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AQP4 Myths and reality in the pathophysiology of CSF disorders

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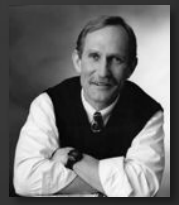
Neurosurgical Fellow
Boston University, Boston Medical Center

ASNR 2014 meeting, IHIWG, Montreal, CANADA, May 23th 2014

AQUAPORINS

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

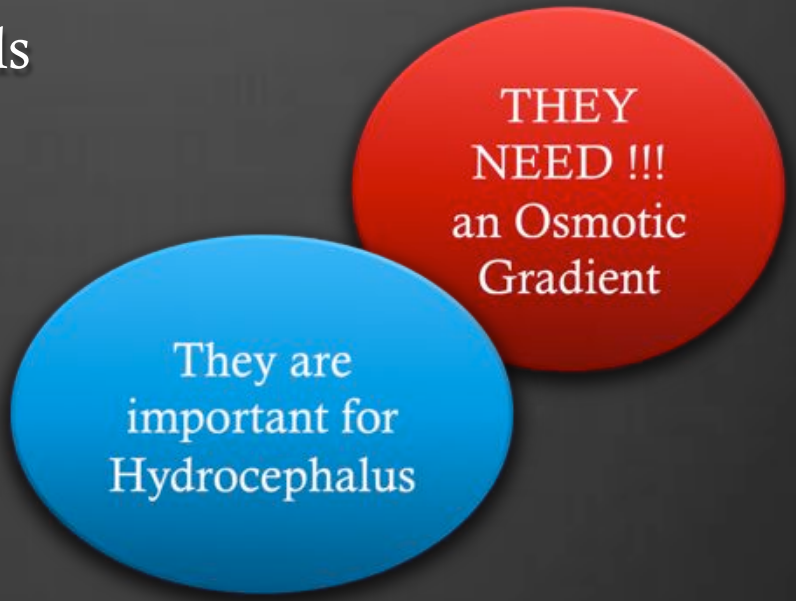


- Aquaporin-4 (AQP4) is the dominant form in the brain, with AQP1 being dominant at the choroid plexus

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

AQP1 LOCALIZATION

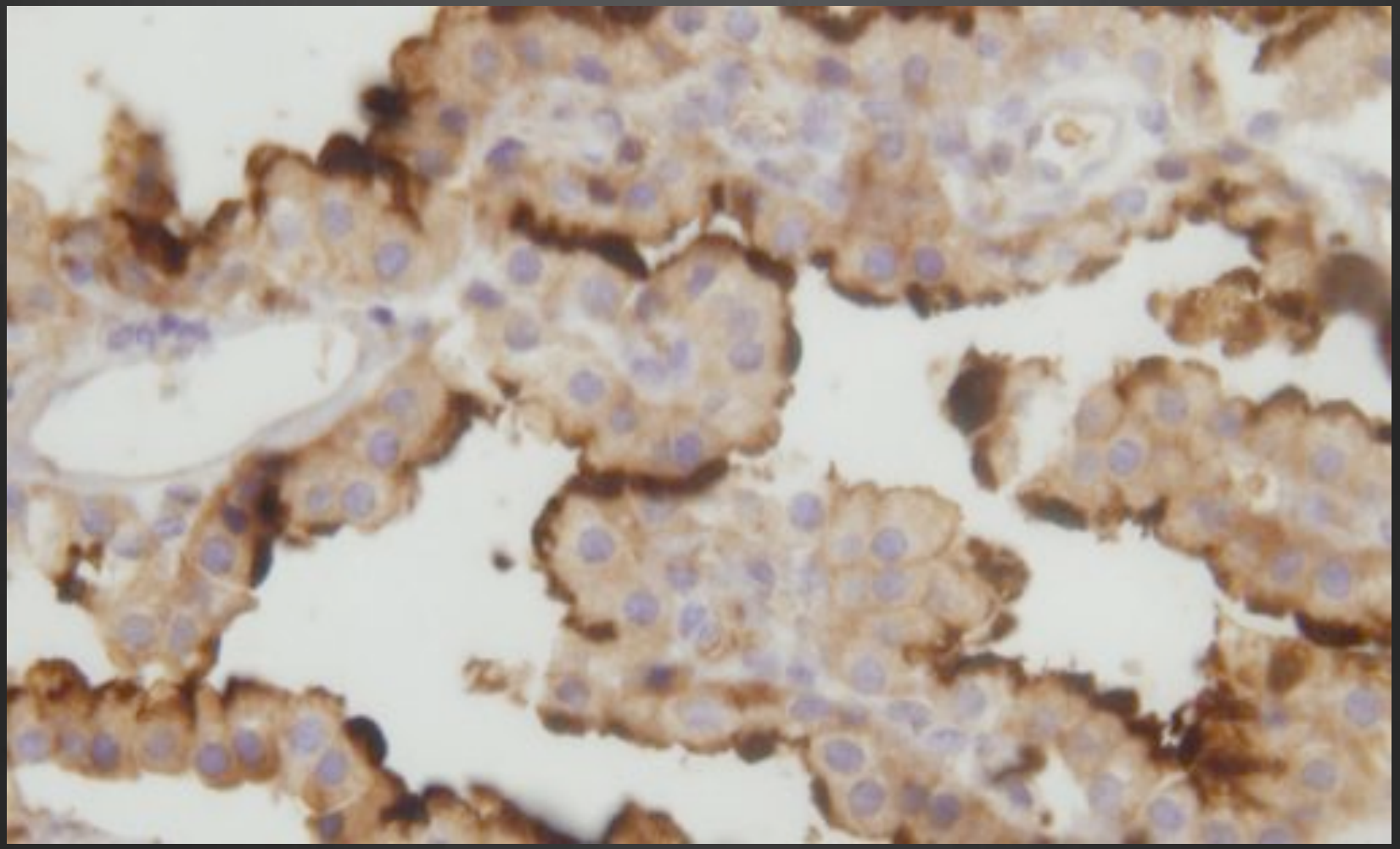
- **Mostly at the apical surface of choroid plexus**
- Some presence at the basolateral surface of the choroid plexus
- Abundant in red blood cells



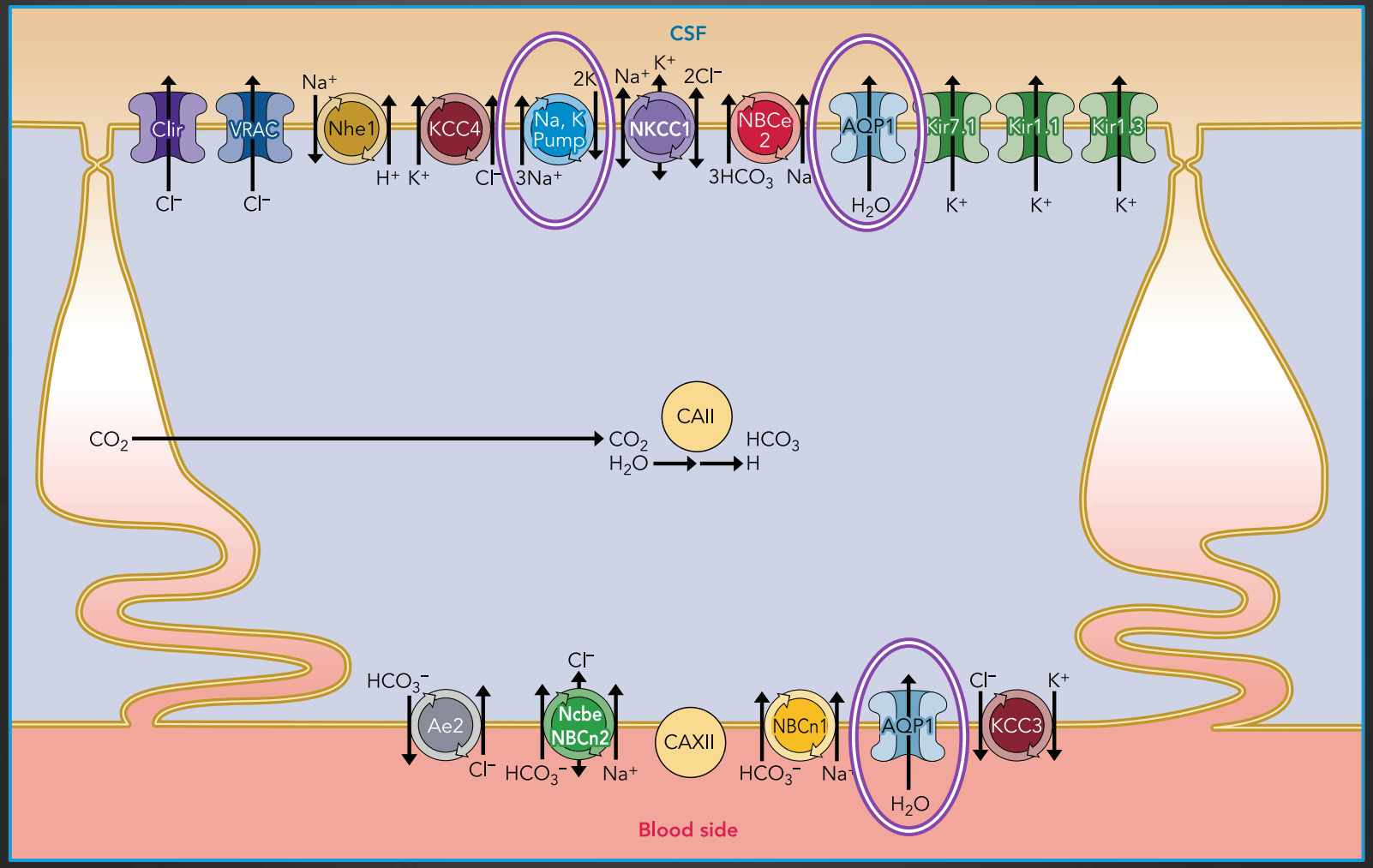
Badaut et al.
Aquaporins in brain: distribution, physiology, and pathophysiology.
J Cereb Blood Flow Metab (2002) vol. 22 (4) pp. 367-78

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



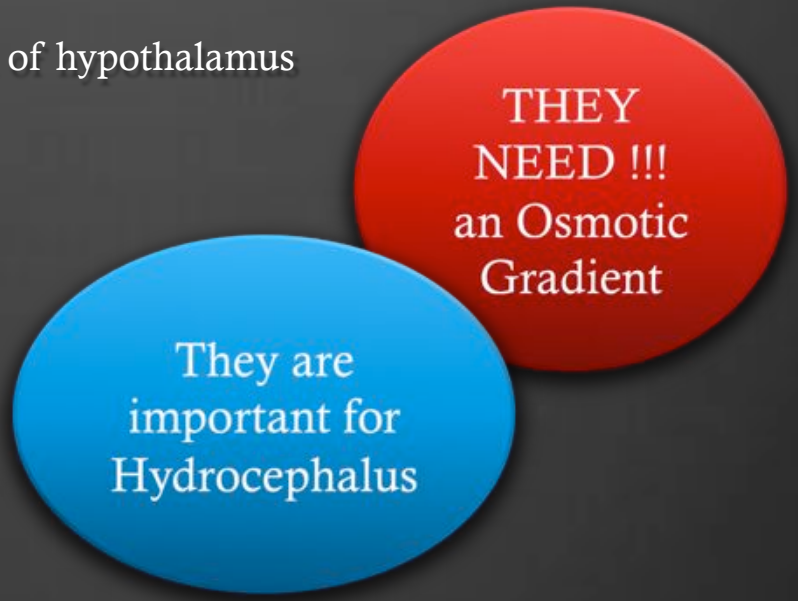
choroid plexus: ion channels



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

AQP4 LOCALIZATION

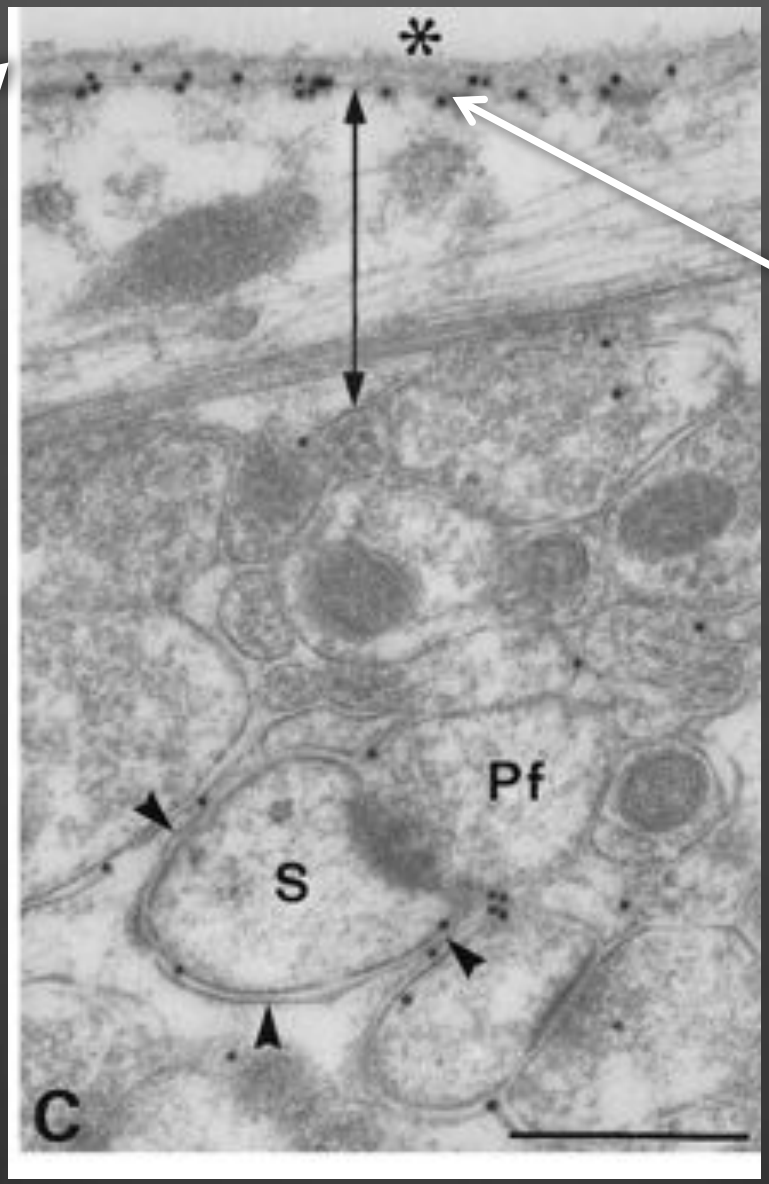
- **Glia Limitans**
- **Astrocyte foot processes around capillaries that form the Blood-Brain-Barrier (BBB)**
- Ependymal cells
- Supraoptic and suprachiasmatic nuclei of hypothalamus
- Cerebellum
- Hippocampal dentate gyrus,
- Hippocampal areas CA1-CA2
- Neocortex
- Nucleus of stria terminalis
- Medial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



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.

