[1] Weiguo Peng,[1] Georg A. Gundersen,[3,4] Helene Benveniste,[5,6] G. Edward Vates,[1] Rashid Deane,[1] Steven A. Goldman,[1,7] Erlend A. Nagelhus,[3,4] Maiken Nedergaard[1]*

Because it lacks a lymphatic circulation, the brain must clear extracellular proteins by an alternative mechanism. The cerebrospinal fluid (CSF) functions as a sink for brain extracellular solutes, but it is not clear how solutes from the brain interstitium move from the parenchyma to the CSF. We demonstrate that a substantial portion of subarachnoid CSF cycles through the brain interstitial space. On the basis of in vivo two-photon imaging of small fluorescent tracers, we showed that CSF enters the parenchyma along paravascular spaces that surround penetrating arteries and that brain interstitial fluid is cleared along paravenous drainage pathways. Animals lacking the water channel aquaporin-4 (AQP4) in astrocytes exhibit slowed CSF influx through this system and a ~70% reduction in interstitial solute clearance, suggesting that the bulk fluid flow between these anatomical influx and efflux routes is supported by astrocytic water transport. Fluorescent-tagged amyloid β, a peptide thought to be pathogenic in Alzheimer's disease, was transported along this route, and deletion of the *Aqp4* gene suppressed the clearance of soluble amyloid β, suggesting that this pathway may remove amyloid β from the central nervous system. Clearance through paravenous flow may also regulate extracellular levels of proteins involved with neurodegenerative conditions, its impairment perhaps contributing to the mis-accumulation of soluble proteins.

www.ScienceTranslationalMedicine.org 15 August 2012 Vol 4 Issue 147 147ra111



Iliff et al, Science Translational Medicine, Aug 2012

Time (hours)

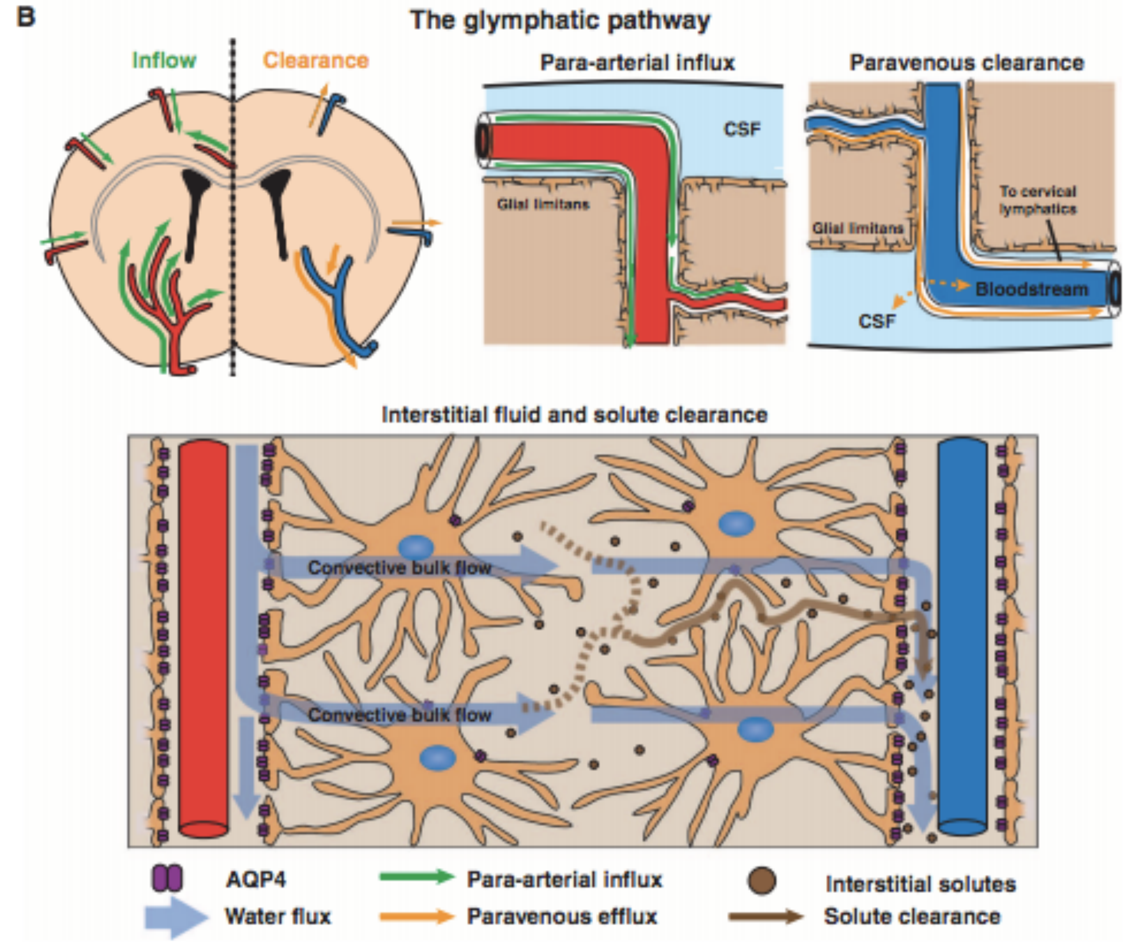


Fig. 5. The glymphatic system supports interstitial solute and fluid clearance from the brain. (**A**) To evaluate the role of the clearance of interstitial solutes, we measured the elimination of intrastriate [^{3}H]mannitol from the brain (for details, see fig. S8A). Over the first 2 hours after injection, the clearance of intrastriate [^{3}H]mannitol from *Aqp4*-null mouse brains was significantly reduced (**P* < 0.01, *n* = 4 per time point) compared to WT controls. (**B**) Schematic depiction of the glymphatic pathway. In this brain-wide pathway, CSF enters the brain along para-arterial routes, whereas ISF is cleared from the brain along paravenous routes. Convective bulk ISF flow between these influx and clearance routes is facilitated by AQP4-dependent astroglial water flux and drives the clearance of interstitial solutes and fluid from the brain parenchyma. From here, solutes and fluid may be dispersed into the subarachnoid CSF, enter the bloodstream across the postcapillary vasculature, or follow the walls of the draining veins to reach the cervical lymphatics.

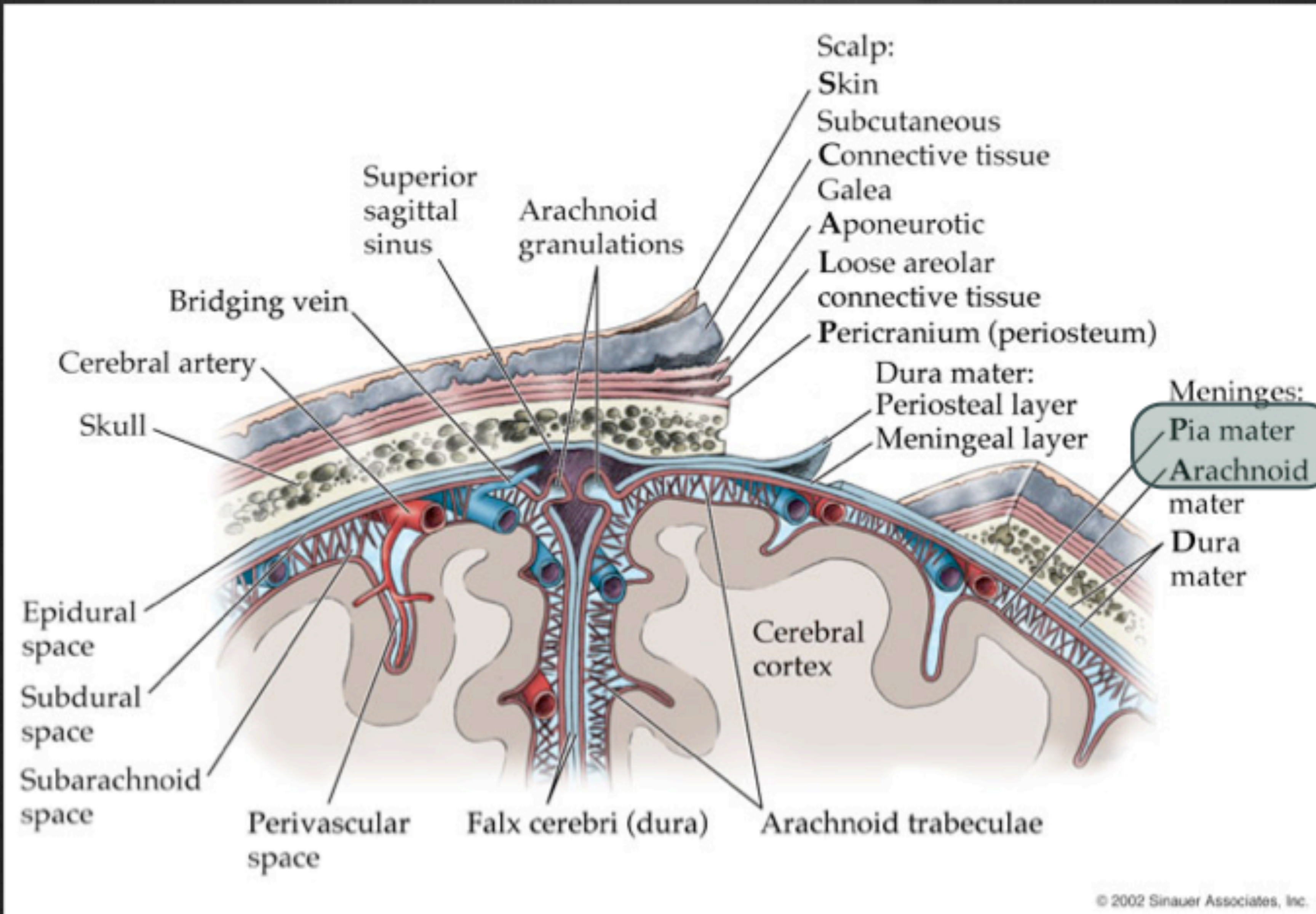
Iliff et al, Science Translational Medicine, Aug 2012

Could this be also the case for CSAS ?

Is Solute-coupled transport of water present ?

