Dept. Physiology, Membrane permeability Lab
Medical School, University of Thessaly, GREECE

current affiliation:
Dept. Neurosurgery, Marmarou Lab, VCU, USA

CSF absorption from the CSAS:

what is known, what is new ?, what can we see
what should we have to see

Aristotelis S. Filippidis M.D., Ph.D.
Postdoctoral Fellow

Hydrocephalus and CSF Flow Study Group, New York, www.ihiwg.org, April 2012

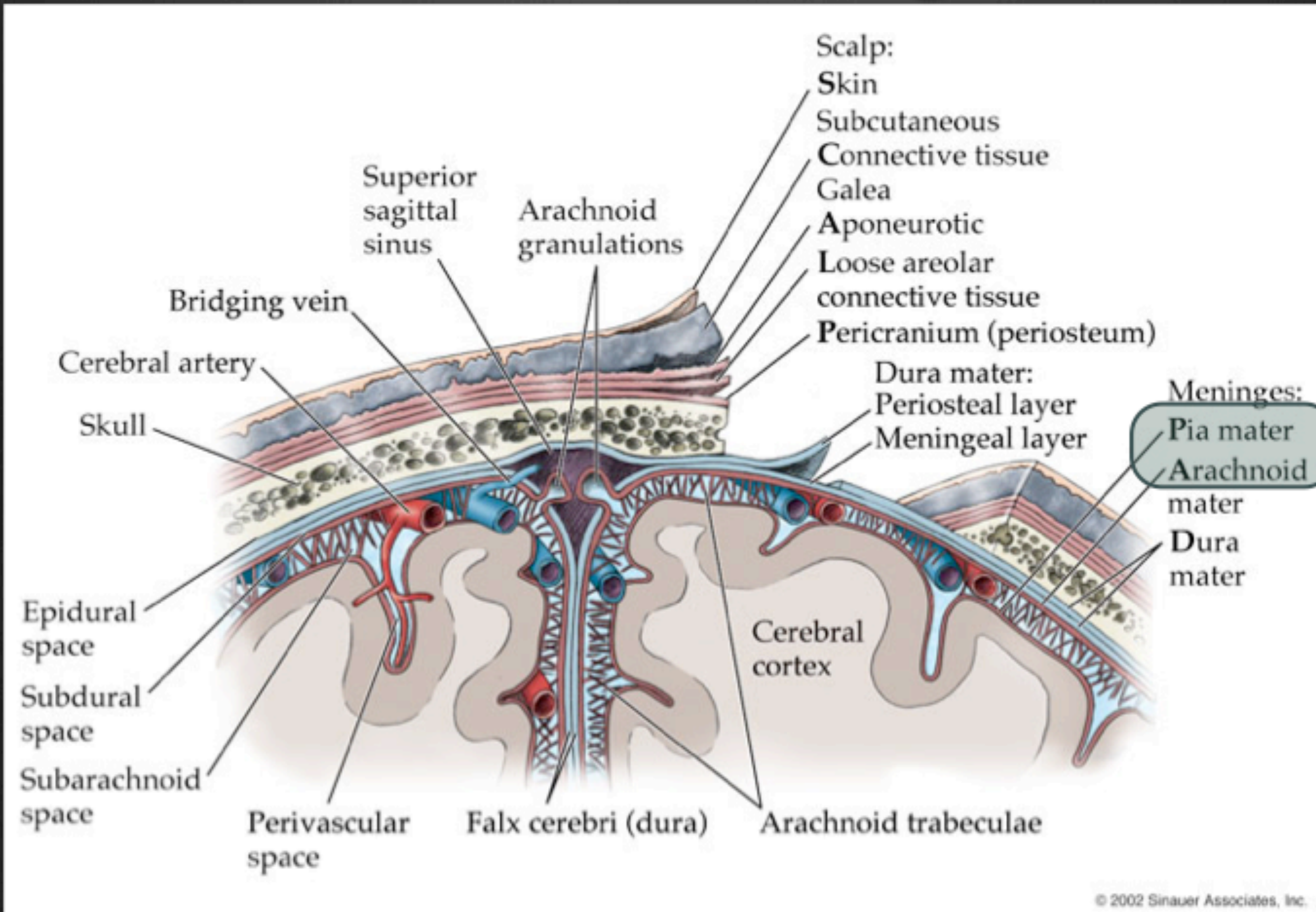
Is CSAS important for neurohydrodynamics and CSF disorders ?