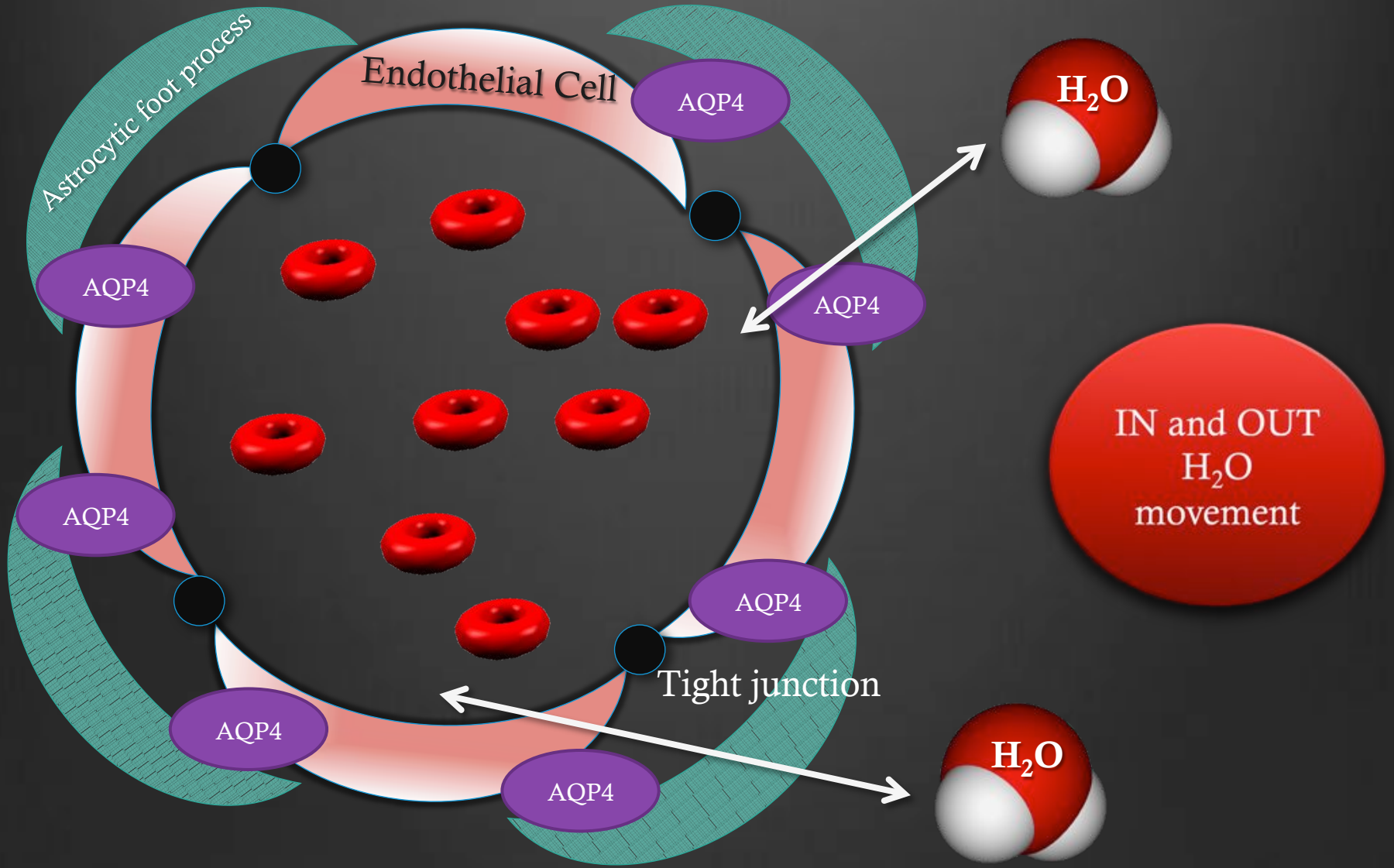
PIA mater - Glia Limitans
lower CSAS interface



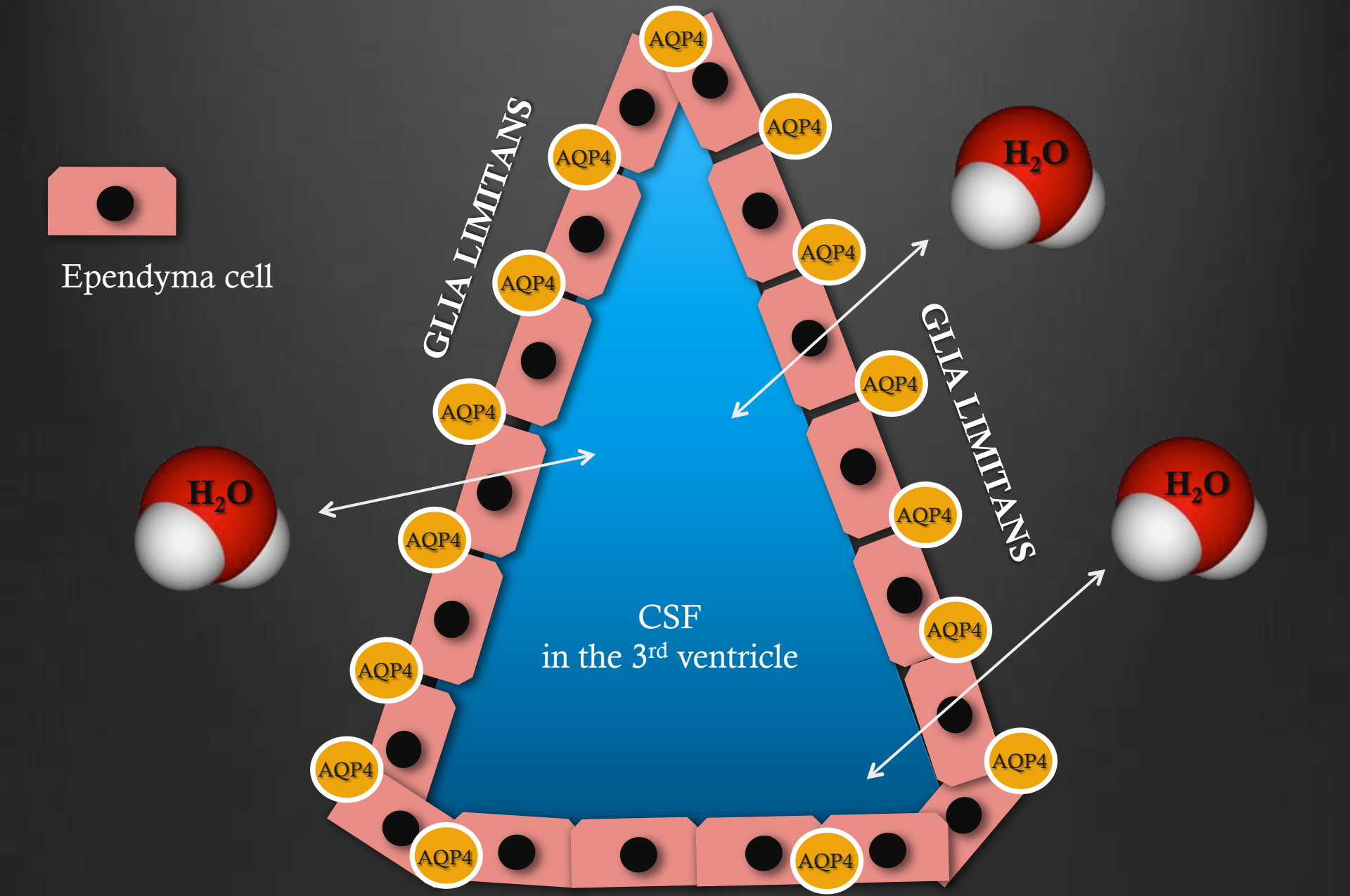
Black dots ?
AQP4

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

AQP4 and the Blood-Brain-Barrier and Cerebral vessels



AQP4 at Ependyma



Is there a role for AQPs?

For HYDROCEPHALUS

For IDIOPATHIC INTRACRANIAL HYPERTENSION

For SYRINGOMYELIA and CHIARI MALFORMATION

FACTS

> AQPs transfer water bidirectionally

> AQPs direction of water transport depends on

local OSMOTIC GRADIENT (at the cellular level)

Literature

Childs Nerv Syst (2011) 27:27–33
DOI 10.1007/s00381-010-1227-6

REVIEW PAPER

Hydrocephalus and aquaporins: lessons learned from the bench

Aristotelis S. Filippidis · M. Yashar S. Kalani · Harold L. Rekate

Table 1 Published studies concerning aquaporins and hydrocephalus

Studies	Species	Method	AQPs studied	Results
Shen et al. [64]	Rats	H-Tx	AQP4	Upregulated
Mao et al. [62]	Rats	Kaolin inj.	AQPs 1,4,9	AQP4 upregulated
Tourdias et al. [63]	Rats	LPS inj. inflammatory HCP	AQP4	Upregulated
Paul et al. [40]	Rats	H-Tx, choroid plexus culture	AQPs 1,4	AQP1 low/AQP4 high
Bloch et al. [45]	Mice	AQP4-KO, kaolin inj.	AQP4	Higher ICP, CSF volume, lower survival in AQP4-KO
Oshio et al. [37]	Mice	AQP1-KO	AQP1	Lower ICP, lower CSF production
Feng et al. [58]	Mice	AQP4-KO	AQP4	Sporadic obstr HCP
Smith et al. [38]	Human	Choroid plexus hyperplasia, case report	AQP1	Downregulated
Longatti et al. [39]	Human	Choroid plexus tumors series	AQP1	Mostly strong expression in tumors with hydrocephalus

AQP aquaporin, *HCP* hydrocephalus, *obstr* obstructive, *AQP4-KO* aquaporin-4 knockouts, *H-Tx* congenital hydrocephalic rats, *ICP* intracranial pressure

Neuropathology and Applied Neurobiology (2013), **39**, 179–191 doi: 10.1111/j.1365-2990.2012.01275.x

Differences in distribution and regulation of astrocytic aquaporin-4 in human and rat hydrocephalic brain

A. D. Skjolding*†‡, A. V. Holst*, H. Broholm†, H. Laursen† and M. Juhler*

*University Clinic of Neurosurgery, †Laboratory of Neuropathology, Copenhagen University Hospital, Rigshospitalet, and ‡Department of Neuroscience and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

In human brain (hydrocephalic and controls) AQP4 immunoreactivity was found on the entire astro- cyte membrane, unlike hydrocephalic rat brain where pronounced endfeet polarization was present.

Skjolding et al. Cerebrospinal Fluid Research 2010, 7:20
http://www.cerebrospinalfluidresearch.com/content/7/1/20

CEREBROSPINAL FLUID RESEARCH

RESEARCH Open Access

Hydrocephalus induces dynamic spatiotemporal regulation of aquaporin-4 expression in the rat brain

Anders D Skjolding[1,4*], Ian J Rowland[2,5], Lise V Søgaard[2], Jeppe Praetorius[3], Milena Penkowa[4], Marianne Juhler[1]

Lateral ventricular volume was significantly increased over control at all time points after induction and the periventricular apparent diffusion coefficient (ADC) value significantly increased after one and two weeks of hydrocephalus. Relative AQP4 density was significantly decreased in both cortex and periventricular region after two days and normalized after one week. After two weeks, periventricular AQP4 expression was significantly increased. Relative periventricular AQP4 density was significantly correlated to lateral ventricular volume. AQP4 immunohistochemical analysis demonstrated the morphological expression pattern of AQP4 in hydrocephalus in astrocytes and ventricular ependyma. AQP4 co-localized with astrocytic glial fibrillary acidic protein (GFAP) in glia limitans. In vascular structures, AQP4 co-localized to astroglia but not to microglia or endothelial cells.

Castañeyra-Ruiz et al. Fluids and Barriers of the CNS 2013, 10:18
http://www.fluidsbarrierscns.com/content/10/1/18

FLUIDS AND BARRIERS OF THE CNS

RESEARCH Open Access

Aquaporin-4 expression in the cerebrospinal fluid in congenital human hydrocephalus

Leandro Castañeyra-Ruiz[1,3], Ibrahim González-Marrero[1], Juan M González-Toledo[1], Agustin Castañeyra-Ruiz[2], Héctor de Paz-Carmona[2], Agustín Castañeyra-Perdomo[1*] and Emilia M Carmona-Calero[1,2]

"We have shown that the AQP4 concentration is higher in the CSF of communicating hydrocephalus infants than in the CSF of non-communicating hydrocephalus patients or controls. It is possible that AQP4 may freely leak from the parenchyma to the CSF during the early stages, when ependymal denudation is occurring and the hydrocephalus remains communicating."

Acta Neurochir (2012) 154:753–759
DOI 10.1007/s00701-011-1241-9

EXPERIMENTAL RESEARCH

Aquaporin-4 expression is not elevated in mild hydrocephalus

Kamran Aghayev · Ercan Bal · Tural Rahimli · Melike Mut · Serdar Balcı · Frank Vrionis · Nejat Akalan

Neurology 82 May 20, 2014

Clinical/Scientific Notes

Stacey L. Clardy, MD, PhD
Claudia F. Lucchinetti, MD
Karl N. Krecke, MD
Vanda A. Lennon, MD, PhD
Orna O'Toole, MD
Brian G. Weinshenker, MD
Clara D. Boyd, MD
Stephen Krieger, MD
Corey McGraw, MD
Yong Guo, MD, PhD
Sean J. Pittock, MD

HYDROCEPHALUS IN NEUROMYELITIS OPTICA

A majority of patients with neuromyelitis optica (NMO) spectrum disorders (NMOSD) have MRI brain abnormalities, some of which are "NMO-typical" with localization in aquaporin 4 (AQP4)–rich circumventricular and periaqueductal regions.[1] Although uncommon in adult patients, symptomatic brain involvement occurs in approximately 50% of NMO-immunoglobulin G (IgG) seropositive children. Here we report the clinical characteristics, type, and frequency of hydrocephalus in NMOSD.

Methods. Obstructive hydrocephalus was identified in the index case. Head MRIs from AQP4-IgG-seropositive patients in the Mayo Clinic NMO database (125 NMO; 45 NMOSD) were reviewed.

follow-up (age 23), the patient had undergone successful third ventriculostomy.

Case 2. A 36-year-old woman had onset of sudden neck and shoulder pain, confusion, bilateral optic neuritis, and quadriparesis. MRI revealed LETM and hydrocephalus (figure, case 2, C.a and C.b). She was treated with steroids and a shunt was placed 5 months after initial symptom onset. LETM recurred in the subsequent 18 years.