Skimming for Evidence…

Cortical Meninges



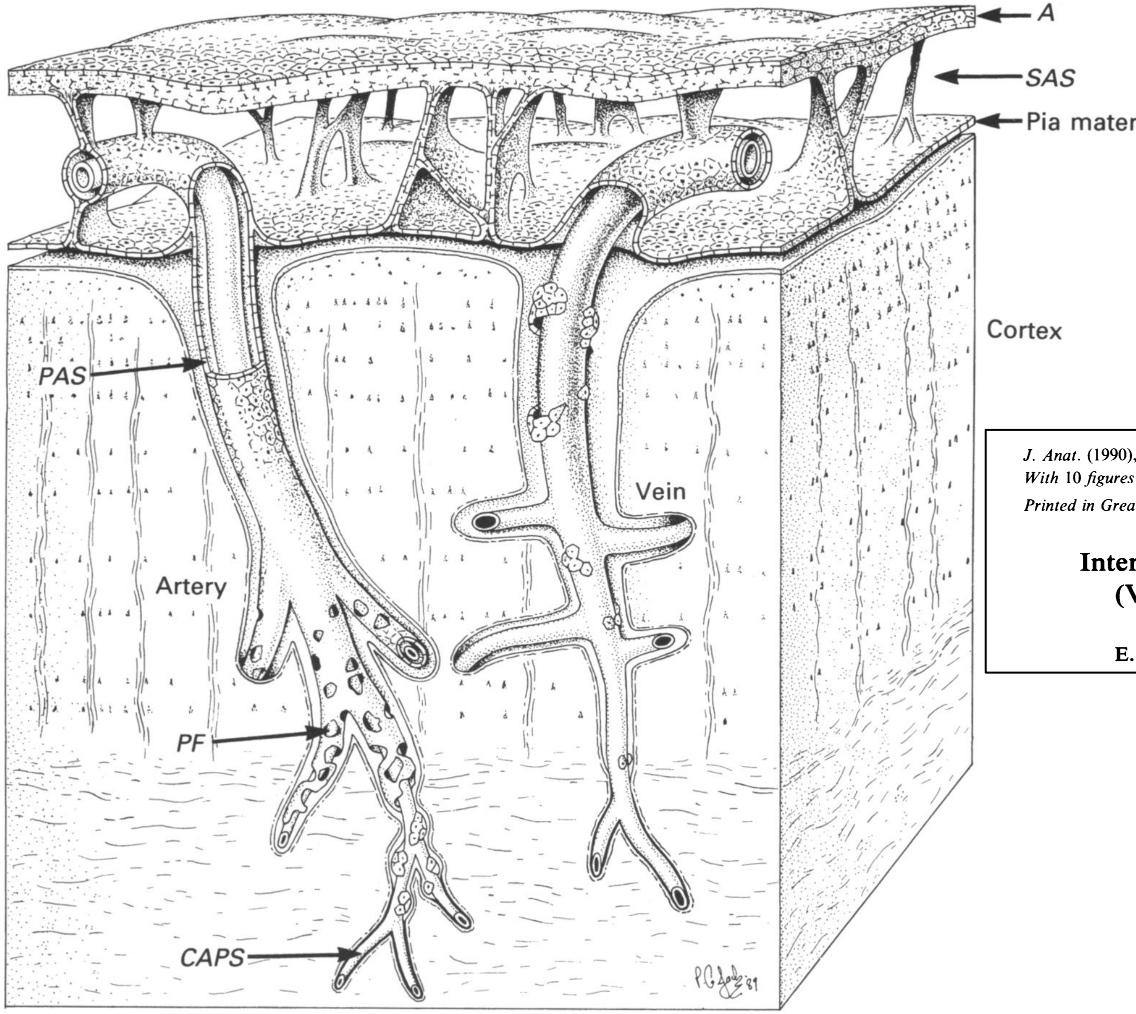
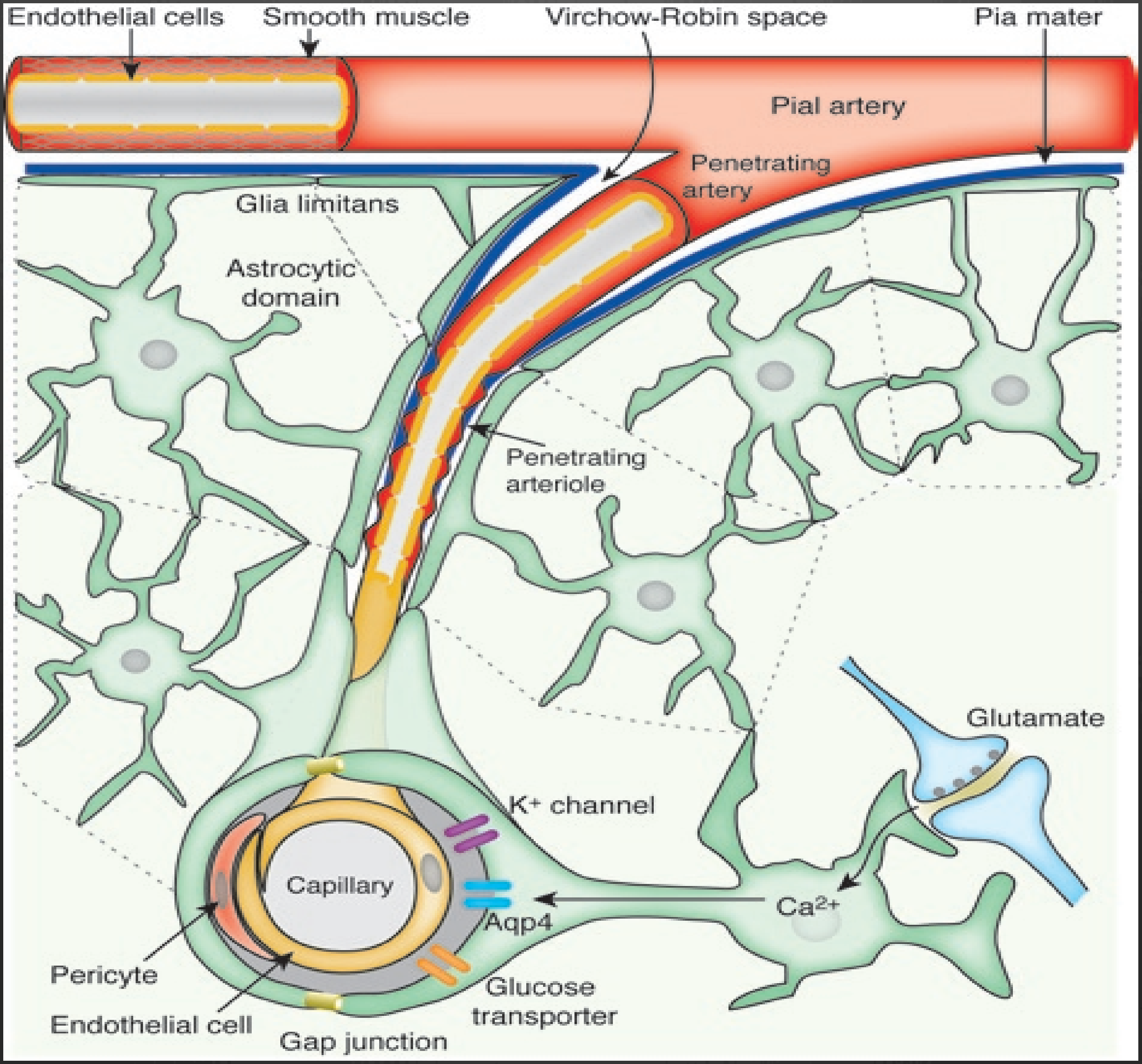


Fig. 10. Diagram demonstrating the relationships of the pia mater and intracerebral blood vessels. Subarachnoid space (*SAS*) separates the arachnoid (*A*) from the pia mater overlying the cerebral cortex. An artery on the left of the picture is coated by a sheath of cells derived from the pia mater; the sheath has been cut away to show that the periarterial spaces (PAS) of the intracerebral and extracerebral arteries are in continuity. The layer of pial cells becomes perforated (*PF*) and incomplete as smooth muscle cells are lost from the smaller branches of the artery. The pial sheath finally disappears as the perivascular spaces are obliterated around capillaries (*CAPS*). Perivascular spaces around the vein (right of picture) are confluent with the subpial space and only small numbers of pial cells are associated with the vessel wall.

J. Anat. (1990), **170**, 111–123 111
With 10 *figures*
Printed in Great Britain

Interrelationships of the pia mater and the perivascular (Virchow–Robin) spaces in the human cerebrum*

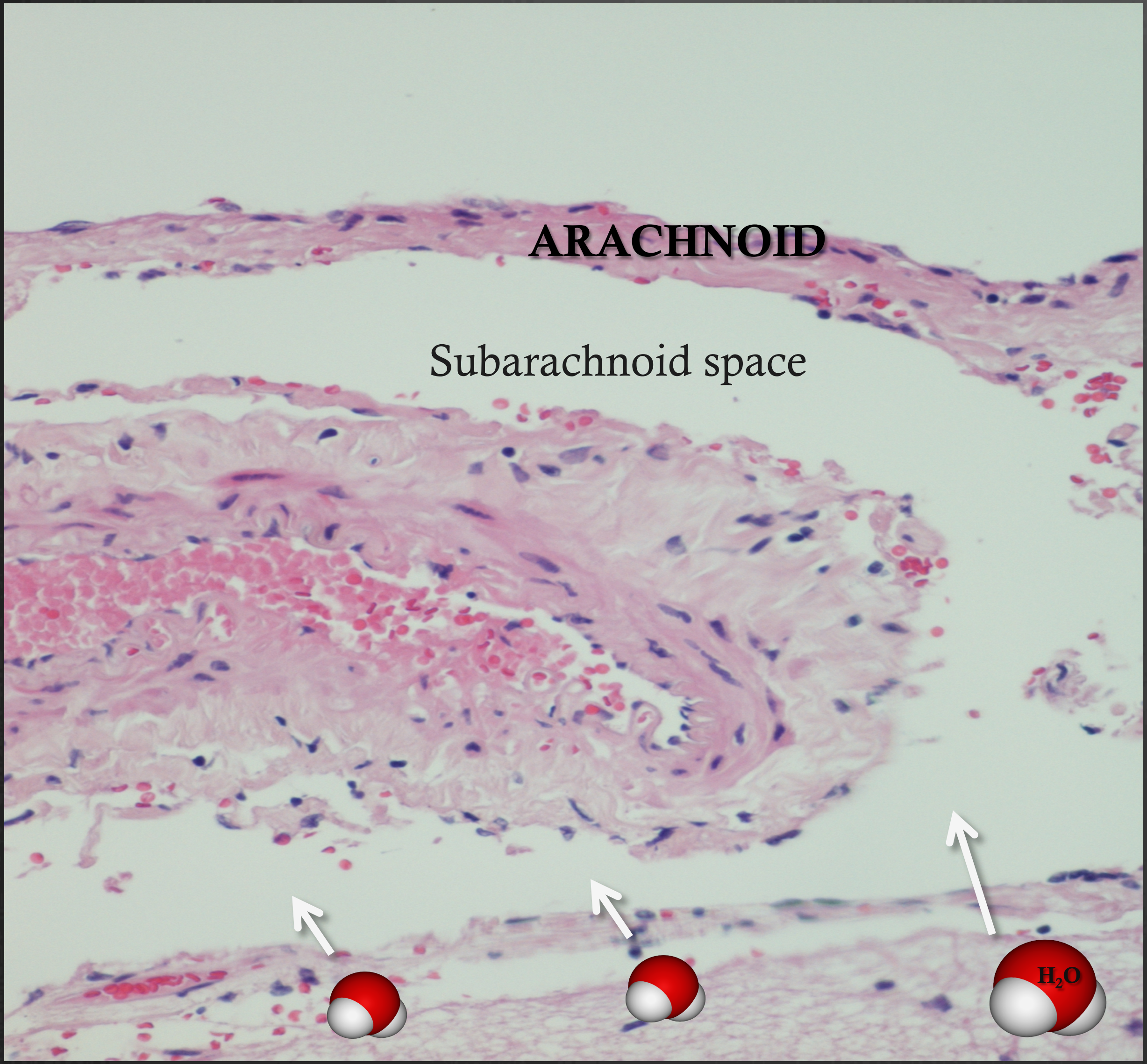
E. T. ZHANG,†‡ C. B. E. INMAN† AND R. O. WELLER†