J Neurosurg Pediatrics 2:1–11, 2008

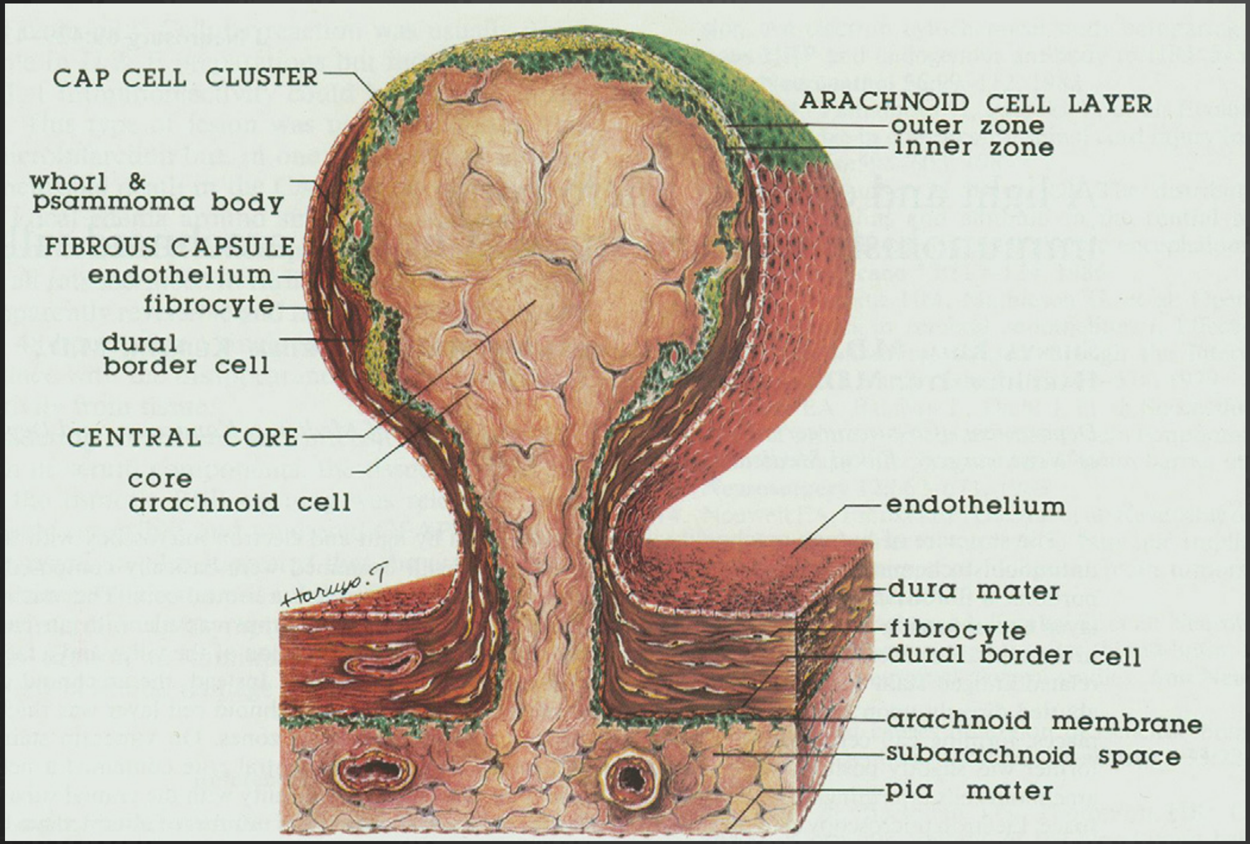
The importance of the cortical subarachnoid space in understanding hydrocephalus

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]

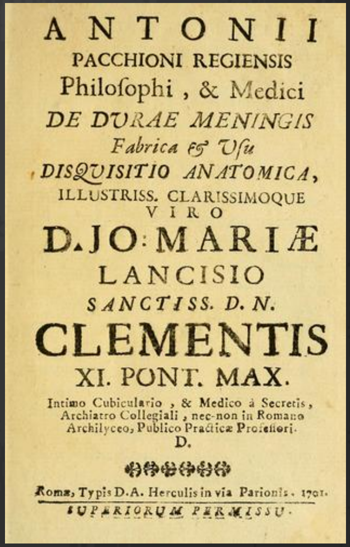
Cortical Meninges



Arachnoid granulations-villi



First described in 1705 by Pacchioni as "peculiar wart-like excrescences"