Discussion. The 1% frequency of obstructive hydrocephalus we observed in patients with NMOSD is far greater than in the general adult population. Larger studies will be required to confirm that this observation is not incidental. The incidence of all types of hydrocephalus, annual numbers of new ventricular shunts recorded in the Nationwide Inpatient Sample database and the Californian population, is 2.95 and 5.5

Pseudotumor or IIH

Aquaporin-4 antibodies are not present in patients with idiopathic intracranial hypertension.
Ekizoglu E, Içoz S, Tuzun E, Birisik O, Kocasoy-Orhan E, Akman-Demir G, Baykan B.
Cephalalgia. 2012 Feb;32(3):198-202. doi: 10.1177/0333102411434167. Epub 2012 Jan 11.
PMID: 22238356

Is the brain water channel aquaporin-4 a pathogenetic factor in idiopathic intracranial hypertension? Results from a combined clinical and genetic study in a Norwegian cohort.
Kerty E, Heuser K, Indahl UG, Berg PR, Nakken S, Lien S, Omholt SW, Ottersen OP, Nagelhus EA.
Acta Ophthalmol. 2013 Feb;91(1):88-91. doi: 10.1111/j.1755-3768.2011.02231.x. Epub 2011 Sep 13.
PMID: 21914143

Absence of aquaporin-4 antibodies in patients with idiopathic intracranial hypertension.
Dhungana S, Waters P, Ismail A, Woodroofe N, Vincent A, Sharrack B.
J Neurol. 2010 Jul;257(7):1211-2. doi: 10.1007/s00415-010-5499-2. Epub 2010 Mar 16. No abstract available. Corrected and republished in: J Neurol. 2010 Jul;257(7):1229-30.
PMID: 20232212

J Neurosurg Spine 17:602–612, 2012

Aquaporin-4 expression and blood–spinal cord barrier permeability in canalicular syringomyelia

Laboratory investigation

Sarah J. Hemley, Ph.D.,[1] Lynne E. Bilston, Ph.D.,[2,3] Shaokoon Cheng, Ph.D.,[2,4] and Marcus A. Stoodley, Ph.D.[1]

[1]The Australian School of Advanced Medicine, Macquarie University; [2]Neuroscience Research Australia; [3]Prince of Wales Clinical School, University of New South Wales; and [4]School of Medical Science, University of New South Wales, Sydney, New South Wales, Australia

Critical review of articles

- We should not approach the AQP4 literature as a cancer related literature (upregulation, downregulation) etc at the mRNA level, how about protein levels, and physiology?
- Also time of expression is important, hydrocephalus is dynamic
- The positive or negative feedback loops. What is the meaning of eg increased AQP4? Good or bad?
- Many studies look for AQP4 levels in CSF, what is the background? Is AQP4 a protein that is secreted in fluids? Are AQP4 bearing cells die and the proteins is released in CSF?
- Why not creating a molecular probe?

"Glymphatics"

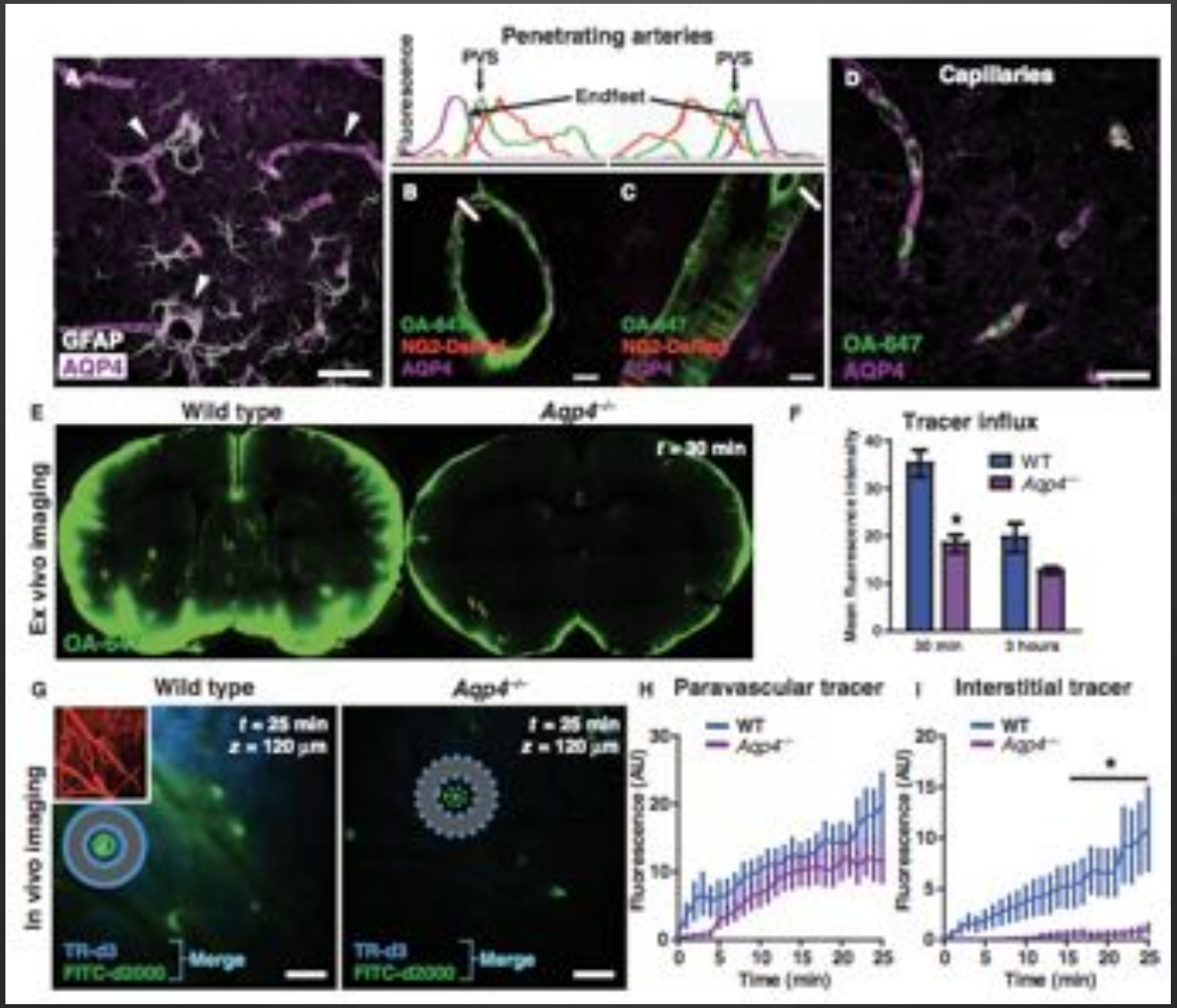
RESEARCH ARTICLE

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.



Iliff et al, Science Translational Medicine, Aug 2012

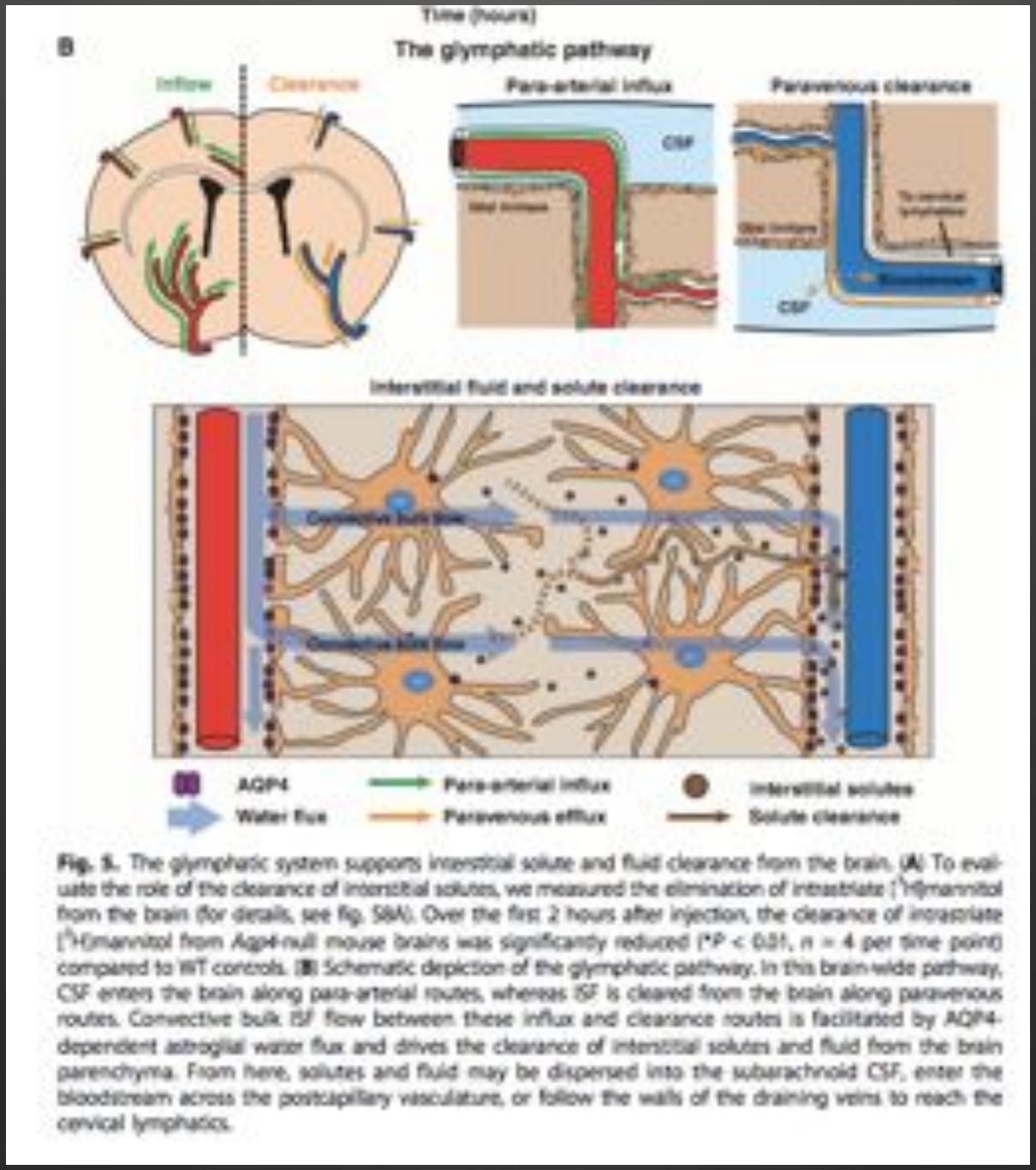


Fig. 5. The glymphatic system supports interstitial solute and fluid clearance from the brain. (**A**) To evaluate the role of the clearance of interstitial solutes, we measured the elimination of intrastriate [^{3}H]mannitol from the brain (for details, see fig. S8A). Over the first 2 hours after injection, 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

BACK TO BASICS: Is there a reason to have osmotic gradients in CSF disorders?

Only THEN AQPs could have a role in the pathophysiology

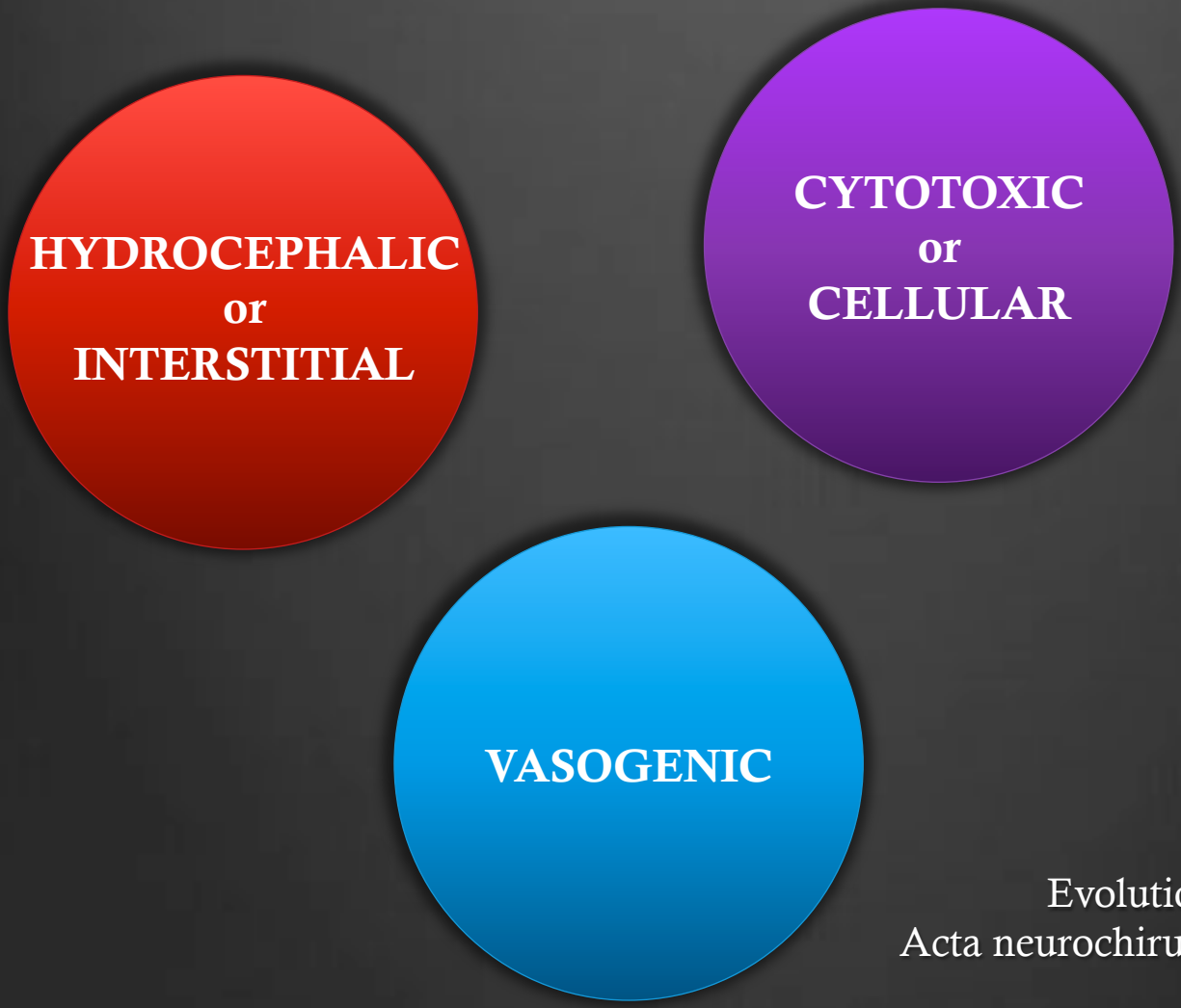
The logic of measuring AQPs only without proving that there is an osmotic gradient would never provide insight into their role in key pathophysiologic steps.

Has the effect of hydrostatic pressure being studied in AQPs?

"No, but results are totally predictable from the biophysics of water permeation. The problem with studying pressure effects is that very large pressures would be needed, and, as soon as water moves in one directly, an osmotic gradients is created that opposes the flow."