Costantino Iadecola & Maiken Nedergaard, *Nature Neuroscience*, 2007

Indirect evidence about brain edema (excess water) clearance at this interface

Glia limitans – subarachnoid space



Tait et al.
Water movements in the brain: role of aquaporins.
Trends Neurosci (2008) vol. 31 (1) pp. 37-43

Reulen et al.
Role of pressure gradients and bulk flow in dynamics of vasogenic brain edema.
J Neurosurg (1977) vol. 46 (1) pp. 24-35

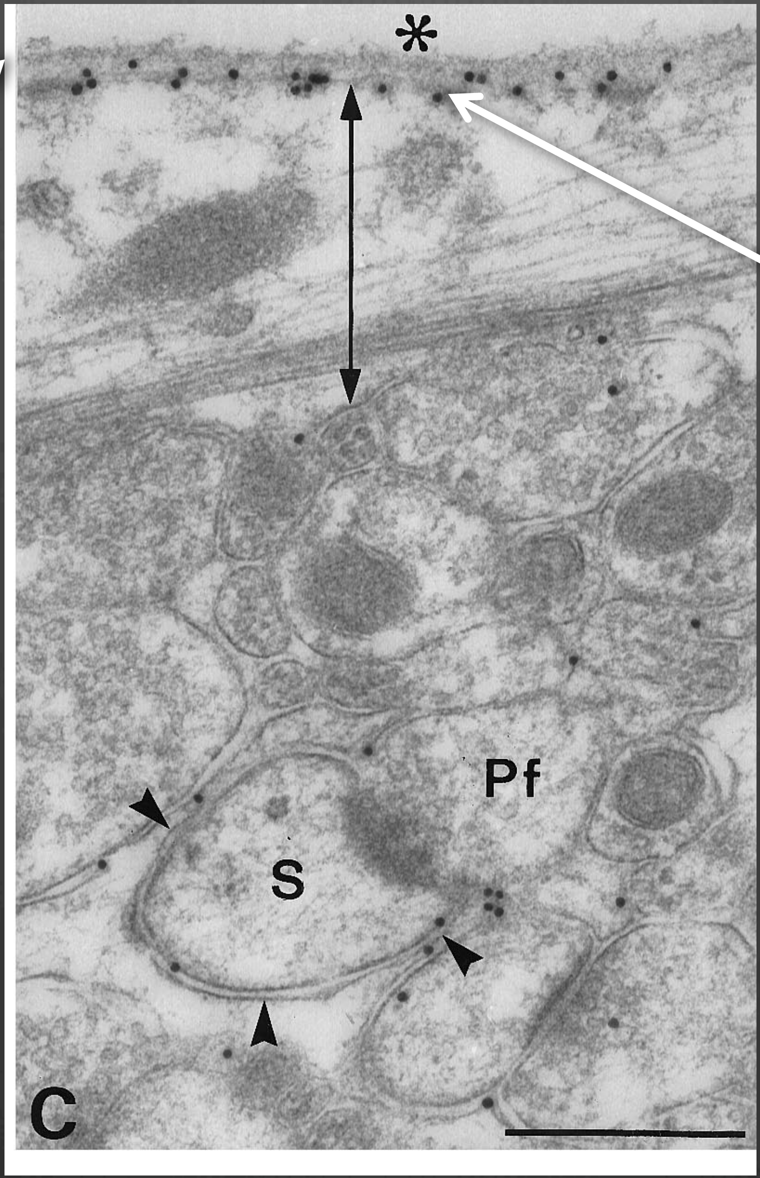
Cerebrospinal Fluid Production by the Choroid Plexus and Brain

Abstract. *The production of cerebrospinal fluid and the transport of ^{24}Na from the blood to the cerebrospinal fluid were studied simultaneously in normal and choroid plexectomized rhesus monkeys. Choroid plexectomy reduced the production of cerebrospinal fluid by an average of 33 to 40 percent and the rate of appearance of ^{24}Na in the cerebrospinal fluid and its final concentration were proportionately reduced. In both normal and plexectomized animals, ^{24}Na levels were found to be markedly greater in the gray matter surrounding the ventricles and in the gray matter bordering the subarachnoid space. That sodium exchanges in these two general areas of the brain may be linked to the formation of the cerebrospinal fluid is discussed here.*

CSAS-
brain surface interface !

Milhorat et al.
Cerebrospinal fluid production by the choroid plexus and brain
Science (1971) vol. 173 (994) pp. 330-332

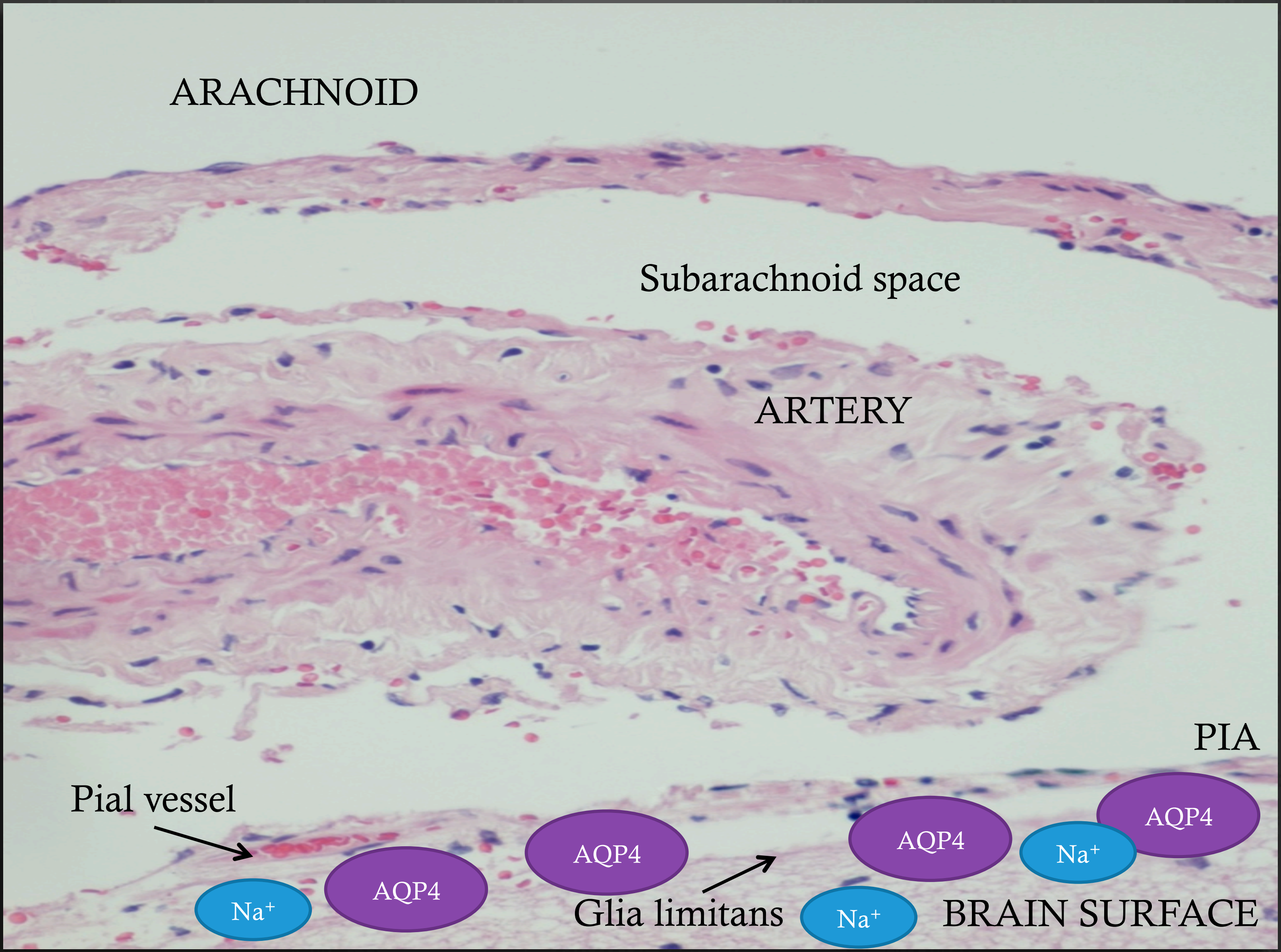
PIA mater - Glia Limitans
lower CSAS interface



Black dots ?
AQP4

Nielsen et al.
Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain
J Neurosci (1997) vol. 17 (1) pp. 171-180

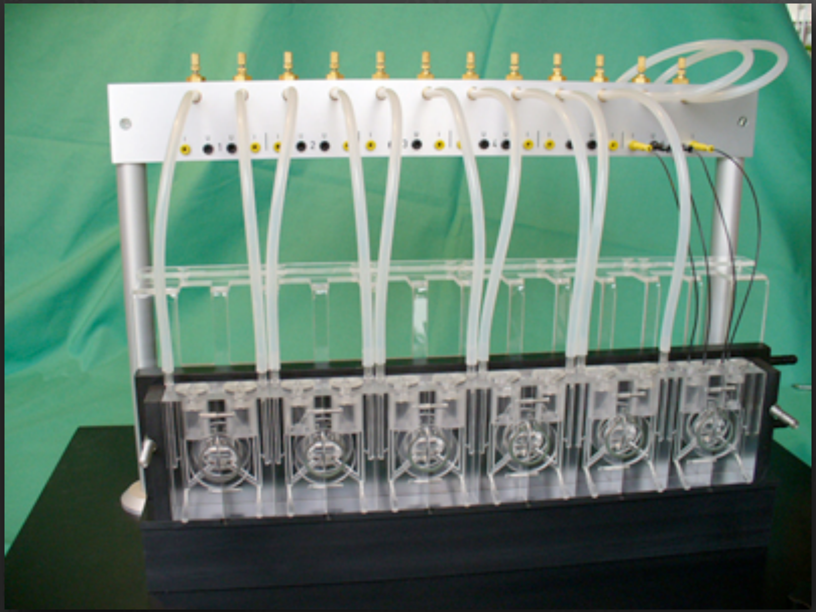
Let us think with teleology in mind !