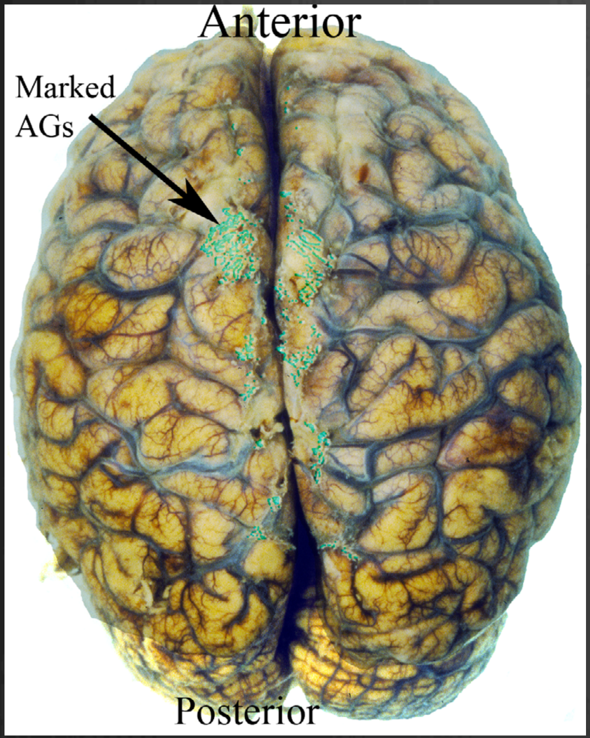


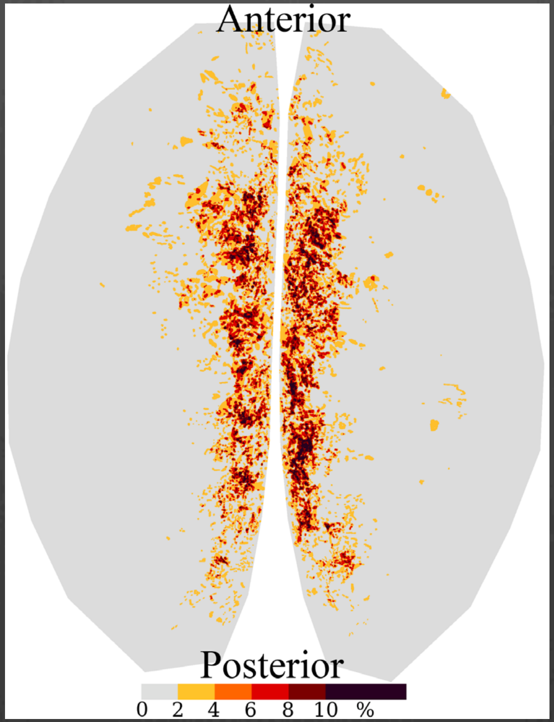
ANTONII
PACCHIONI REGIENSIS
Philoſophi, & Medici
DE DURAE MENINGIS
Fabrica & Uſu
DISQUISITIO ANATOMICA,
ILLUSTRISS. CLARISSIMOQUE
VIRO
D. JO: MARIÆ
LANCISIO
SANCTISS. D. N.
CLEMENTIS
XI. PONT. MAX.
Intimo Cubiculario, & Medico à Secretis, Archiatro Collegiali, nec-non in Romano Archilyceo, Publico Practicæ Professori.
D.

Romæ, Typis D.A. Herculis in via Parionis. 1701.
SUPERIORUM PERMISSU.

Image from :Brain Res Bull. 2008 Dec 16;77(6):327-34. Epub 2008 Sep 13.
Cerebrospinal fluid outflow: an evolving perspective.
Kapoor KG, Katz SE, Grzybowski DM, Lubow M.

Arachnoid granulations-villi





Can be found:

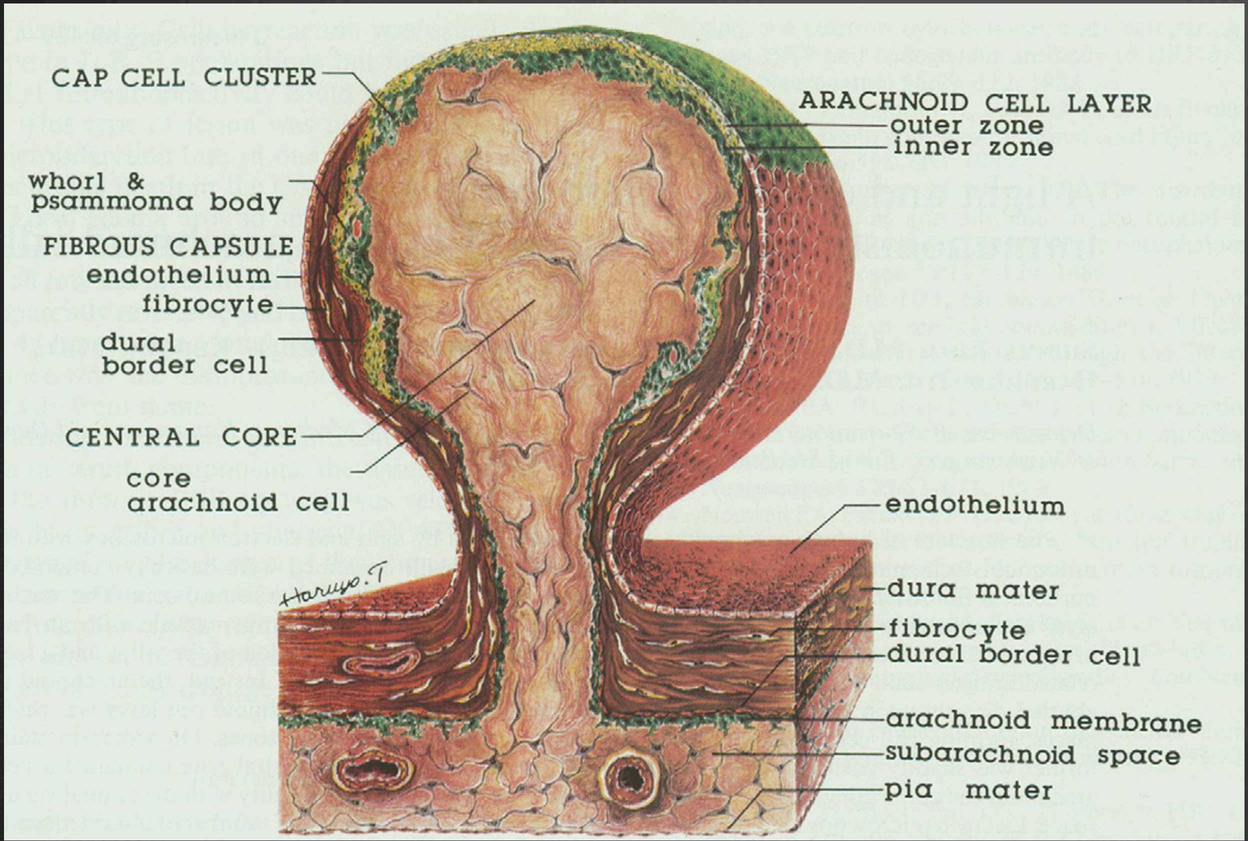
SUPERIOR SAGITTAL SINUS (SSS) area
Transverse sinus
Skull base (sella tursica)
Spine

MAJOR CSF OUTFLOW PATHWAY IN ADULTS

Brain average surface:
78.53 ± 13.13 mm^2

Image from :Brain Res Bull. 2008 Dec 16;77(6):327-34. Epub 2008 Sep 13.
Cerebrospinal fluid outflow: an evolving perspective.
Kapoor KG, Katz SE, Grzybowski DM, Lubow M.