Alan Verkman, 4/8/2014, personal communication

Brain edema types
(revised Klatzo classification)



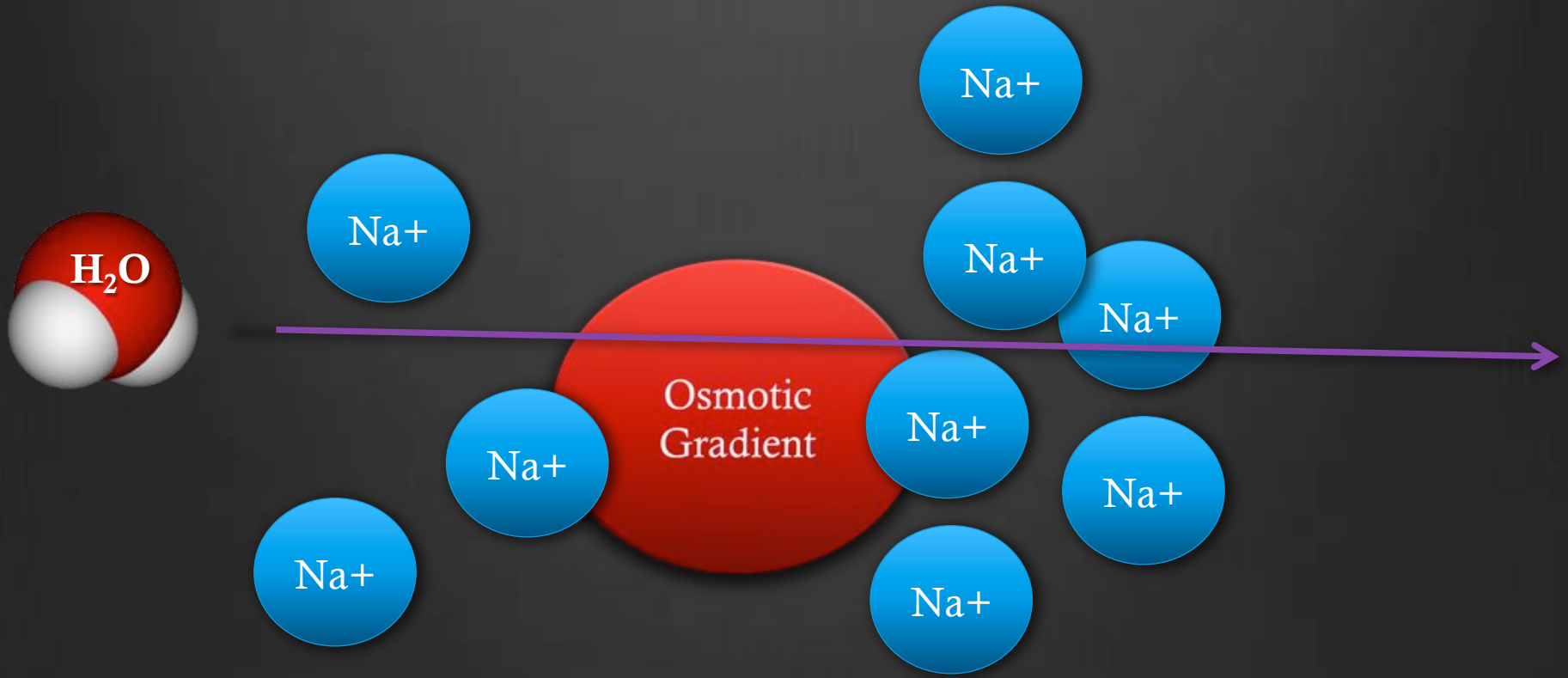
Klatzo.
Evolution of brain edema concepts.
Acta neurochirurgica Supplementum (1994)
vol. 60 pp. 3-6

Thinking beyond structures → "Ion & Water mechanics"

Water FOLLOWS Sodium in polarized epithelia

$\sigma = 1$

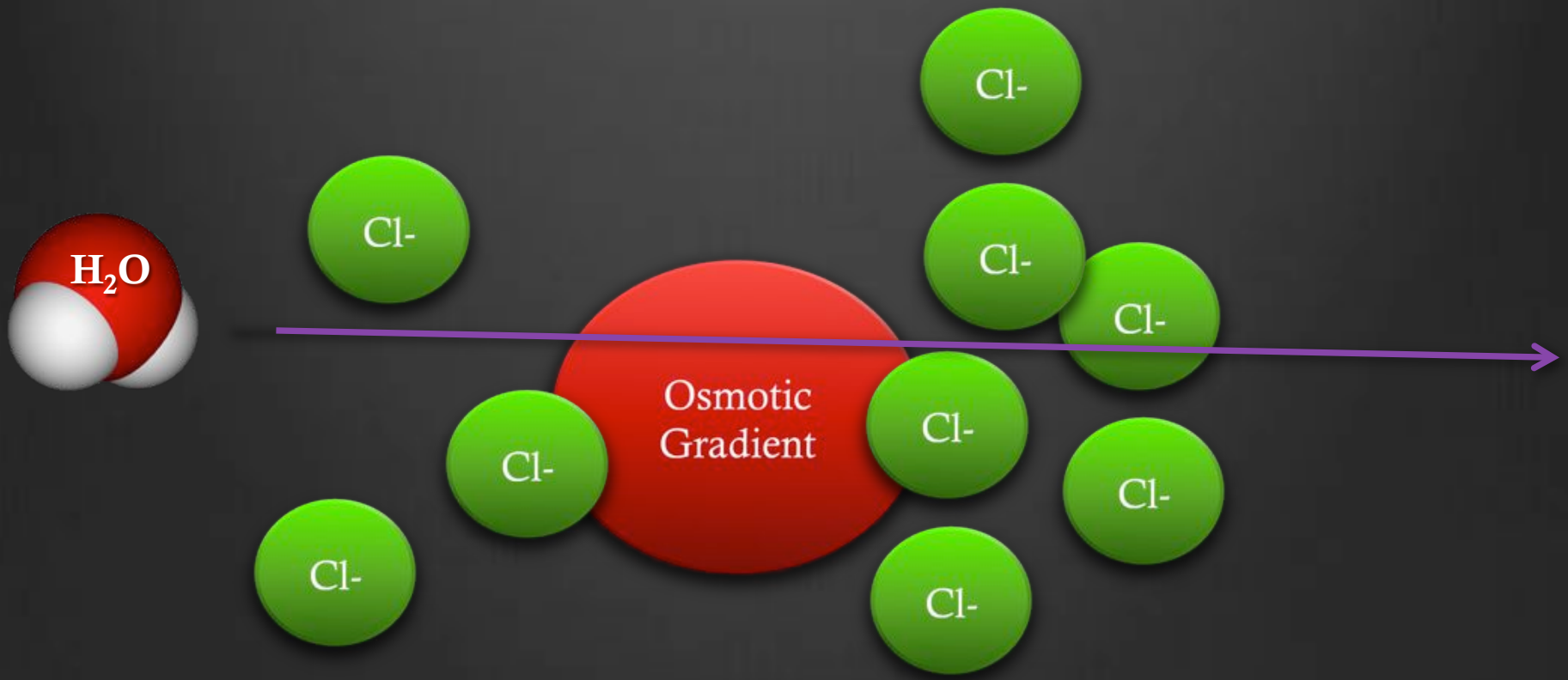
(e.g. choroid plexus, pleura, pericardium, omentum, nephron)



Thinking beyond structures → "Ion & Water mechanics"

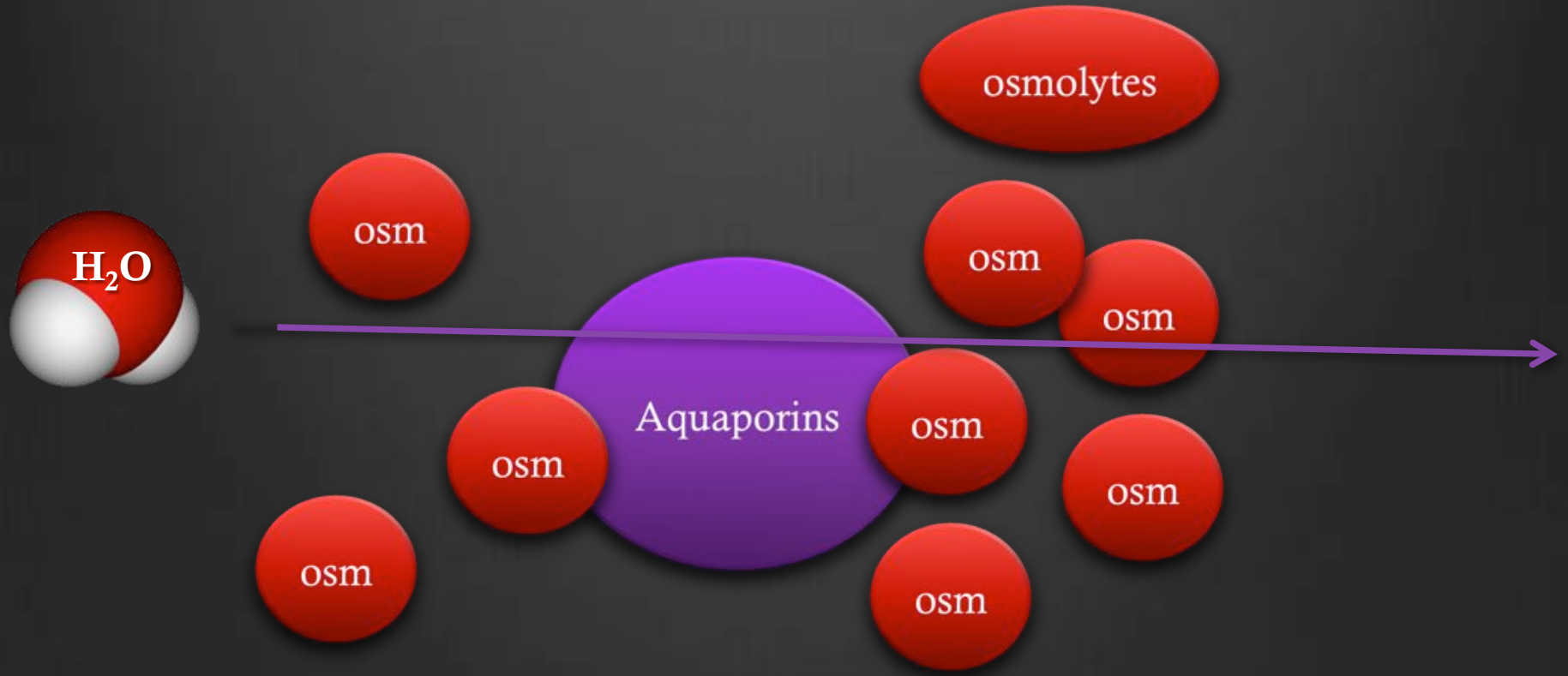
Water FOLLOWS Chloride in polarized epithelia

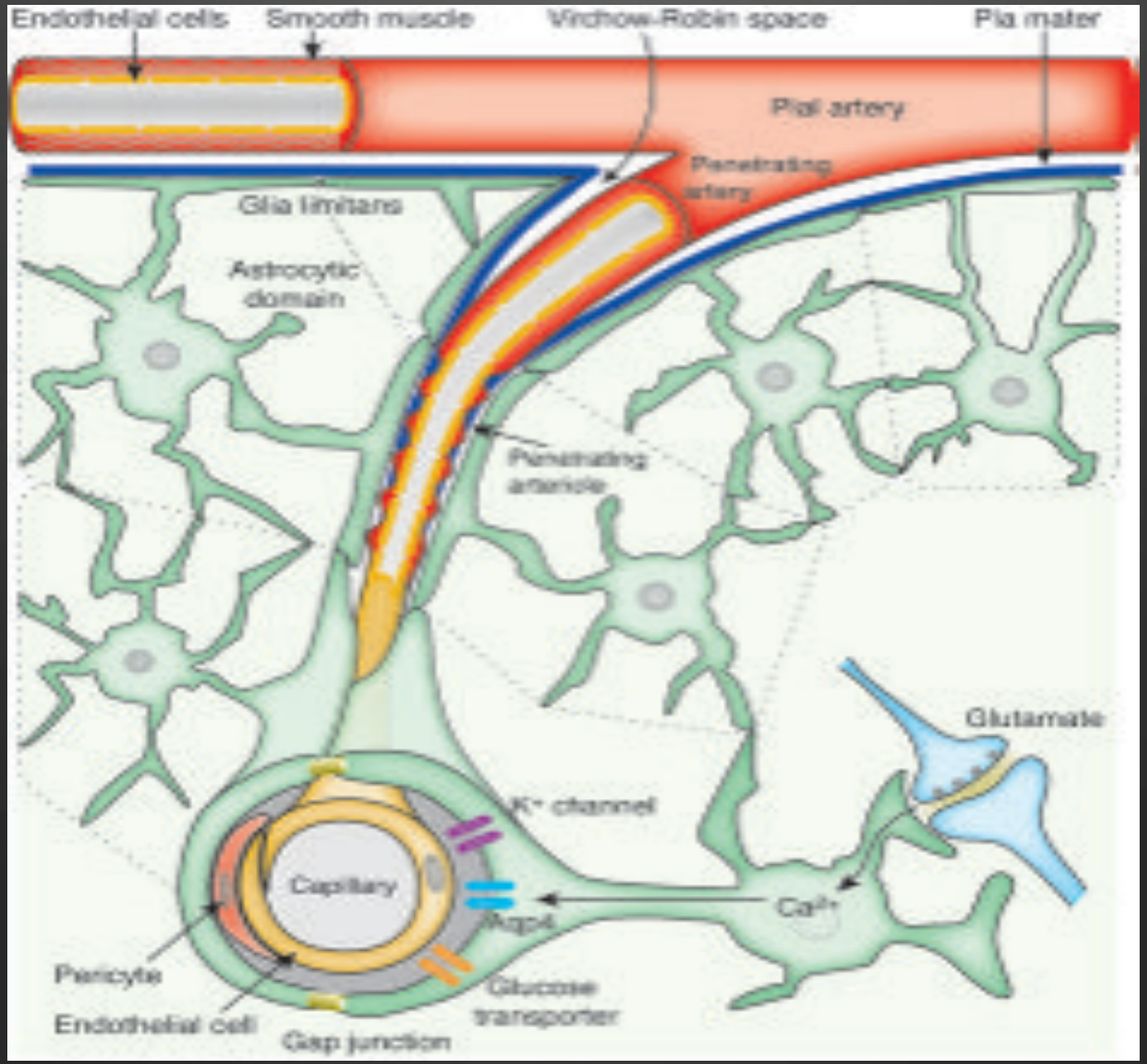
(e.g. sweat glands, salivary glands, bronchi)



Thinking beyond structures → "Ion & Water mechanics"