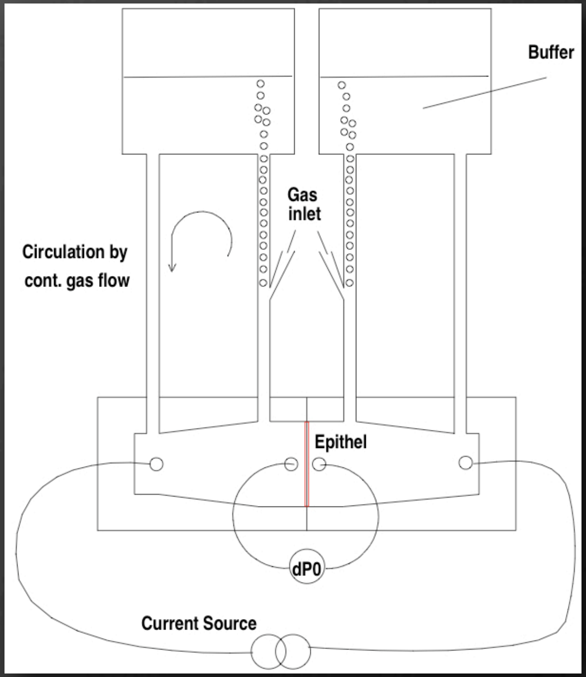


CSAS Ion channels

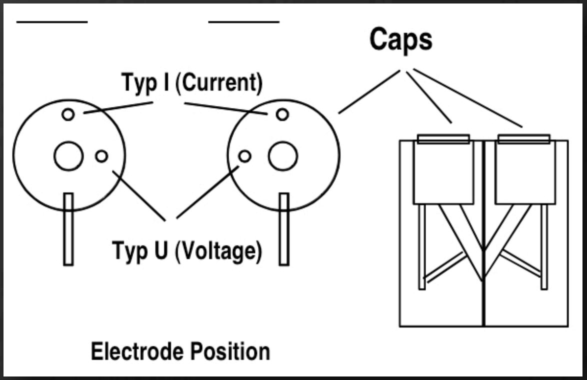
Solute-coupled transport

Membrane Electrophysiology
"Hans Ussing" chambers

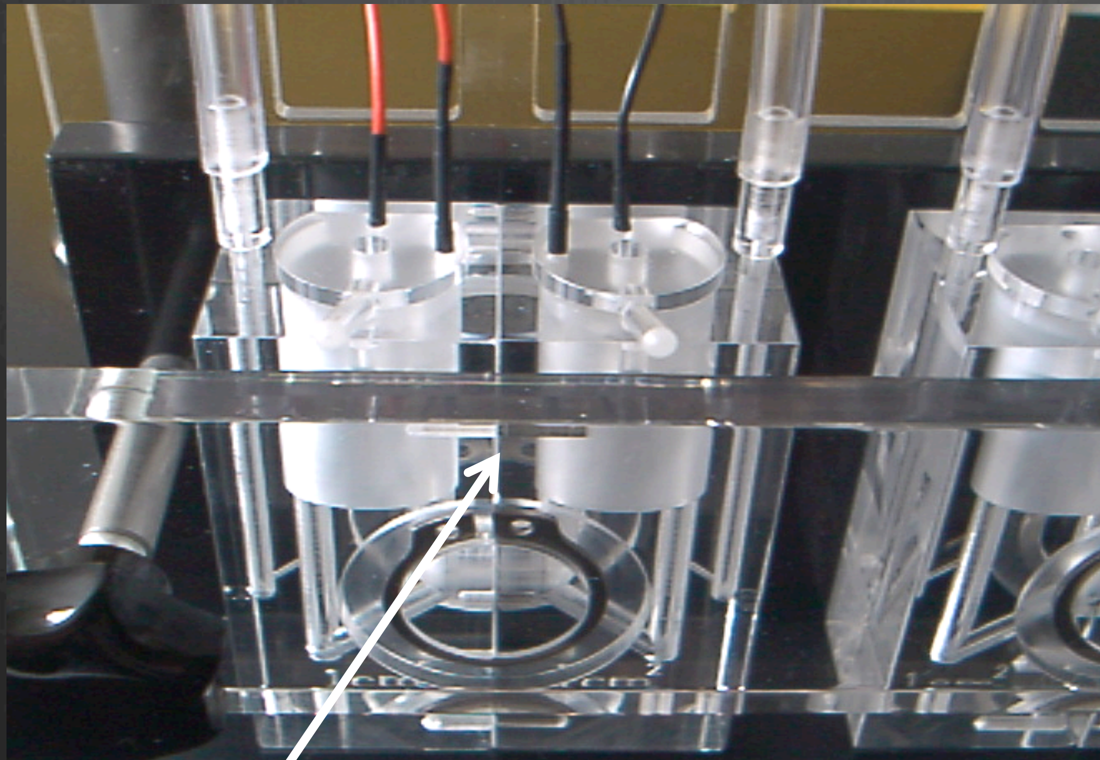




ESTABLISHED METHOD
FOR SOLUTE-COUPLED TRANSPORT STUDIES



Ussing HH, Zerahn K.
Active transport of sodium as the source of electric current in the short-circuited isolated frog skin.
Acta Physiol Scand. 1951 Aug 25;23(2-3):110-27.



Ex vivo CSAS model

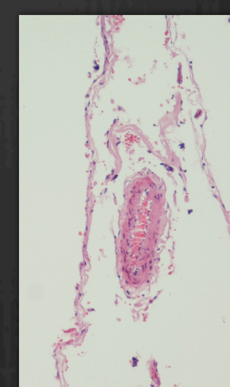
We get the:

Transmembrane Resistance

$\boldsymbol{R}$ **(Ω*cm²)**

HIGH transmembrane resistance = LOW ionic permeability
LOW transmebrane resistance = HIGH ionic permeability

CSAS tissue profiles (facing hemichamber)



Orientation in between hemichambers

RAPID COMMUNICATION

Transmembrane resistance and histology of isolated sheep leptomeninges

Aristotelis Filippidis*, Sotirios Zarogiannis*, Maria Ioannou†, Konstantinos Gourgoulianis‡, Paschalis-Adam Molyvdas* and Chrissi Hatzoglou*

Neurol Res. 2010 Mar;32(2):205-8. Epub 2009 May 8.