Arachnoid granulations - physiology



Average ***cellular*** hydraulic conductivity (L_p)
4.5 μ l/min per mmHg/cm^2

Average ***tissue perfusion in vitro*** hydraulic conductivity (L_p)
92.5 μ l/min per mmHg/cm^2

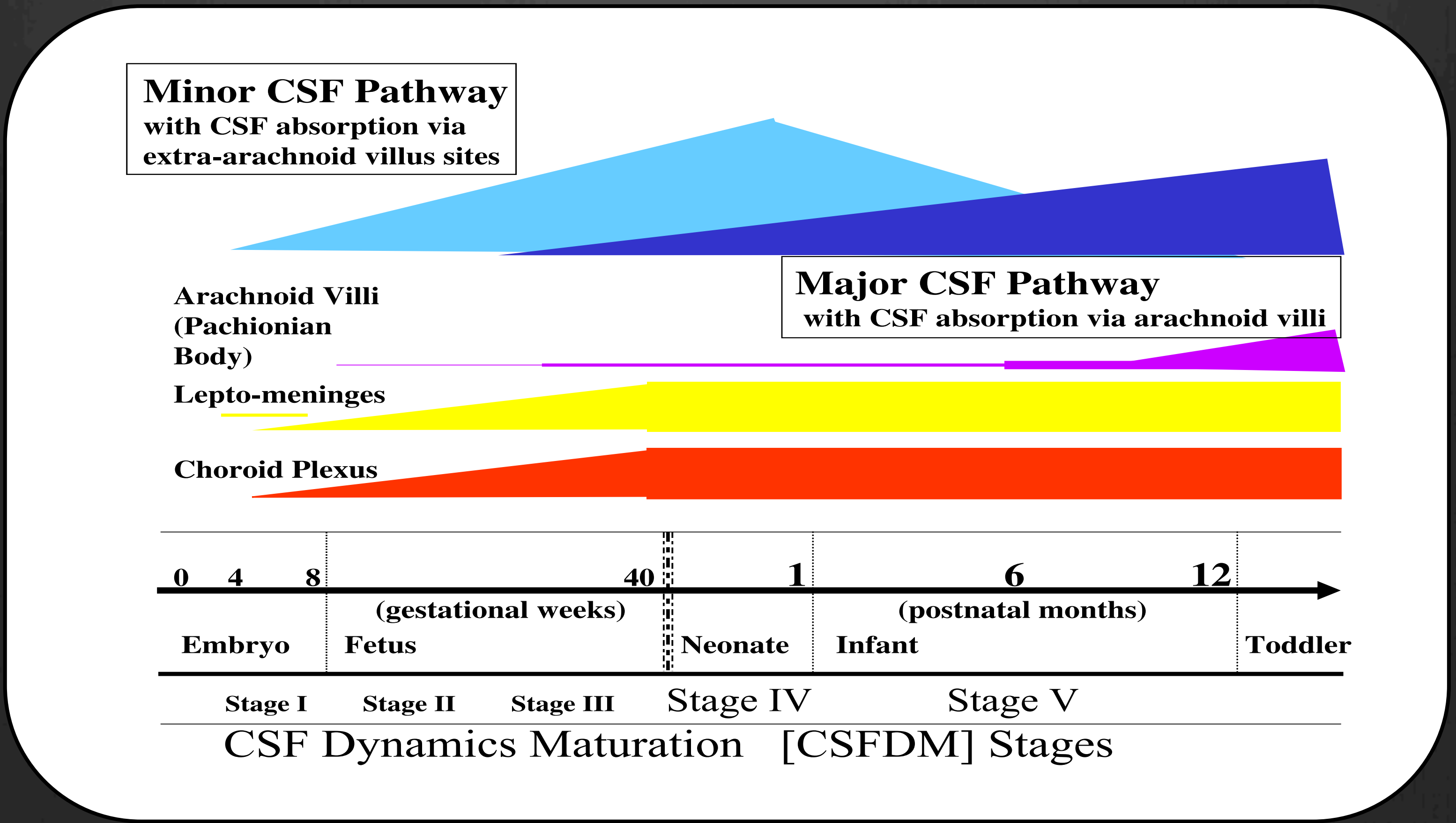
Average ***in vivo MRI based*** hydraulic conductivity (L_p)
130.9 μ l/min per mmHg/cm^2

In vitro model of cerebrospinal fluid outflow through human arachnoid granulations.
Grzybowski DM, Holman DW, Katz SE, Lubow M.
Invest Ophthalmol Vis Sci. 2006 Aug;47(8):3664-72.

Cerebrospinal fluid dynamics in the human cranial subarachnoid space: an overlooked mediator of cerebral disease. II. In vitro arachnoid outflow model.
Holman DW, Kurtcuoglu V, Grzybowski DM.
J R Soc Interface. 2010 Aug 6;7(49):1205-18. Epub 2010 Mar 24.