Water FOLLOWS osmotic gradient of osmolytes through AQPs





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

“the short circuit”
AQP4 at the BBB

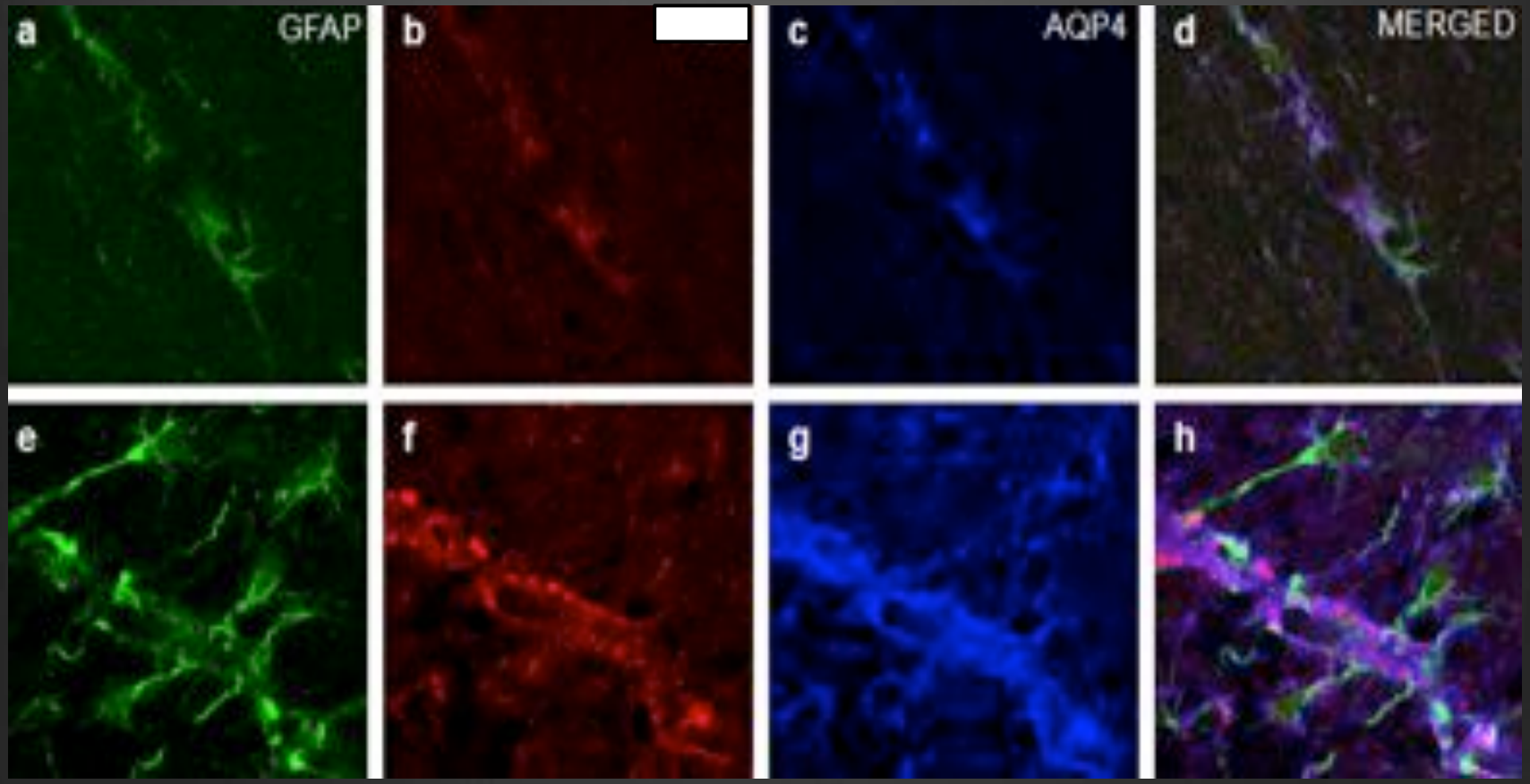


Image courtesy of Marmarou Lab

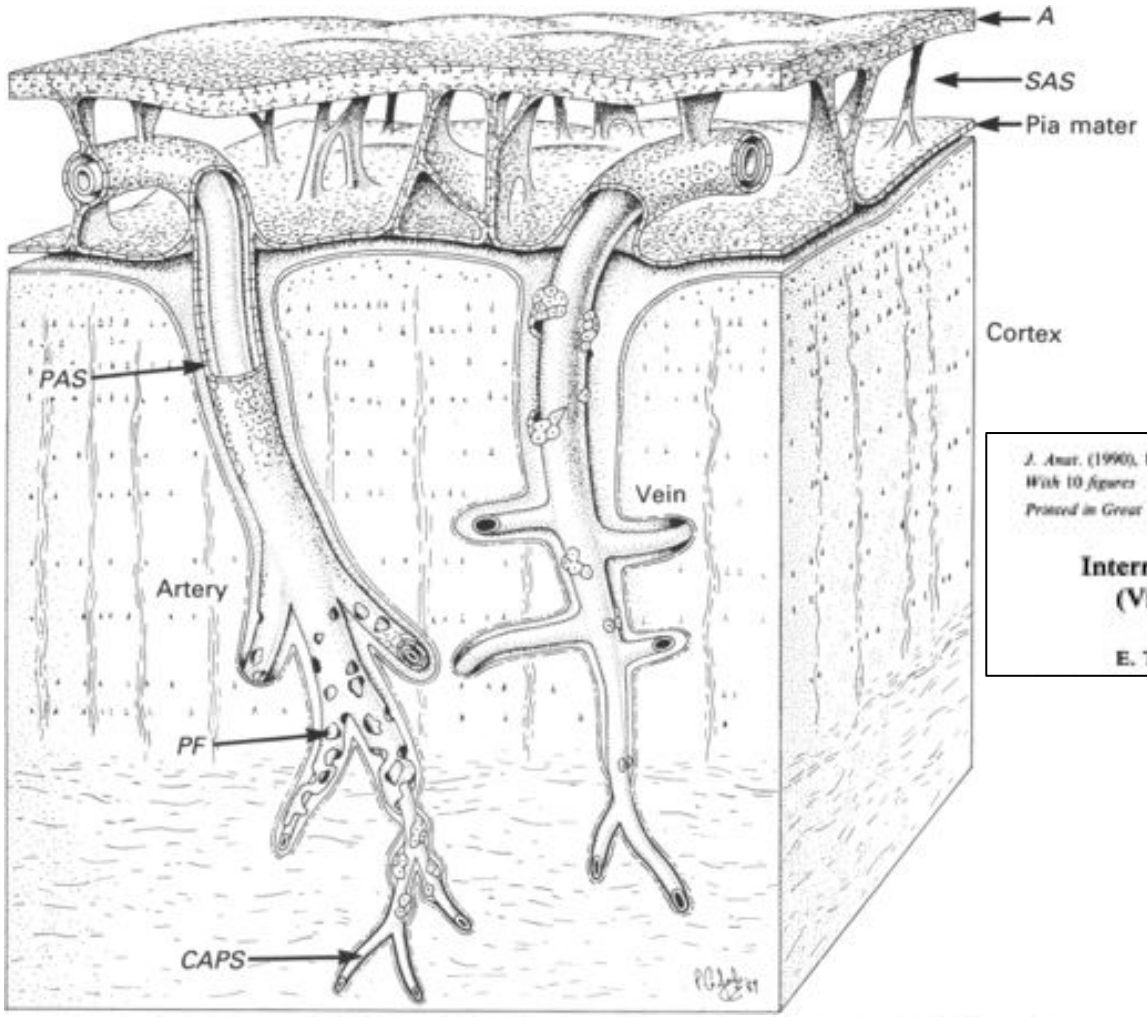


Fig. 10. Diagram demonstrating the relationships of the pia mater and intracerebral blood vessels. Subarachnoid space (*SAS*) separates the arachnoid (*A*) from the pia mater overlying the cerebral cortex. An artery on the left of the picture is coated by a sheath of cells derived from the pia mater; 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.

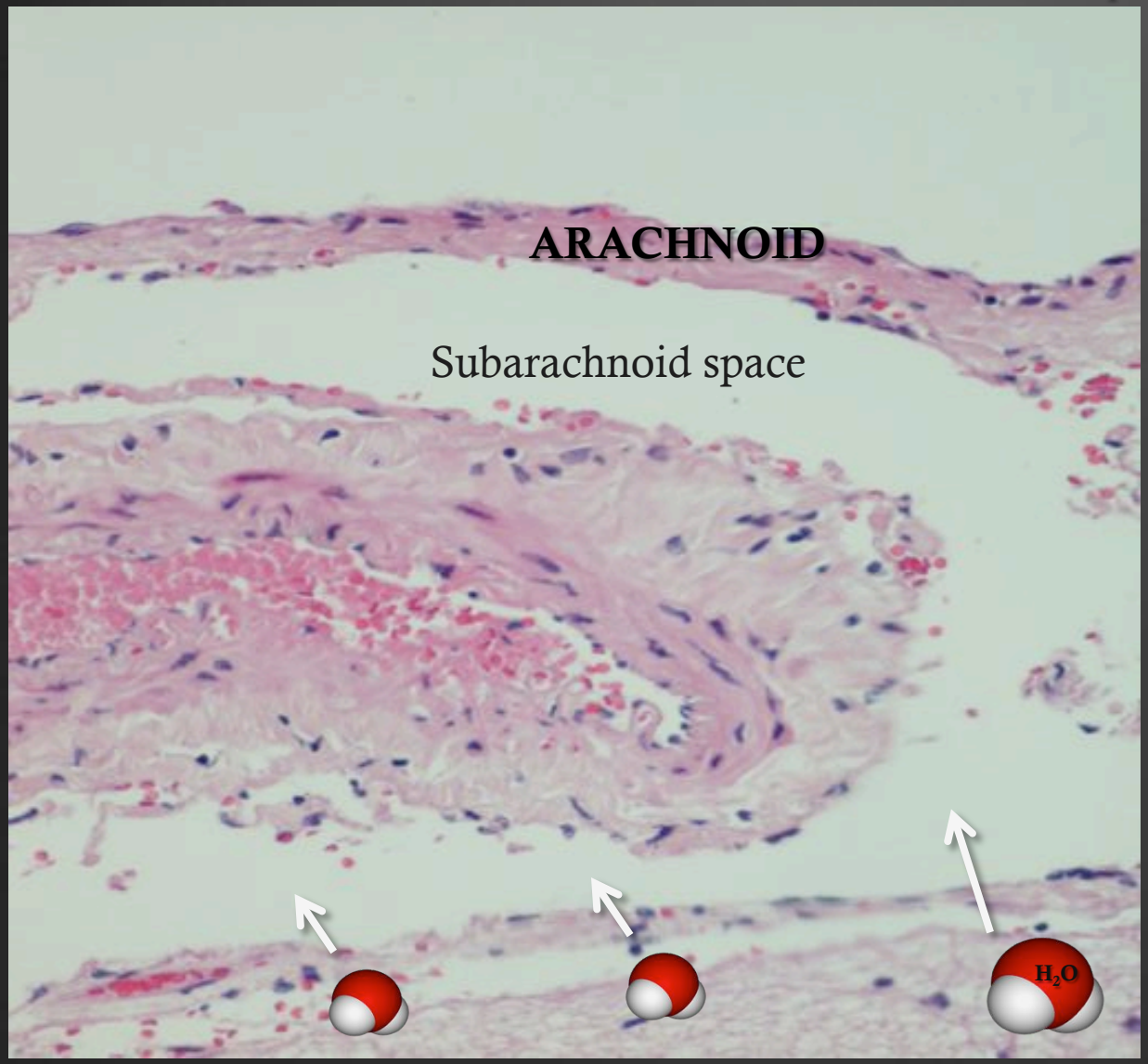
J. Anat. (1990), **170**, 111-123
With 10 figures
Printed in Great Britain

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†

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

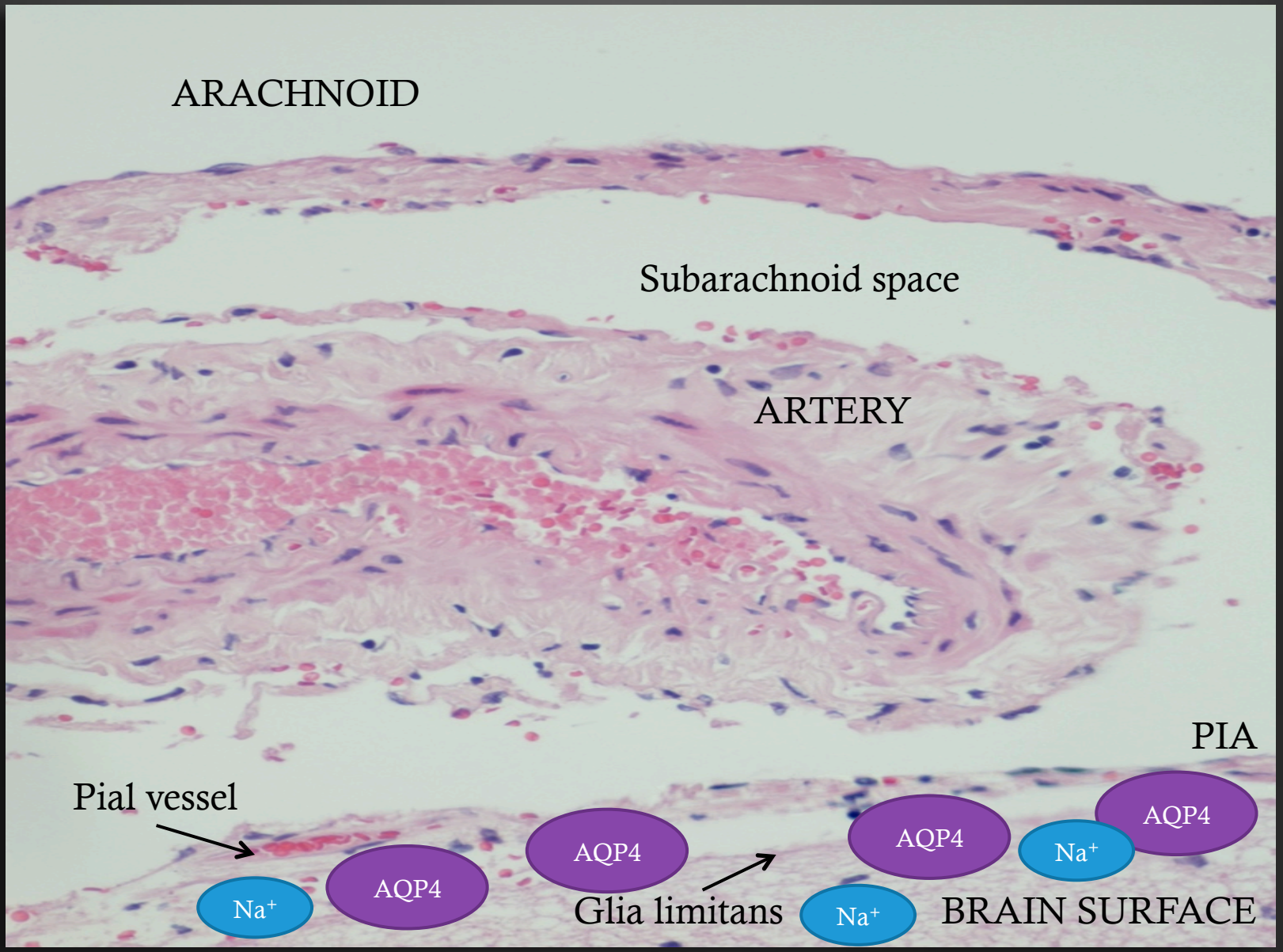
Reulen et al.
Role of pressure gradients and bulk flow in dynamics of vasogenic brain edema.
J Neurosurg (1977) vol. 46 (1) pp. 24-35

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

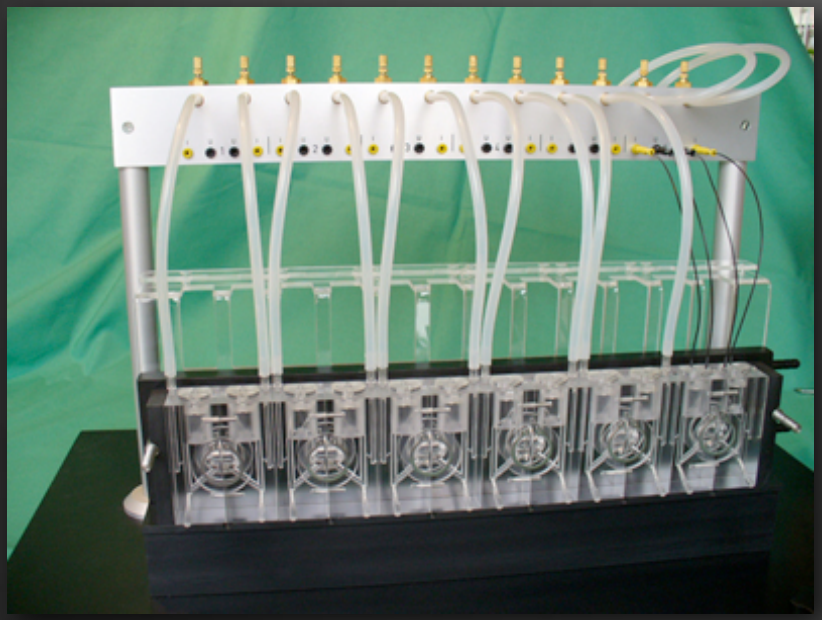

ARACHNOID
Subarachnoid space
ARTERY
PIA
Pial vessel
AQP4
AQP4
AQP4
AQP4
Na+
Na+
Na+
Glia limitans
BRAIN SURFACE