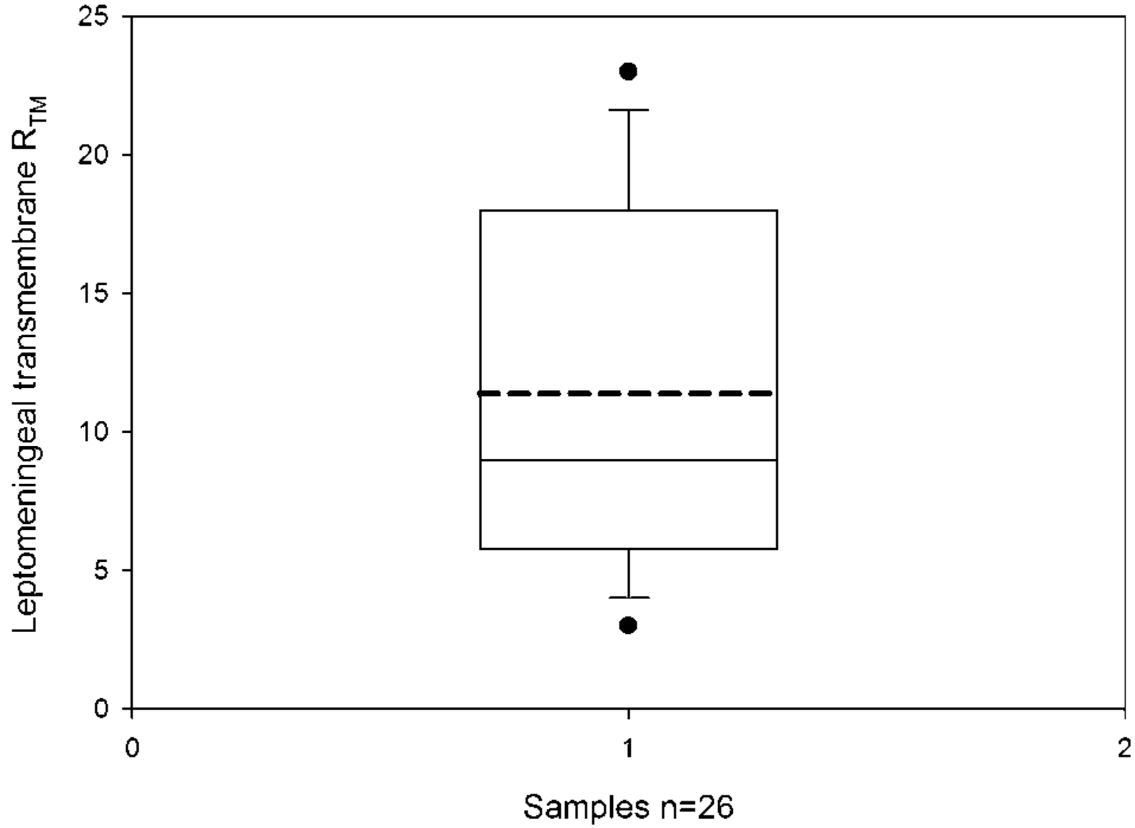
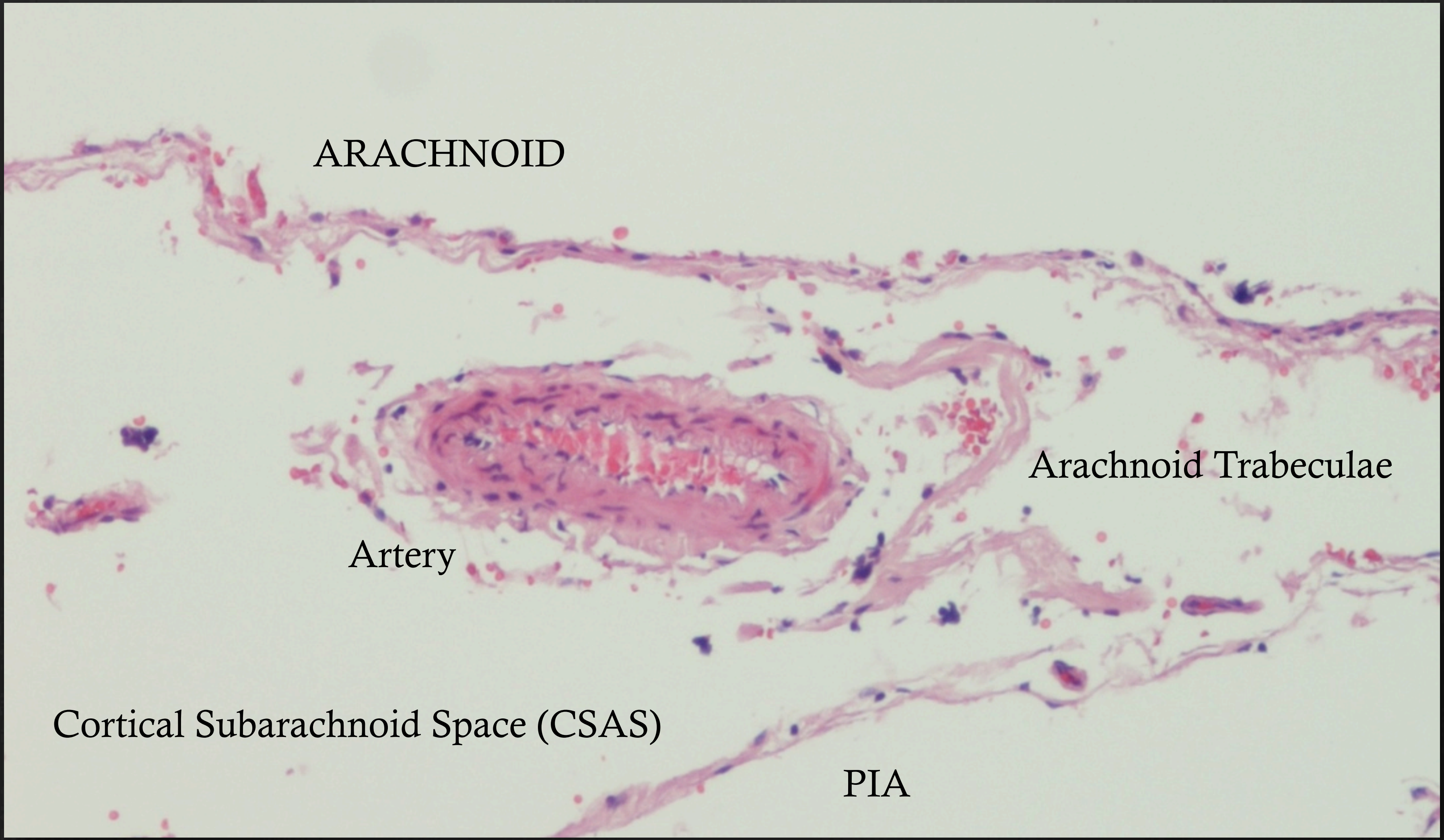


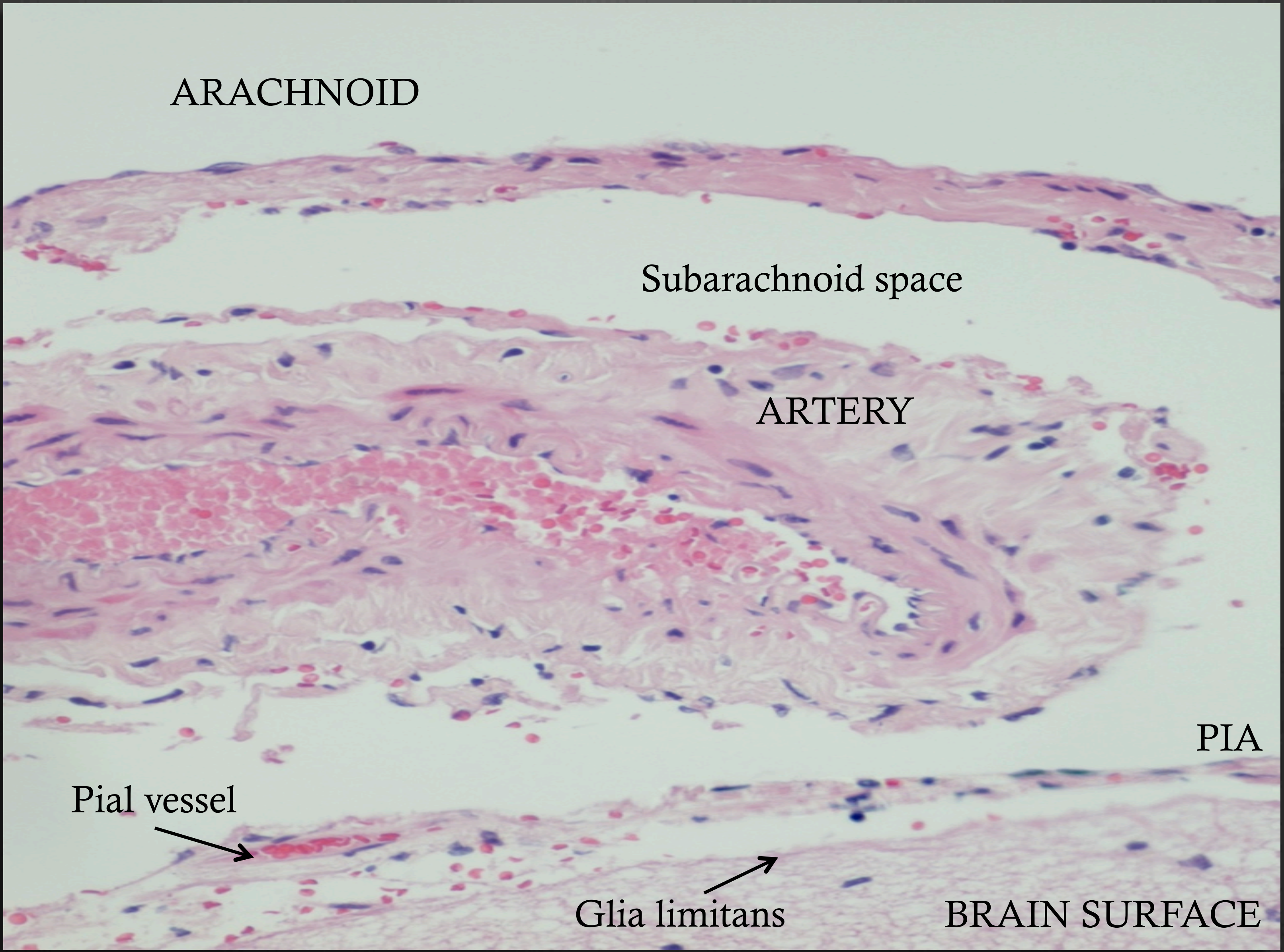
Figure 1 **Boxplot diagram describing the distribution of measured values of leptomeningeal transmembrane resistance in sheep along with mean value and outliers. Dotted line in the box represents the mean value of 11·38 Ω cm^2 obtained from 26 experiments**

PIAL SURFACE

ARACHNOID SURFACE



Filippidis et al.
Transmembrane resistance and histology of isolated sheep leptomeninges
Neurological Research (2010) vol. 32 (2) pp. 205



Filippidis et al.
Transmembrane resistance and histology of isolated sheep leptomeninges
Neurological Research (2010) vol. 32 (2) pp. 205

Is CSAS important for neurohydrodynamics and CSF disorders ?

J Neurosurg Pediatrics 2:1–11, 2008

The importance of the cortical subarachnoid space in understanding hydrocephalus

Harold L. Rekate, M.D.,[1,2] Trimurti D. Nadkarni, M.Ch.,[3]
and Donna Wallace, R.N., M.S., C.P.N.P.[1]