Developmental orchestration of CSF absorption pathways

Childs Nerv Syst. 2006 Jul;22(7):662-9. Epub 2006 May 10.
Proposal of "evolution theory in cerebrospinal fluid dynamics" and minor pathway hydrocephalus in developing immature brain.
Oi S, Di Rocco C. *(courtesy of image)*



Lymphatics

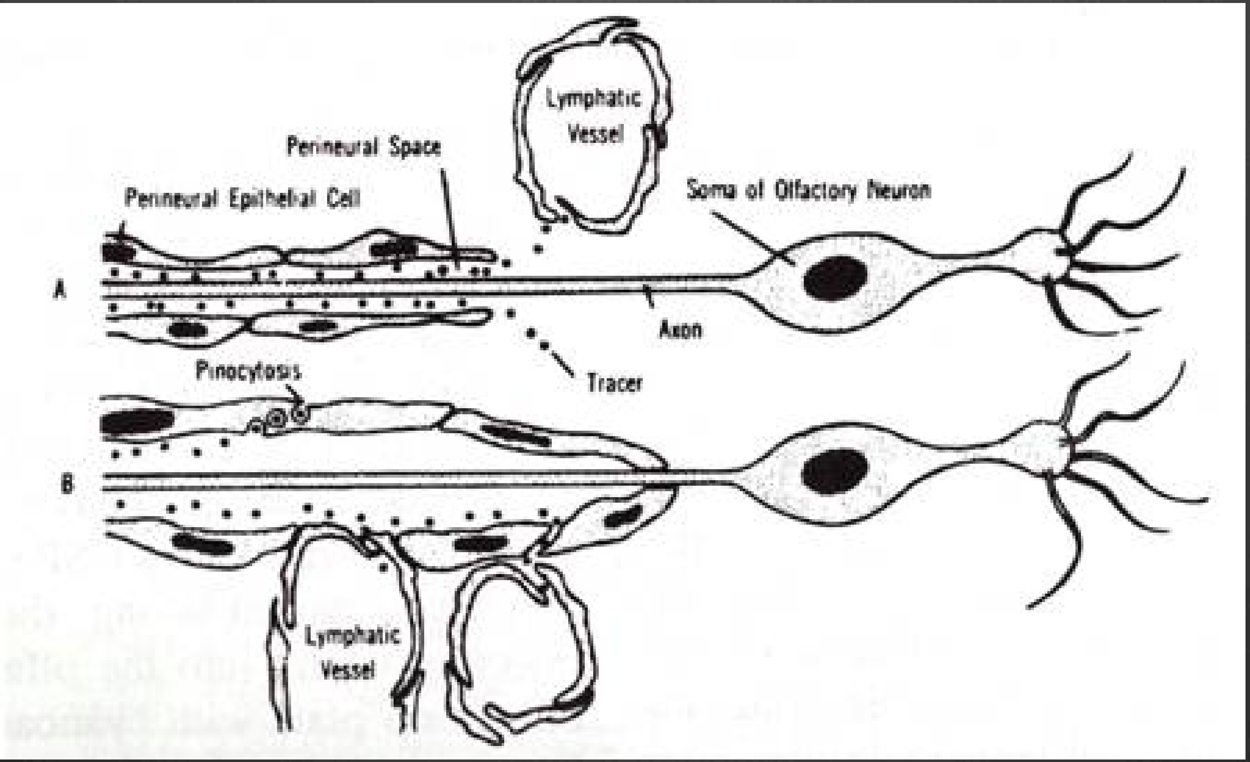


Figure 8 Diagrammatic representations of two models of olfactory perineural pathway to nasal lymphatic outflow system (reproduced with permission from Jackson *et al* [48]).

Schwab in 1869:
observed that substances injected into SAS could be found later in the cervical lymph nodes

The function and structure of the cerebrospinal fluid outflow system.
Pollay M.
Cerebrospinal Fluid Res. 2010 Jun 21;7:9.

Cerebrospinal Fluid Res. 2004 Dec 10;1(1):2.

Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species.

Johnston M, Zakharov A, Papaiconomou C, Salmasi G, Armstrong D.