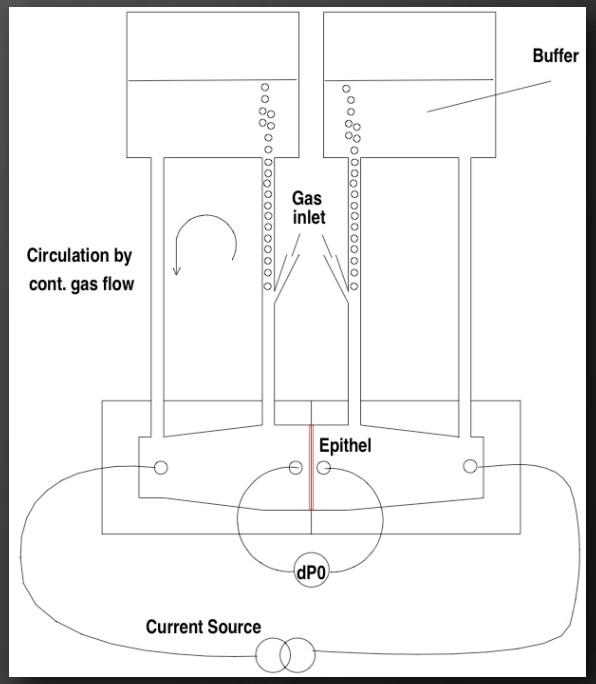
CSAS Ion channels

Solute-coupled transport

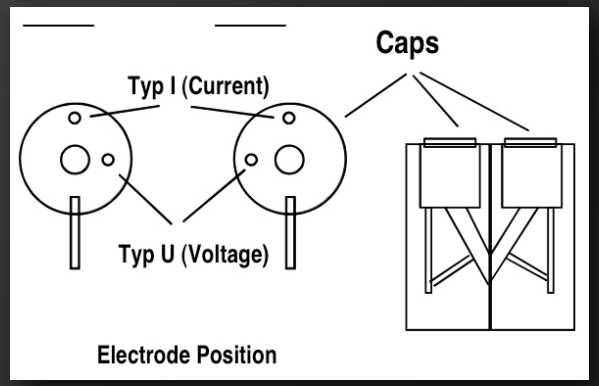
Membrane Electrophysiology

"Hans Ussing" chambers

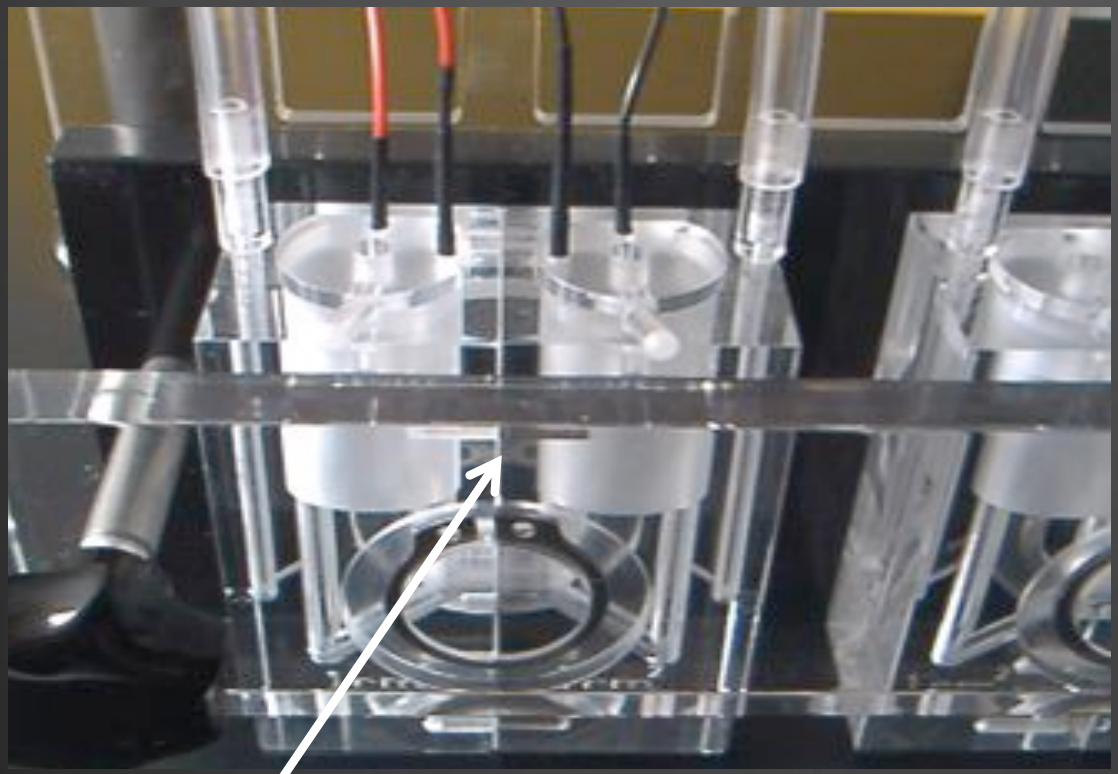




ESTABLISHED METHOD
FOR SOLUTE-COUPLED TRANSPORT STUDIES



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

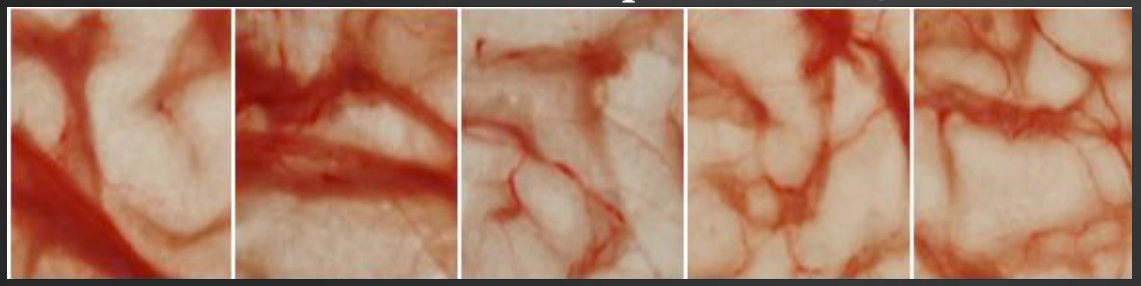
We get the:

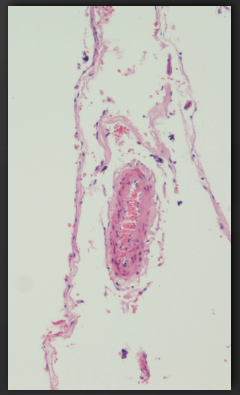
Transmembrane Resistance

R ($\Omega * cm^2$)

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.

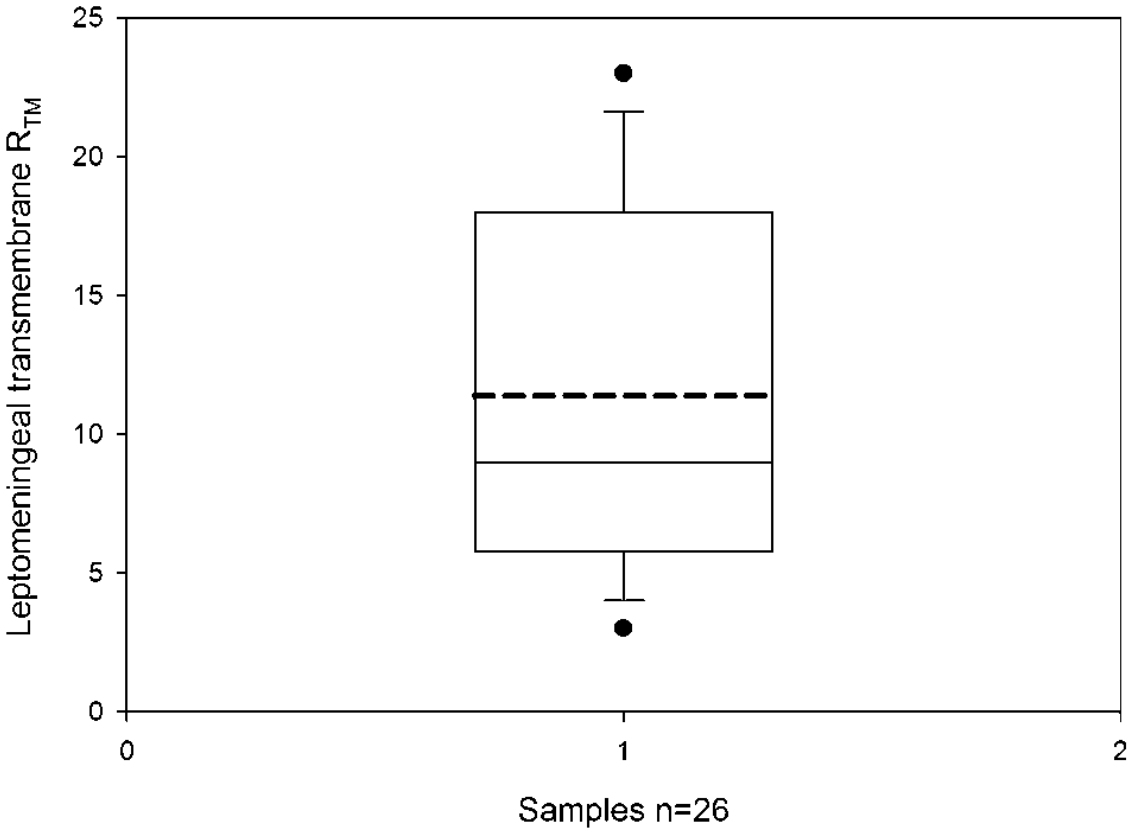
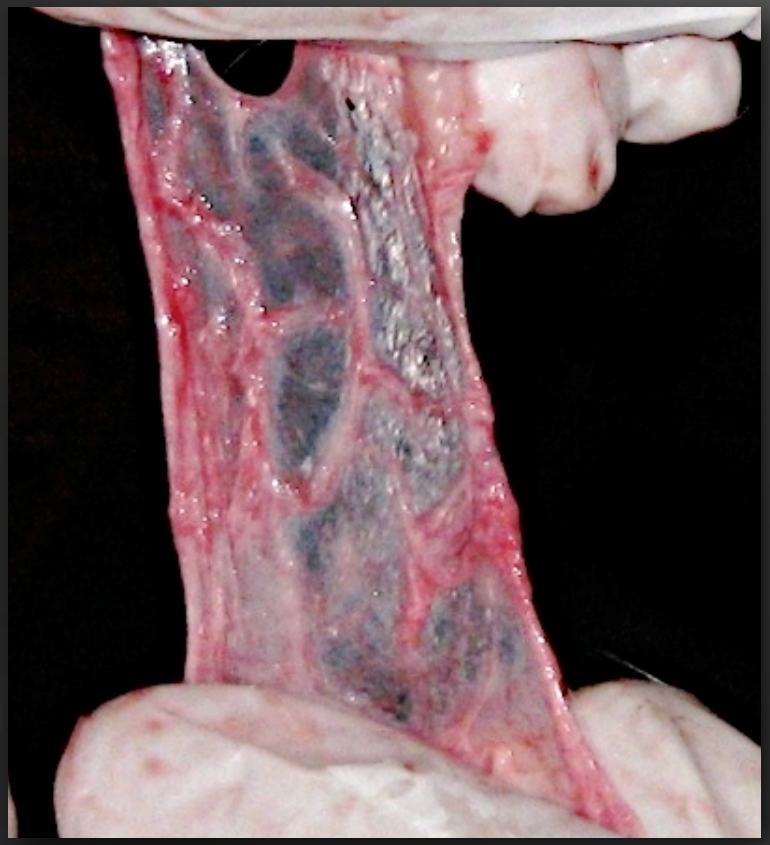
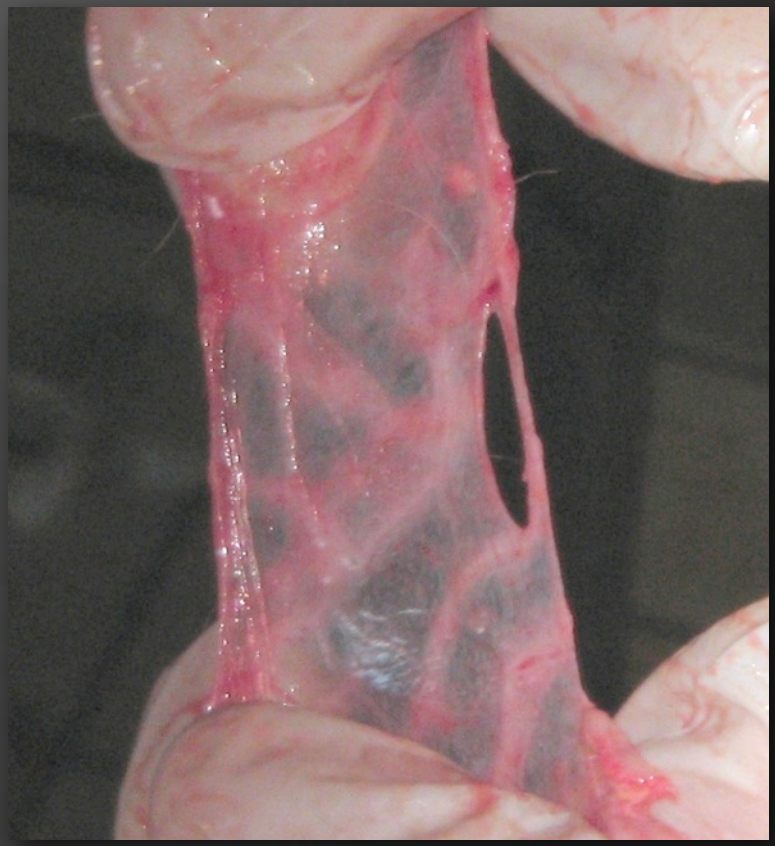