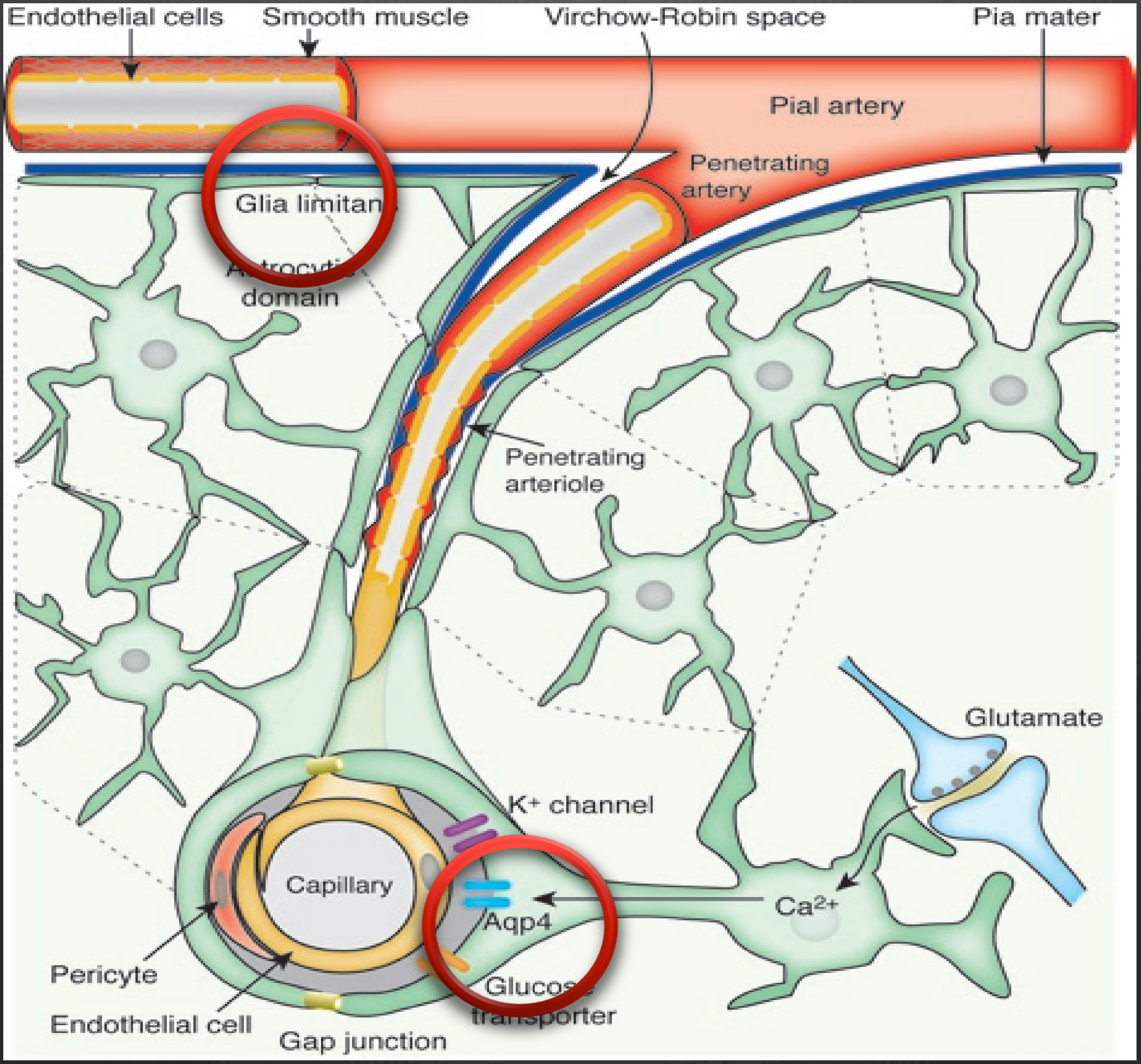
So…

The arachnoid and pia mater, line a preformed space (CSAS), with a biological fluid (CSF) which consists of 99% WATER.

We need to address the relationship of CSAS with Water…

Let’s move on to the cellular level

Let’s talk about **solute-coupled transport of water**



Costantino Iadecola & Maiken Nedergaard, ***Nature Neuroscience***, 2007

CSAS

"It is a "leaky" epithelium which bears properties of mesothelium"

Filippidis A, Zarogiannis S, Ioannou M, Gourgoulianis K, Molyvdas PA, Hatzoglou C.
Transmembrane resistance and histology of isolated sheep leptomeninges.
Neurol Res. 2010 Mar;32(2):205-8. Epub 2009 May 8.

Childs Nerv Syst
DOI 10.1007/s00381-012-1688-x

ORIGINAL PAPER

Permeability of the arachnoid and pia mater. The role of ion channels in the leptomeningeal physiology

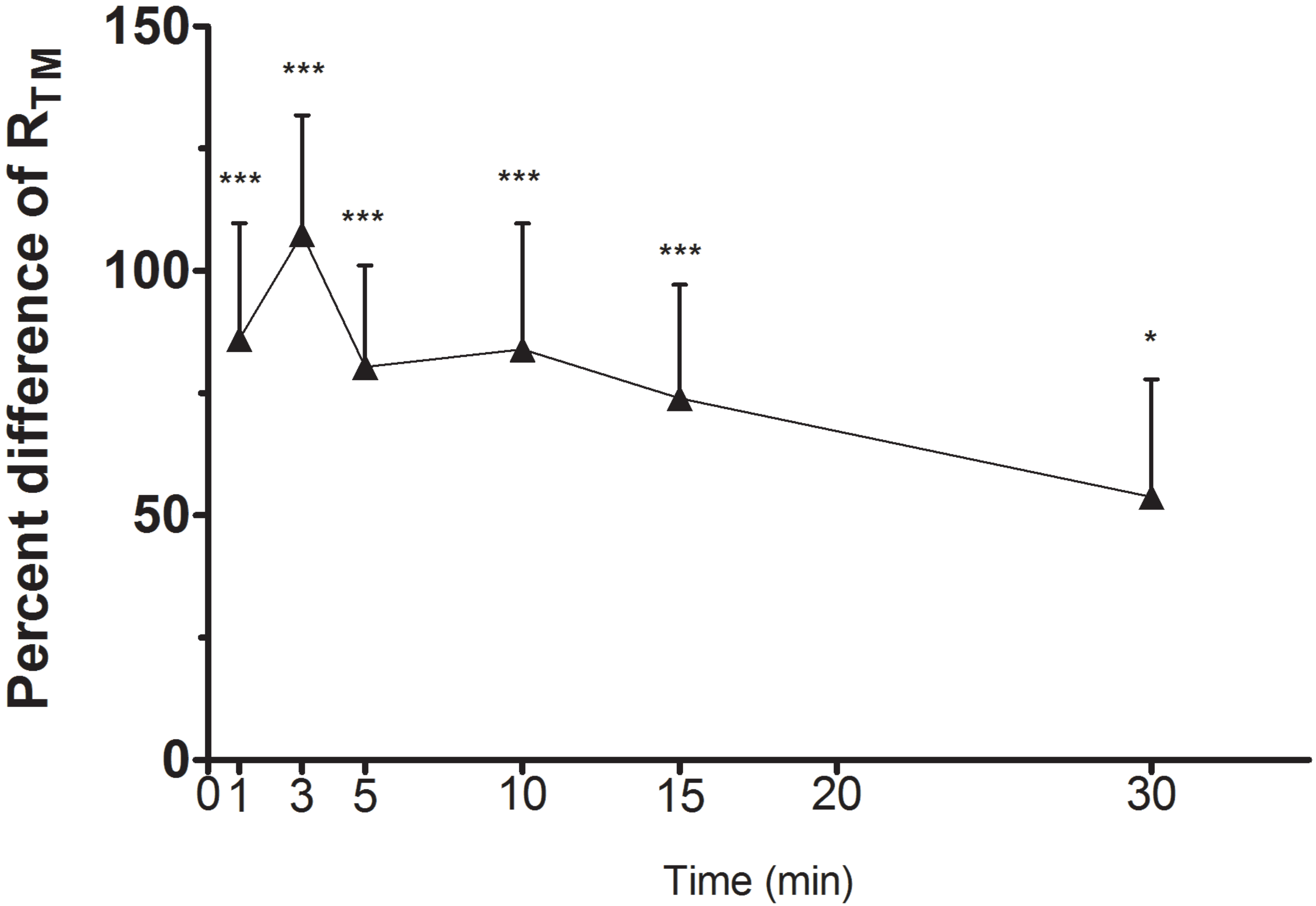
Aristotelis S. Filippidis • Sotirios G. Zarogiannis • Maria Ioannou • Konstantinos Gourgoulianis • Paschalis-Adam Molyvdas • Chrissi Hatzoglou