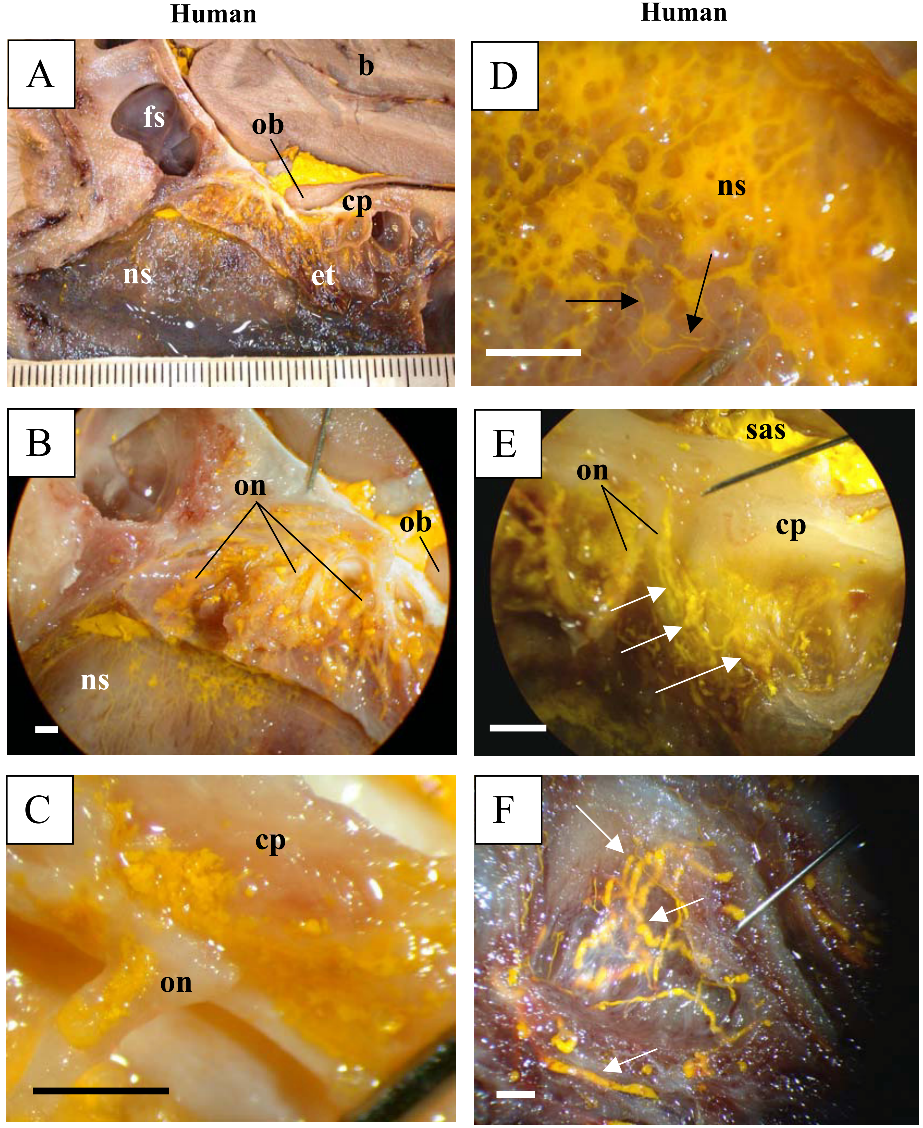
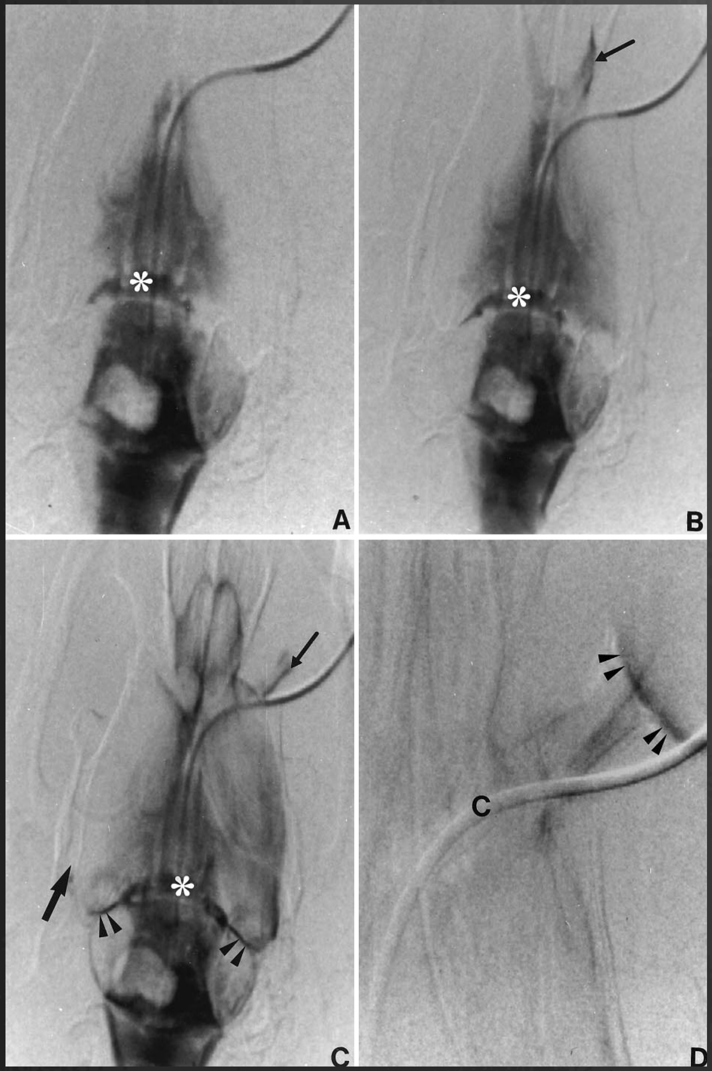


Figure 4
Microfil distribution patterns in the head of a human (A-F). All images are presented in sagittal plane with gradual magnification of the olfactory area adjacent to the cribriform plate. Reference scales are provided either as a ruler in the image (mm) or as a longitudinal bar (1 mm). As in the other species, Microfil introduced into the subarachnoid space was observed around the olfactory bulb (A), in the perineurial spaces of the olfactory nerves (B, C) and in the lymphatics of the nasal septum (D), ethmoid labyrinth (E) and superior turbinate (F). Due to tissue deterioration, some of the lymphatic vessels had ruptured and Microfil was noted in the interstitium of the submucosa of the nasal septum (D). In (E), Microfil is observed in the subarachnoid space and the perineurial space of olfactory nerves. The perineurial Microfil is continuous with that in lymphatic vessels (arrows). Intact lymphatic vessels containing Microfil are outlined with arrows (D, E, F). b – brain; fs – frontal sinus; cp – cribriform plate; et – ethmoid turbinates; ob – olfactory bulbs; on – olfactory nerves; ns – nasal septum; sas – subarachnoid space.