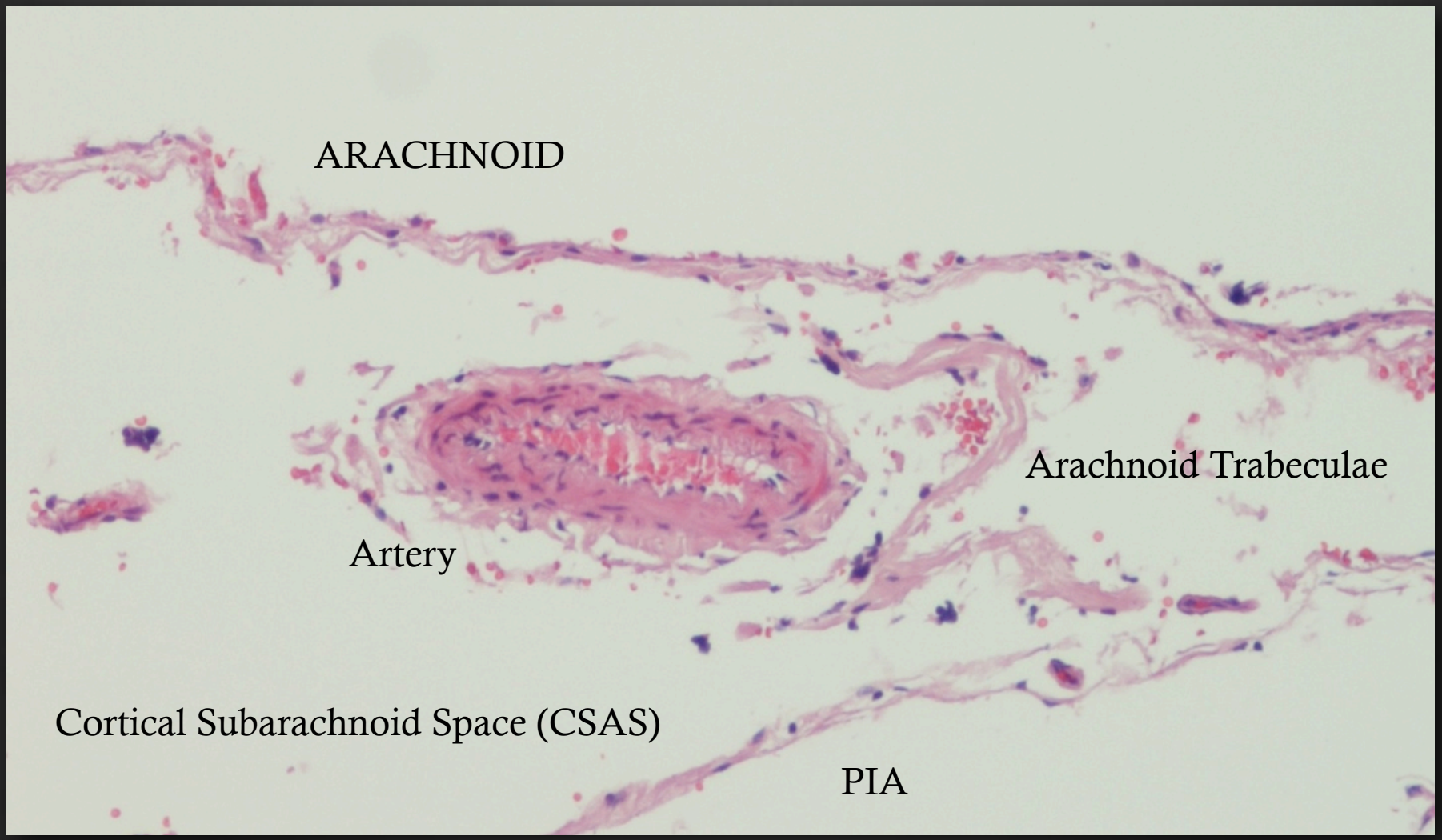
Figure 1 **Boxplot diagram describing the distribution of measured values of leptomeningeal transmembrane resistance in sheep along with mean value and outliers. Dotted line in the box represents the mean value of 11·38 Ω cm^2 obtained from 26 experiments**

PIAL SURFACE

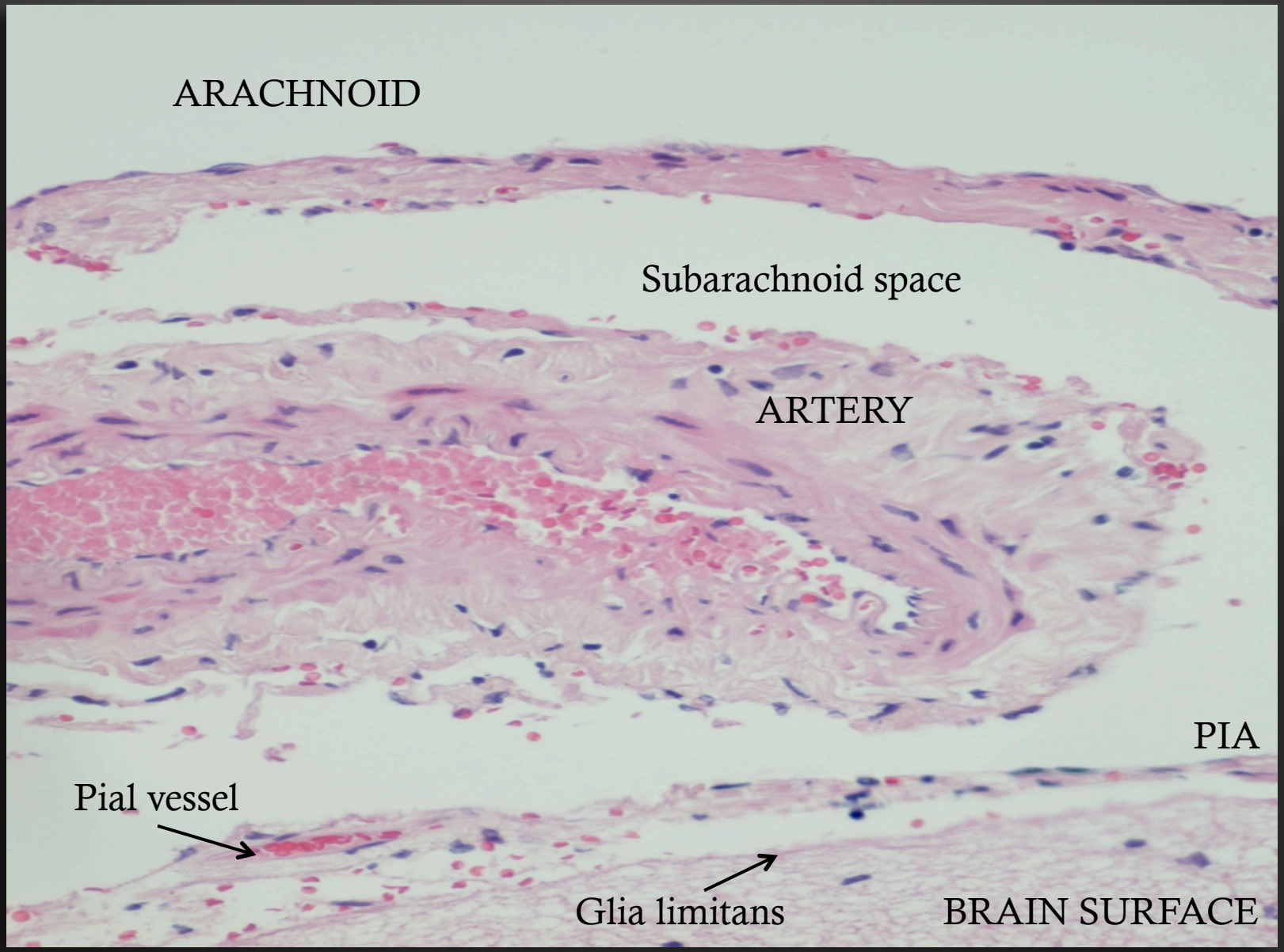


ARACHNOID SURFACE





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



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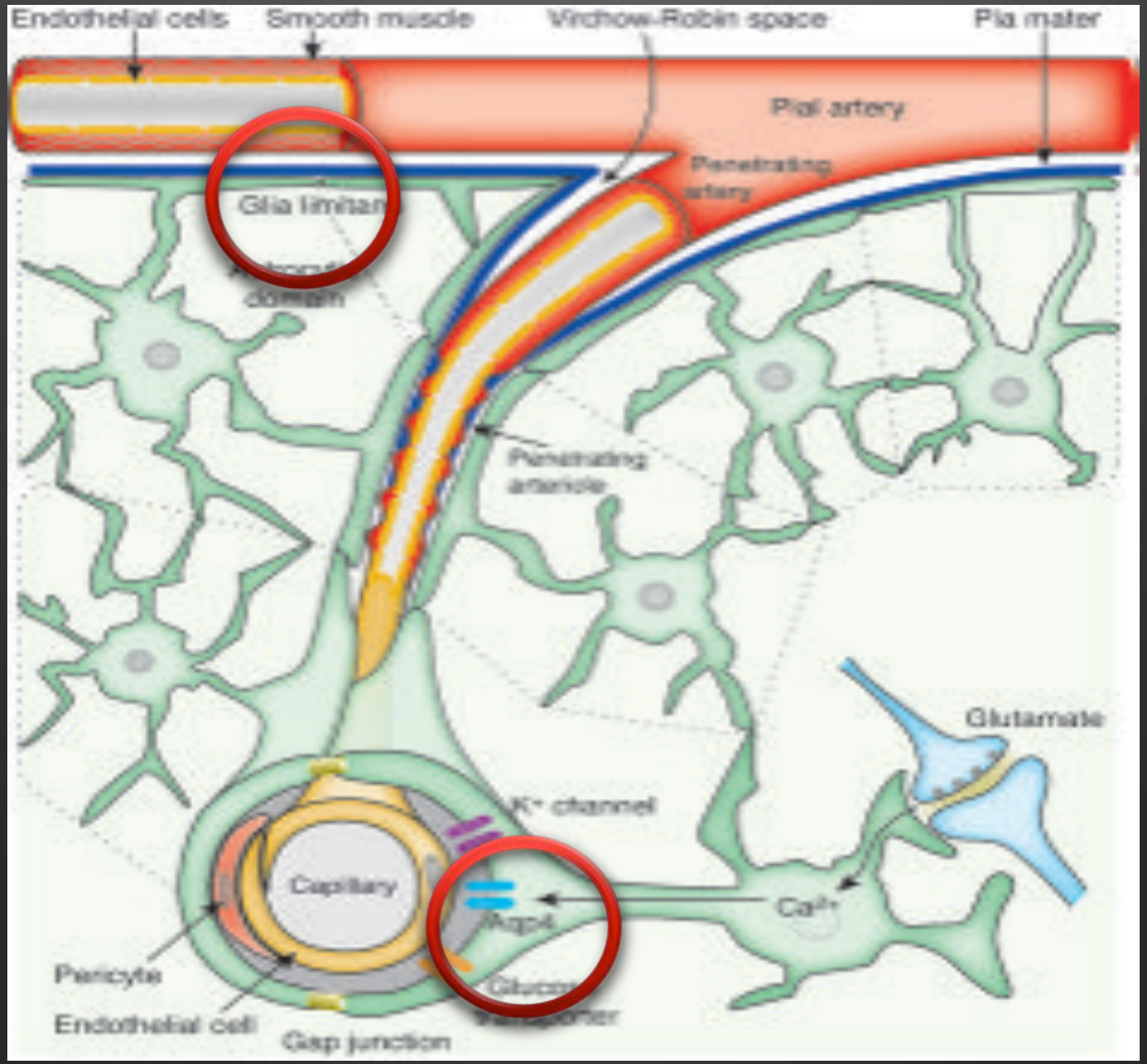
So…

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.
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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

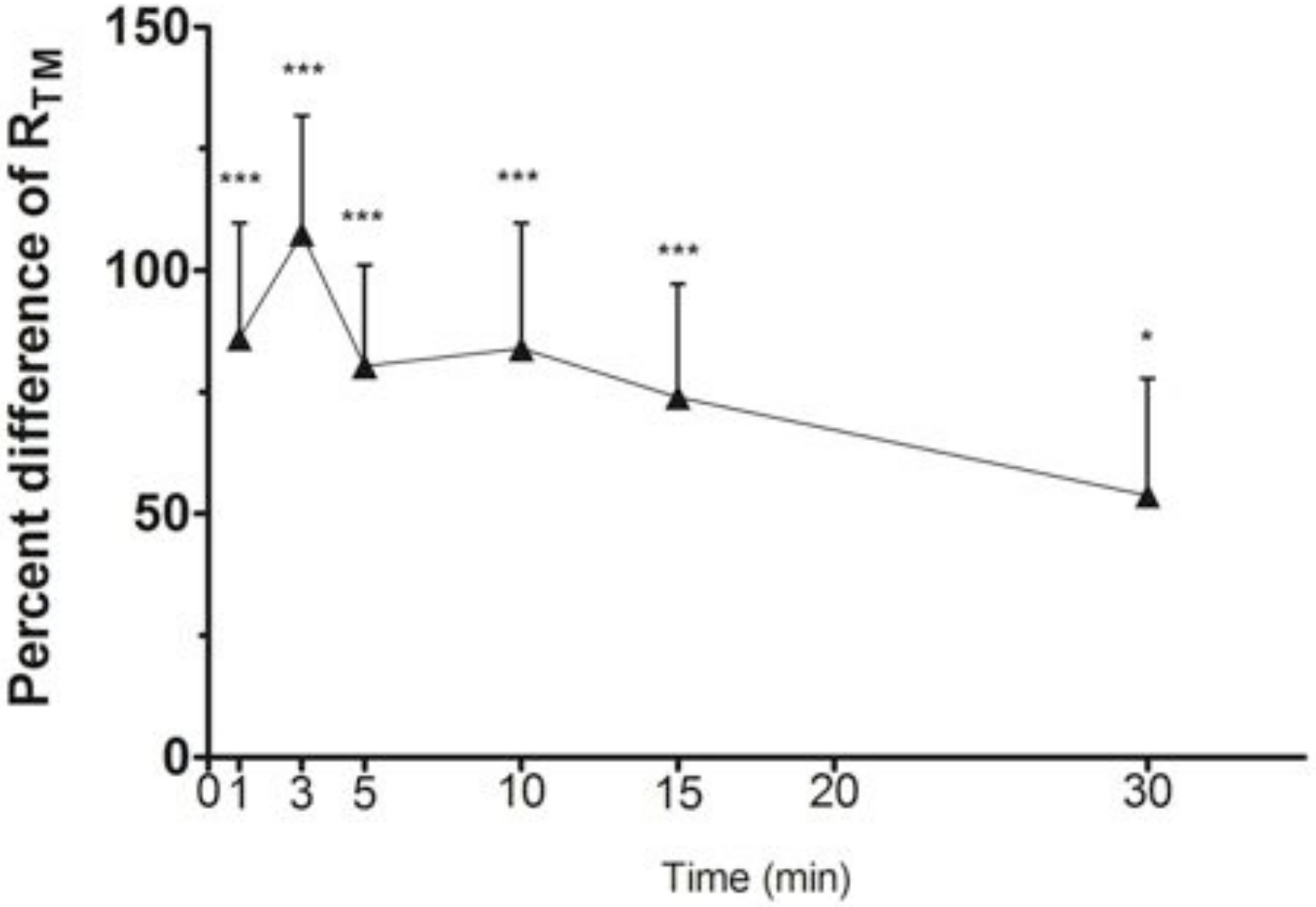
Aristotelis S. Filippidis · Sotirios G. Zarogiannis · Maria Ioannou · Konstantinos Gourgoulianis · Paschalis-Adam Molyvdas · Chrissi Hatzoglou