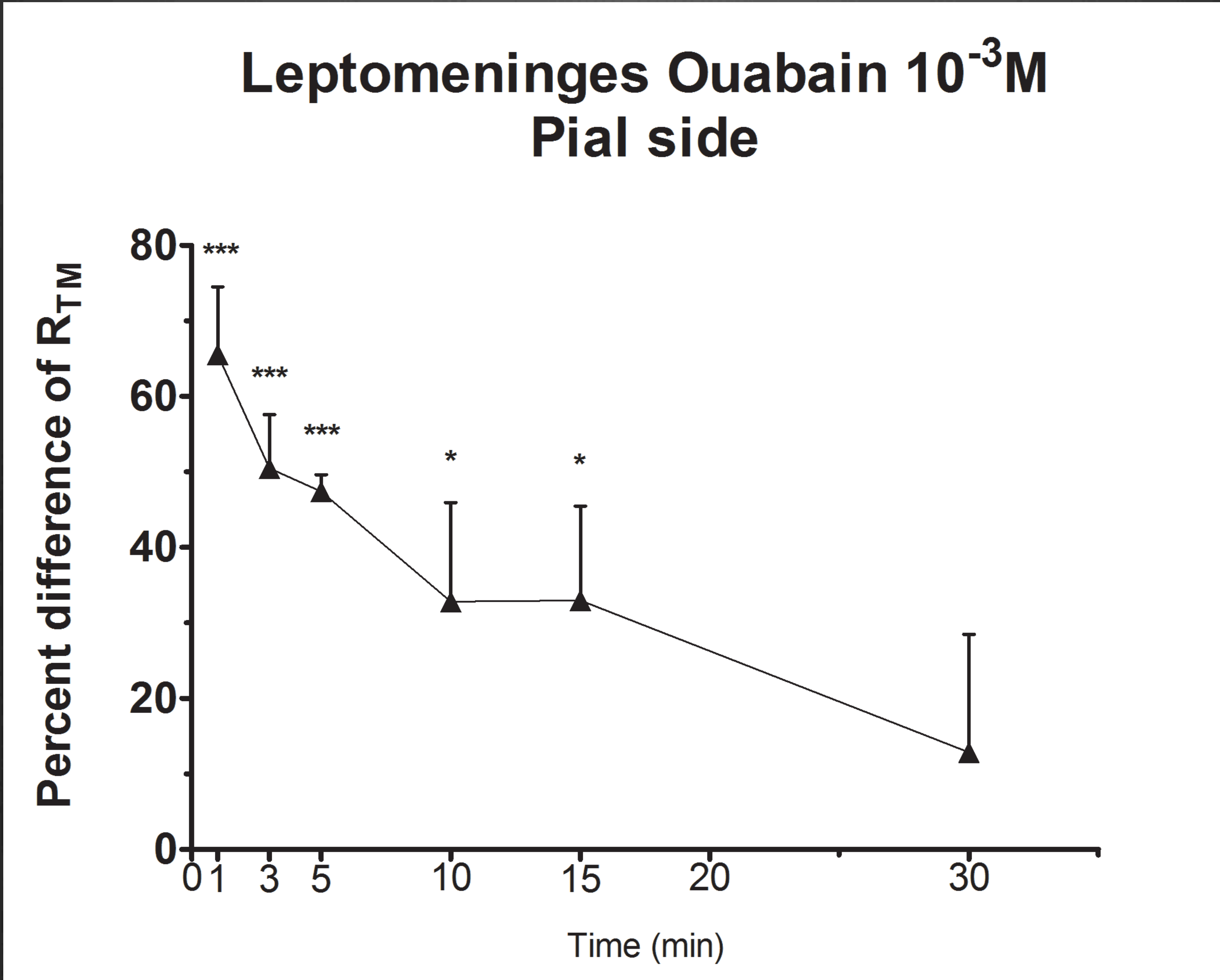
Sodium-Potassium-ATPase

Main source of extracellular Sodium

We tested inhibition with OUABAIN

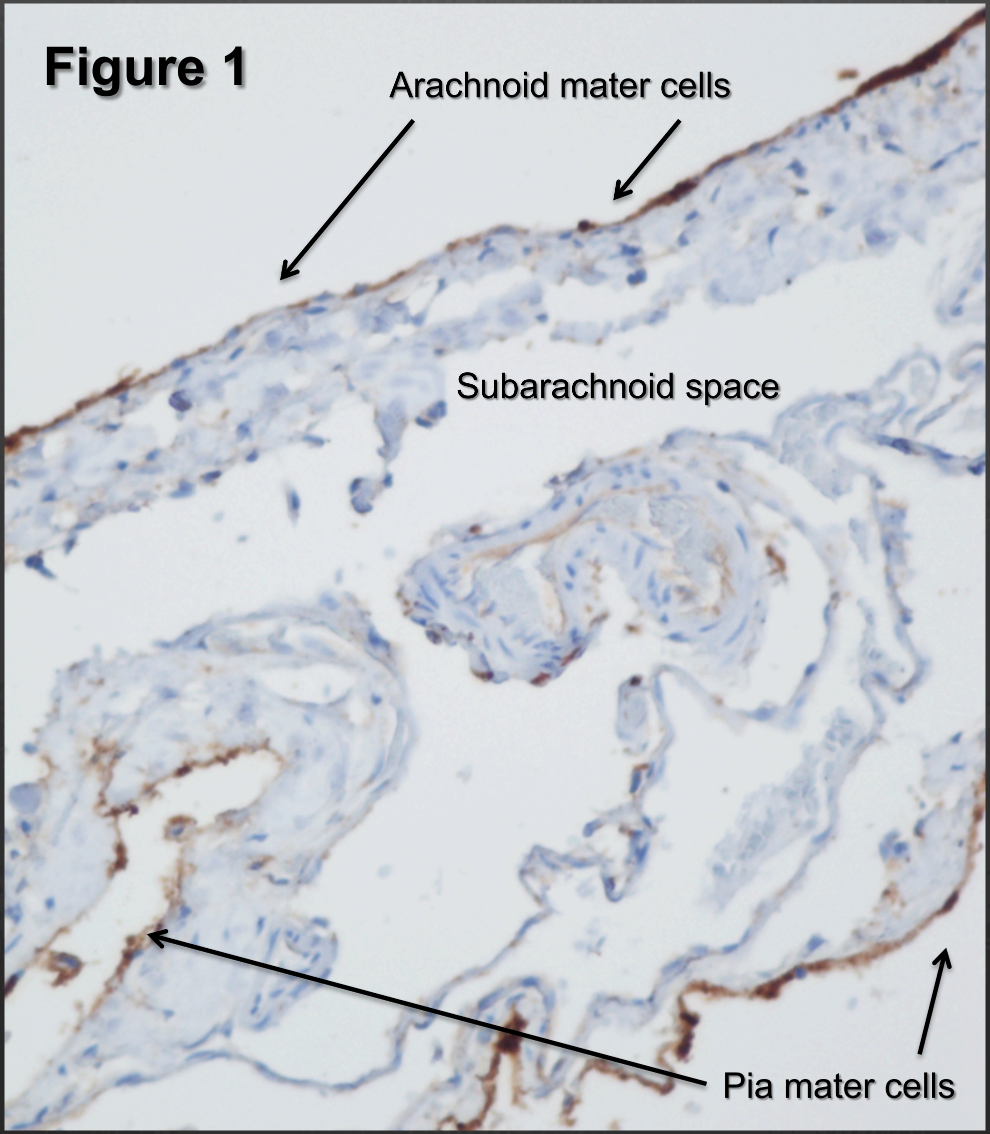
Leptomeninges Ouabain 10-3M
Arachnoidal side





Leptomeninges Ouabain 10-3M
Pial side
Percent difference of RTM
80
60
40
20
0
***
***
***
*
*
0 1 3 5 10 15 20 30
Time (min)