Lymphatics



Early functional imaging studies
10-20 mm/s velocity of particle movement in cats

Acta Neuropathol. 1997 Nov;94(5):493-8.
Dynamic properties of lymphatic pathways for the absorption of cerebrospinal fluid.
Brinker T, Lüdemann W, Berens von Rautenfeld D, Samii M.

Lymphatics - physiology

Cserr in rabbit, sheep, cat : 14-47% of injected tracer into the brain goes to the lymphatics

Weller: supported that lymphatics drain in preference the cerebral interstitial fluid into the CSF

Boulton: At physiologic pressures the microfil injected failed to go through the ARACHNOID VILLI! (sheep)

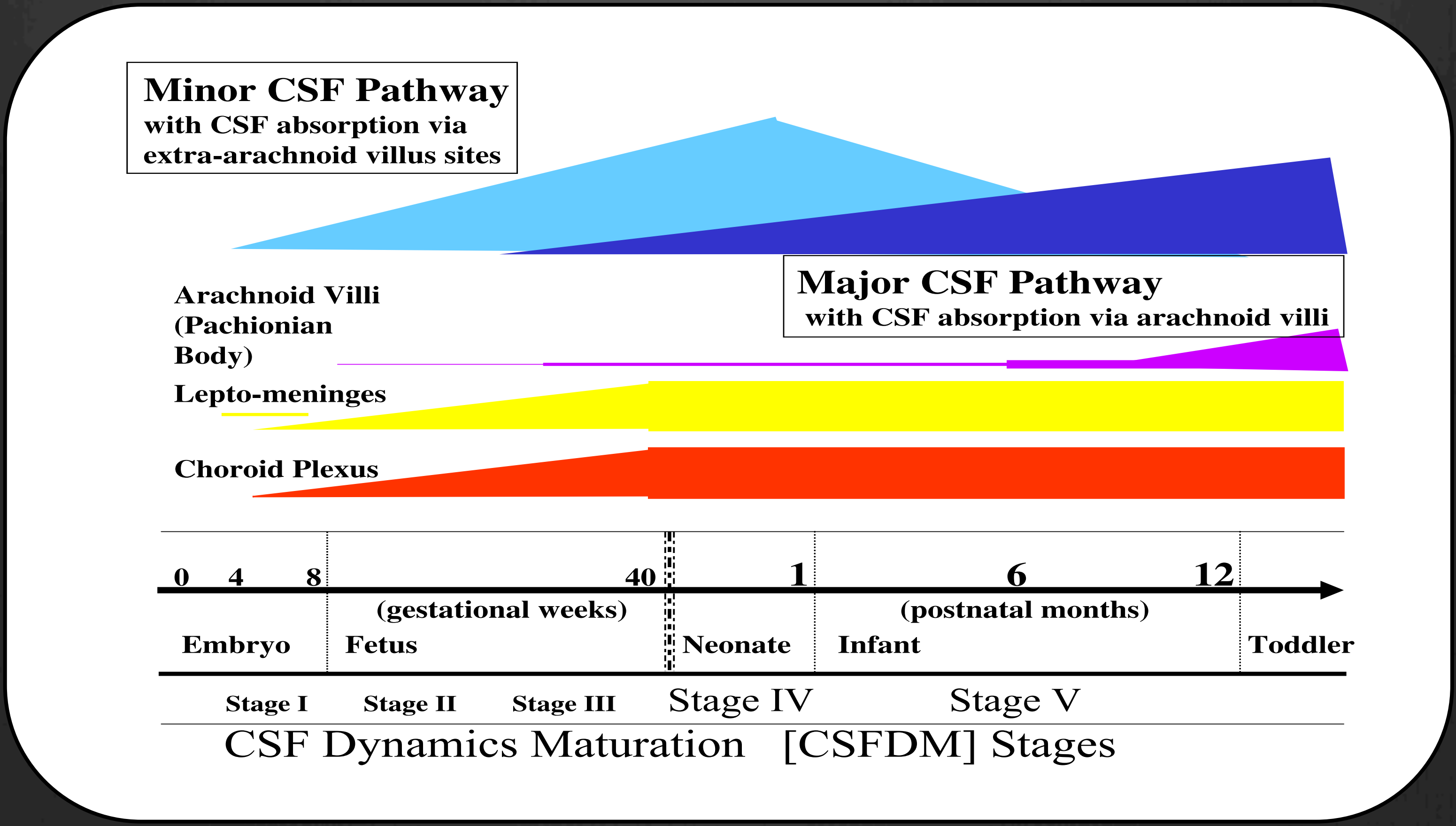
10 cm H_2O increase raises AV and CSF lymphatic clearance by 2.7 and 3.9 respectively
Total (AV + lymphatics) outflow in animal models: 3.48 ml/h

Nagra et al and Rammling et al: The blockage of the lymphatics can lead to HYDROCEPHALUS

The function and structure of the cerebrospinal fluid outflow system.
Pollay M.
Cerebrospinal Fluid Res. 2010 Jun 21;7:9.