Sodium-Potassium-ATPase

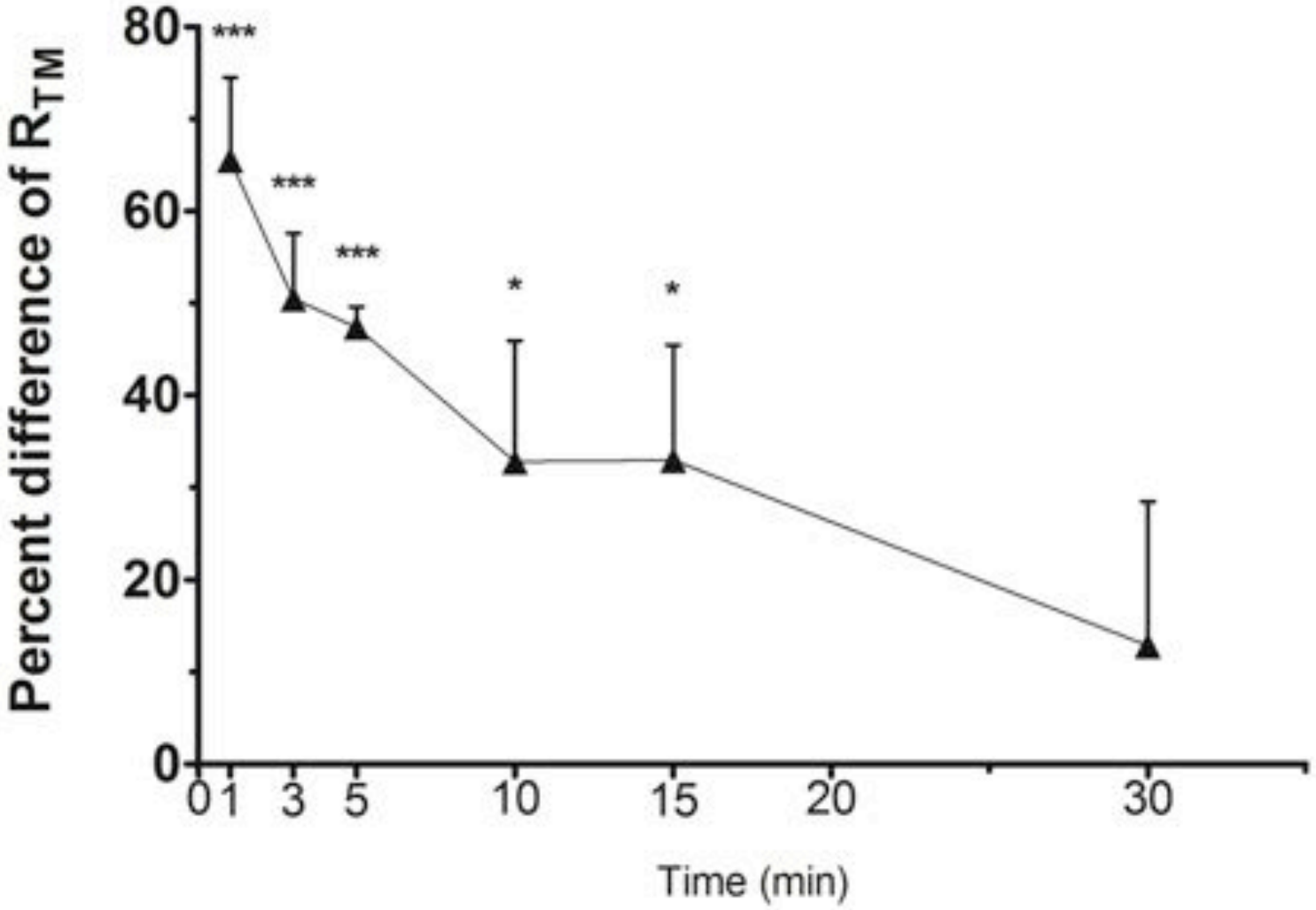
Main source of extracellular Sodium

We tested inhibition with OUABAIN

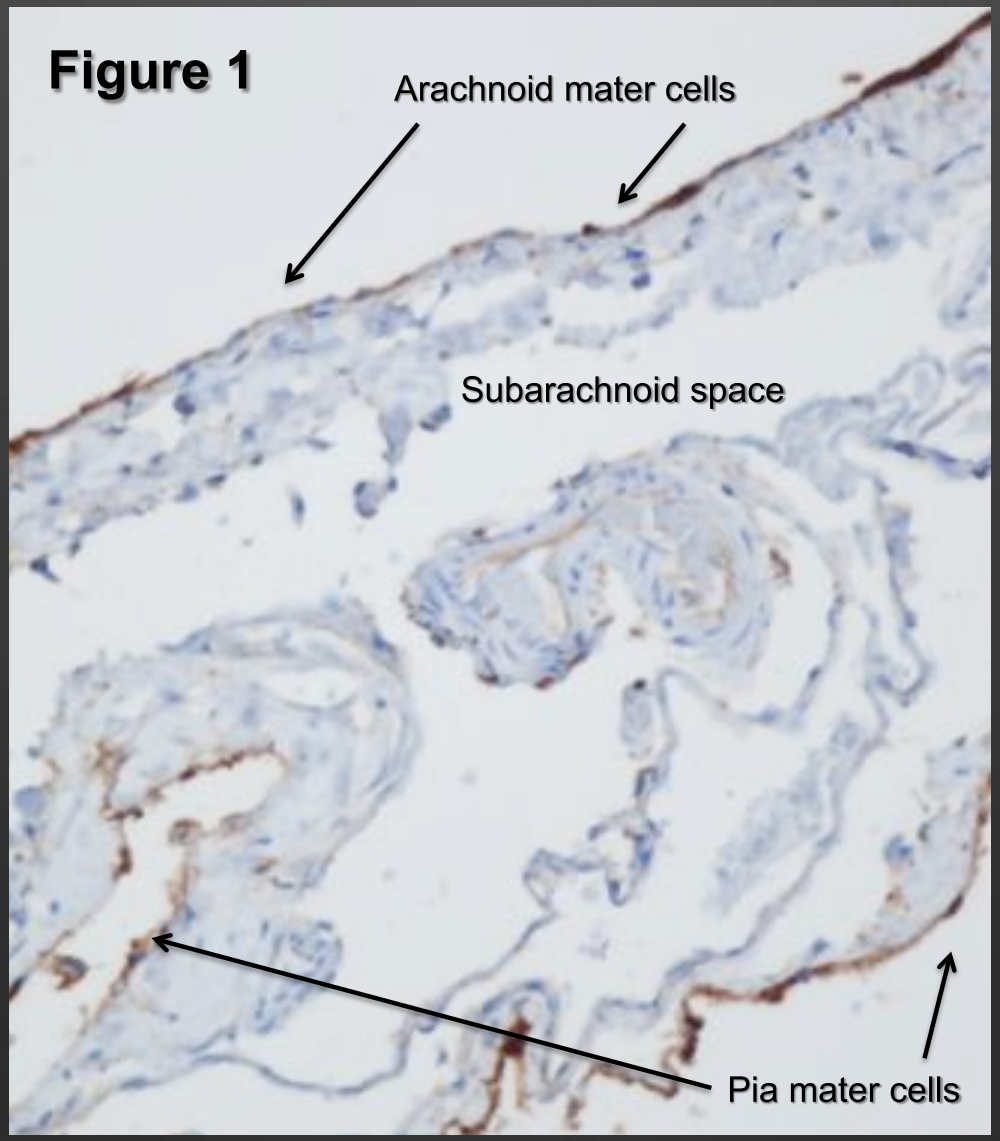
Leptomeninges Ouabain 10-3M Arachnoidal side



Leptomeninges Ouabain 10^{-3}M
Pial side



a1 subunit Sodium-Potassium-ATPase

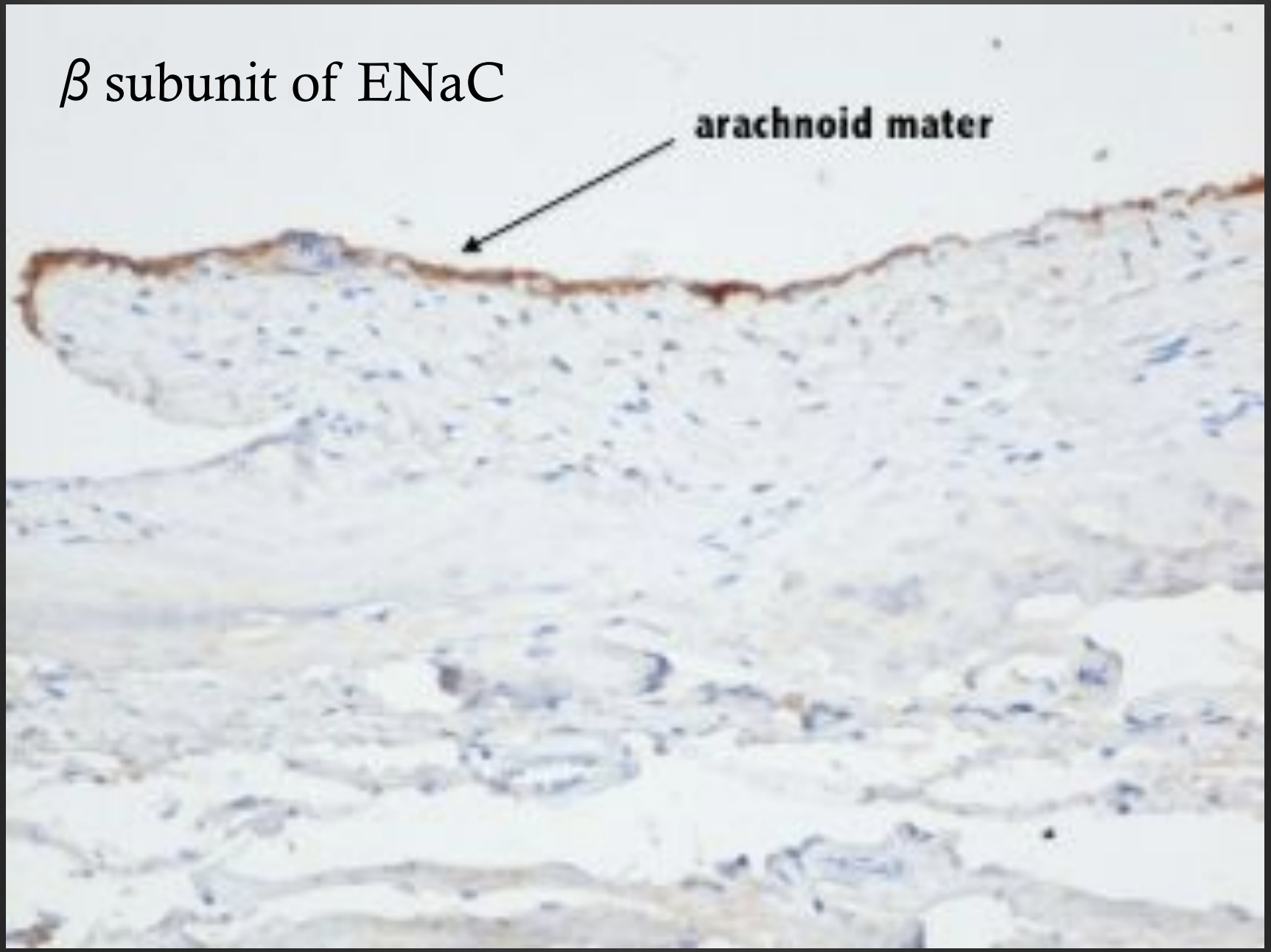


ENaC

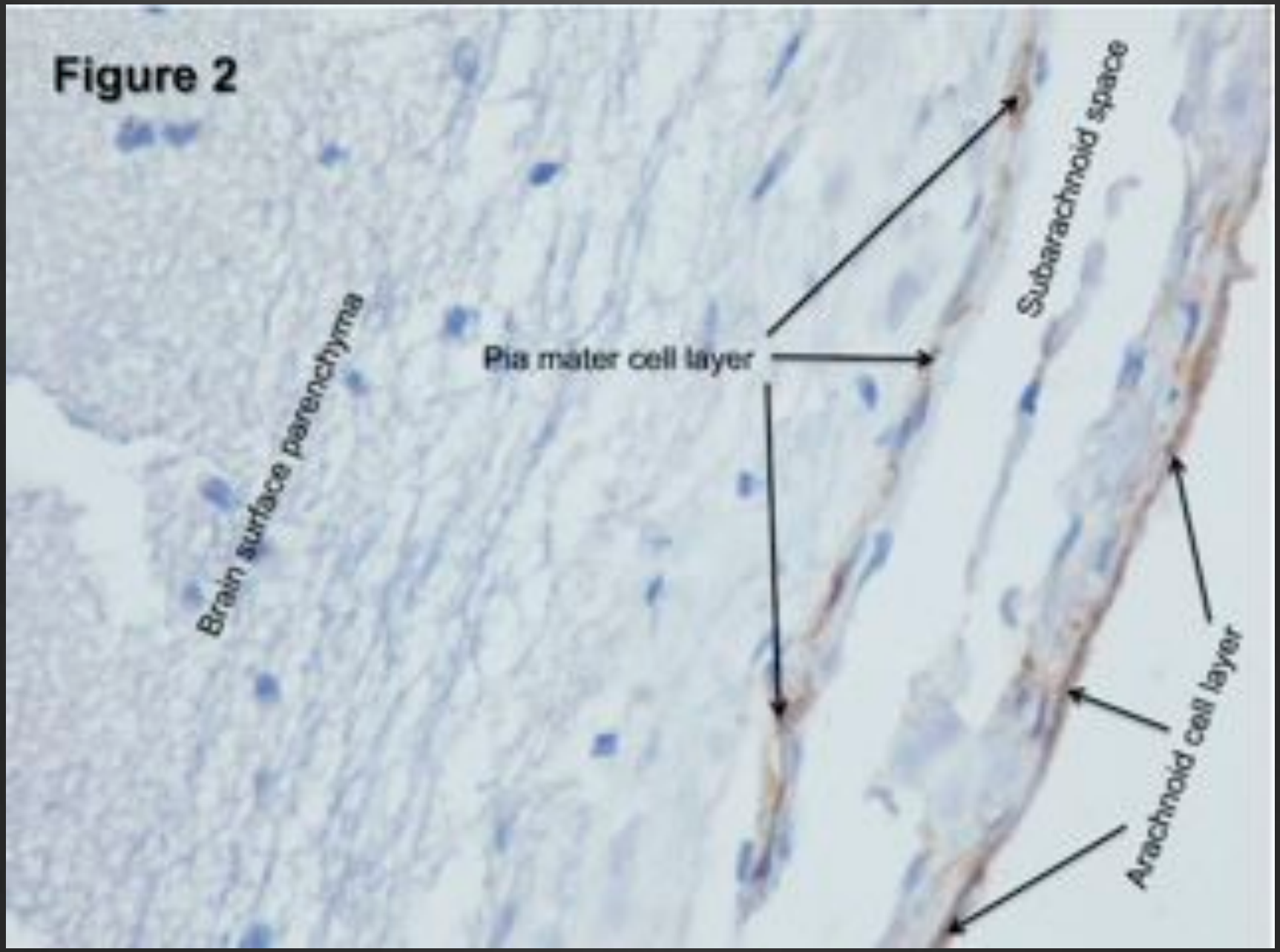
Epithelial Sodium Channel

We tested inhibition with AMILORIDE

β subunit of ENaC



δ subunit - ENaC



Conclusions

- There IS a role for AQPs and hydrocephalus. Is this the most important?
- Possibly deserves a "point of obstruction" reference in Dr. Rekate's hydrocephalus model
- A new approach is needed for AQP research in hydrocephalus
- Key is OSMOSIS at the cellular level
- Responsible to identify if Osmotic gradients exist in Hydrocephalus microcosmos
- If so then we have to explain how pressure (hydrostatic or ICP) affect the physiology
- 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

Invitation for contribution, FRONTIERS journal,

Title: "CSF disorders, advances in pathophysiology, modeling, diagnosis, and treatment"