a1 subunit Sodium-Potassium-ATPase

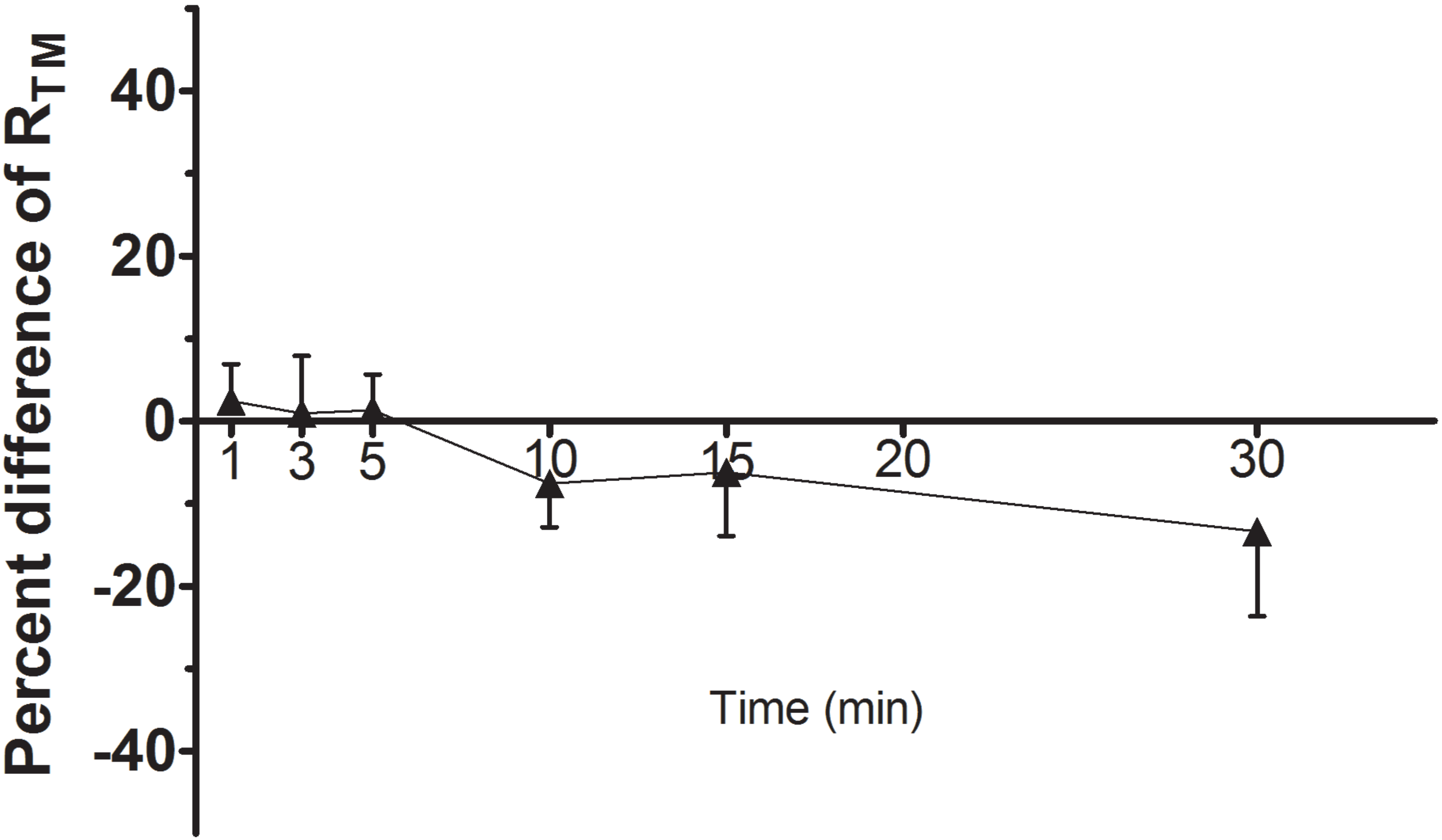


ENaC

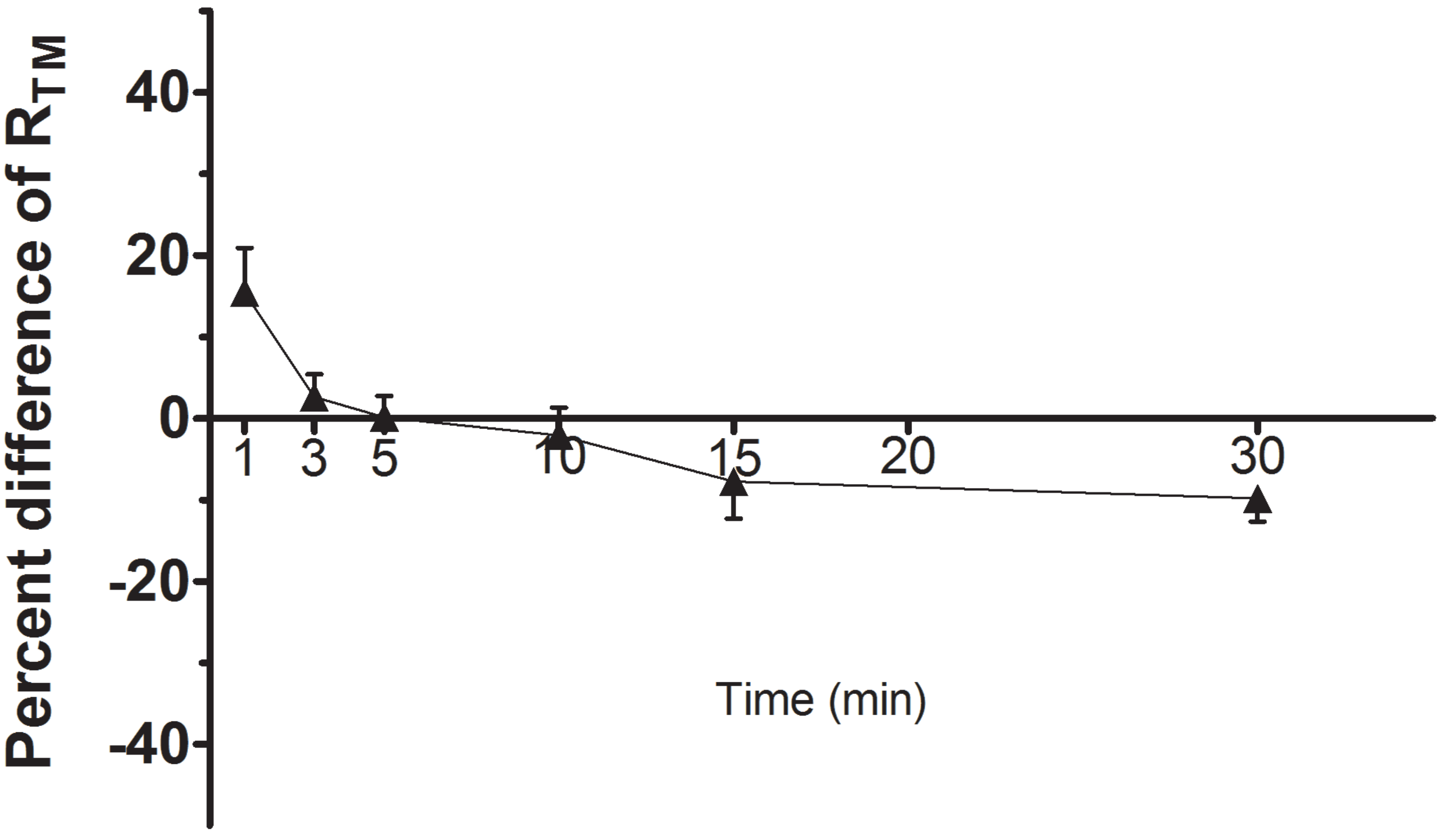
Epithelial Sodium Channel

We tested inhibition with AMILORIDE

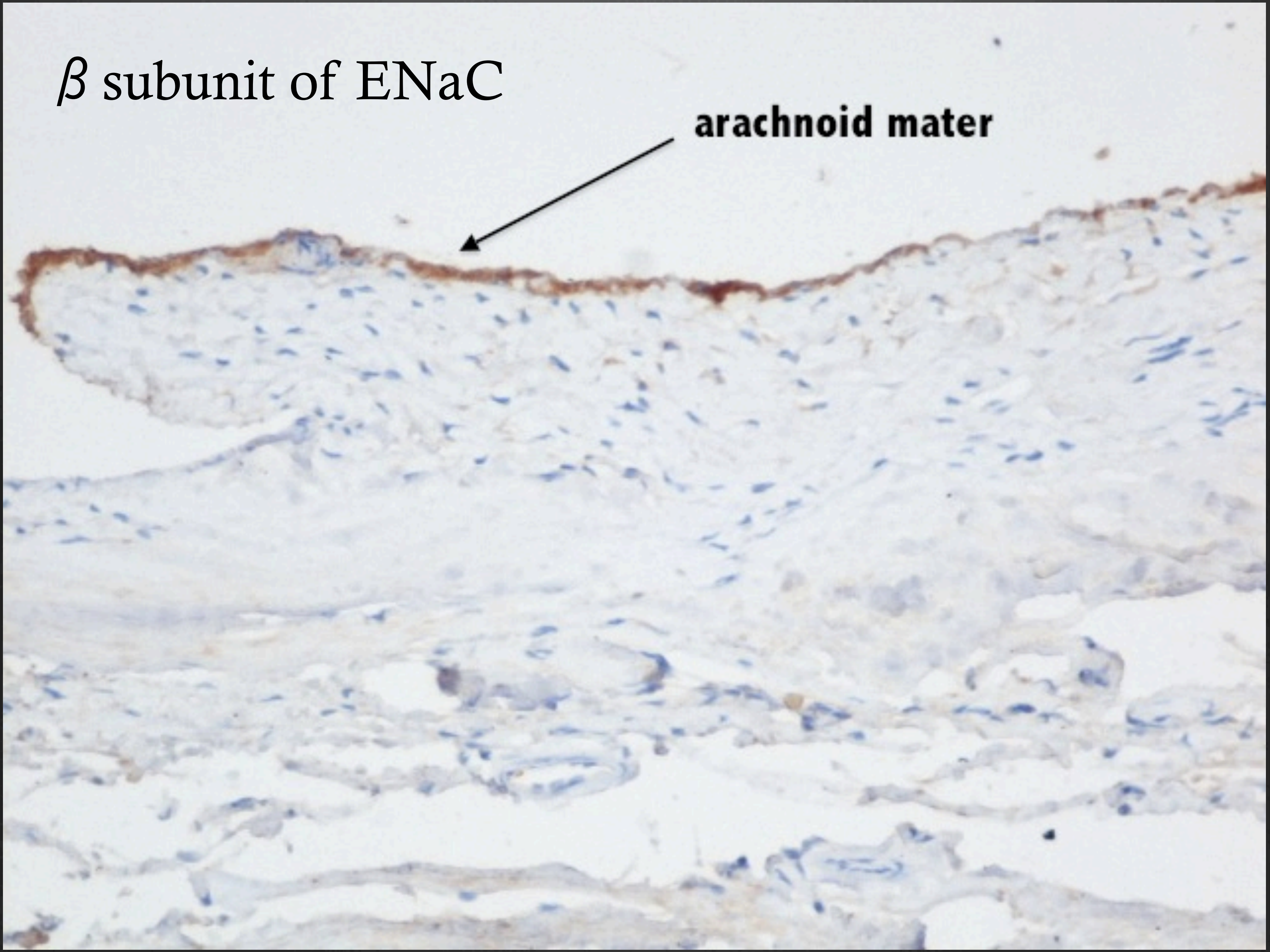
Leptomeninges Amiloride 10^{-5}M
Arachnoidal side



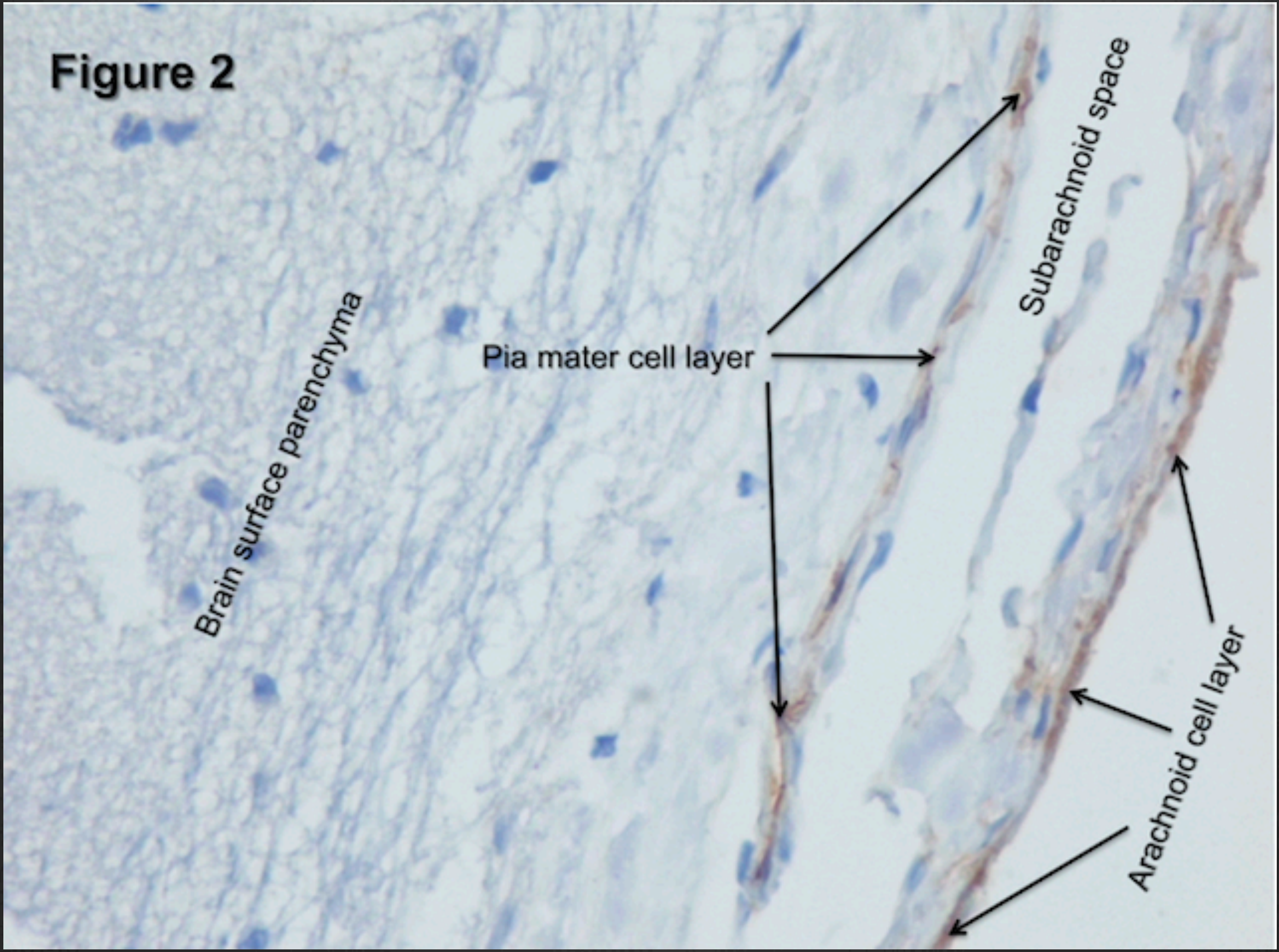
Leptomeninges Amiloride 10^{-5}M
Pial side



β subunit of ENaC



δ subunit - ENaC



Conclusions

- Solute-coupled transport can potentially occur at this interface since key structures such AQP4 exist
- Polarity of ion channels signifies the direction of flow at the cellular level
- Solute coupled transport can be involved both to CSF production or absorption.
- More studies needed to explore this new field.

Hydrocephalus is a multifactorial disease that bears mysteries in both the macroscopic and microscopic world. A combined approach is needed to identify the weight and degree of involvement of each key element.
The role of AQP4 and solute-coupled transport are an important addition to the research field

Plato and Aristotle, "The school of Athens", Raphael 1509

"Η γὰρ γένεσις ἕνεκα τῆς οὐσίας ἐστίν, ἀλλ' οὐχ ἡ οὐσία ἕνεκα τῆς γενέσεως."

"For the process of evolution is for the sake of the thing evolved, and not this for the sake of the process."

It is all about "Teleology" in the end

THANK YOU !

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