Developmental orchestration of CSF absorption pathways

Childs Nerv Syst. 2006 Jul;22(7):662-9. Epub 2006 May 10.
Proposal of "evolution theory in cerebrospinal fluid dynamics" and minor pathway hydrocephalus in developing immature brain.
Oi S, Di Rocco C. *(courtesy of image)*



Solute-coupled transport

A neglected part of the equation ?

So…

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

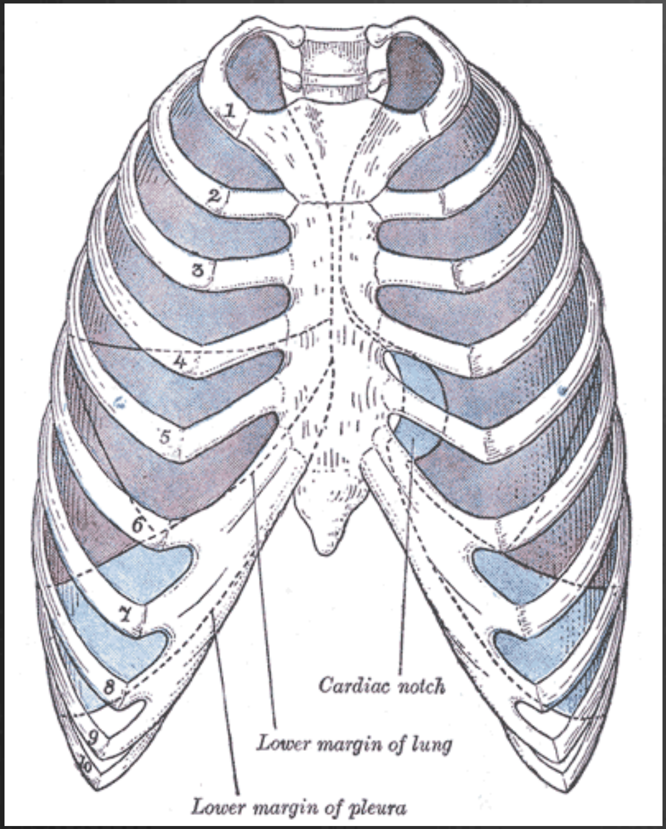
We <u>need</u> to address the relationship of CSAS with Water…

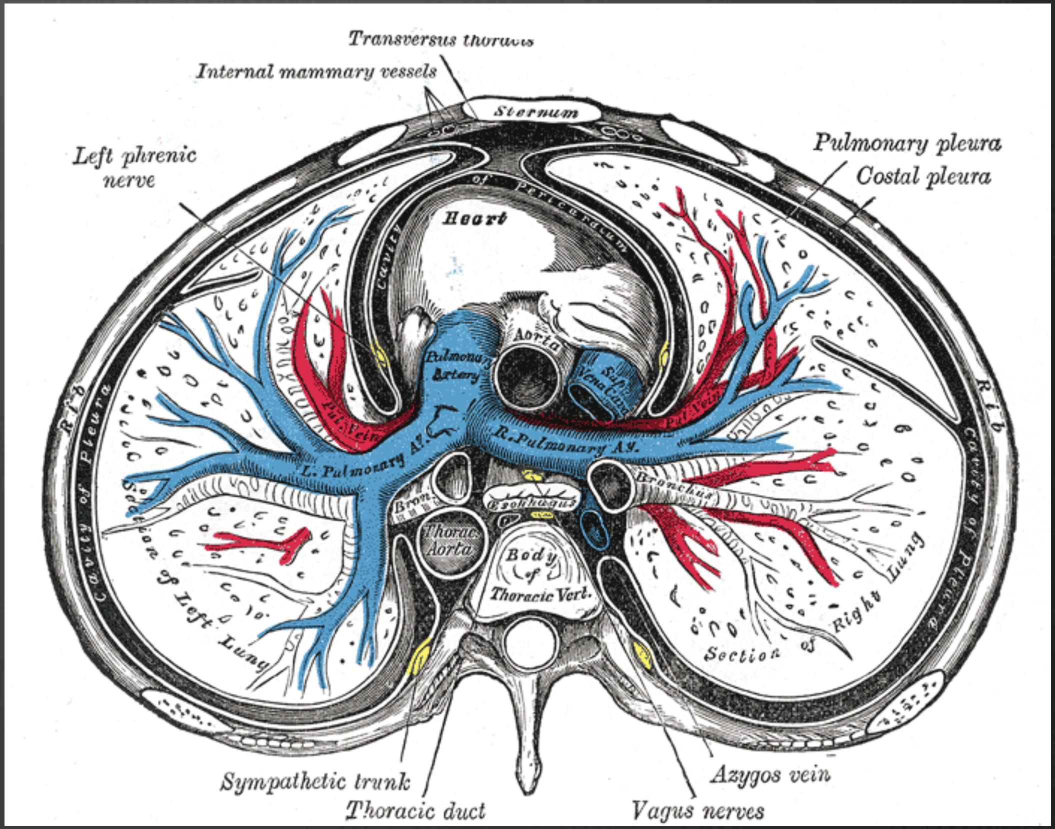
Let’s move on to the cellular level

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

Let’s think of what is known in other tissues

Pleura and mesothelial tissues: analogy with CSAS ?

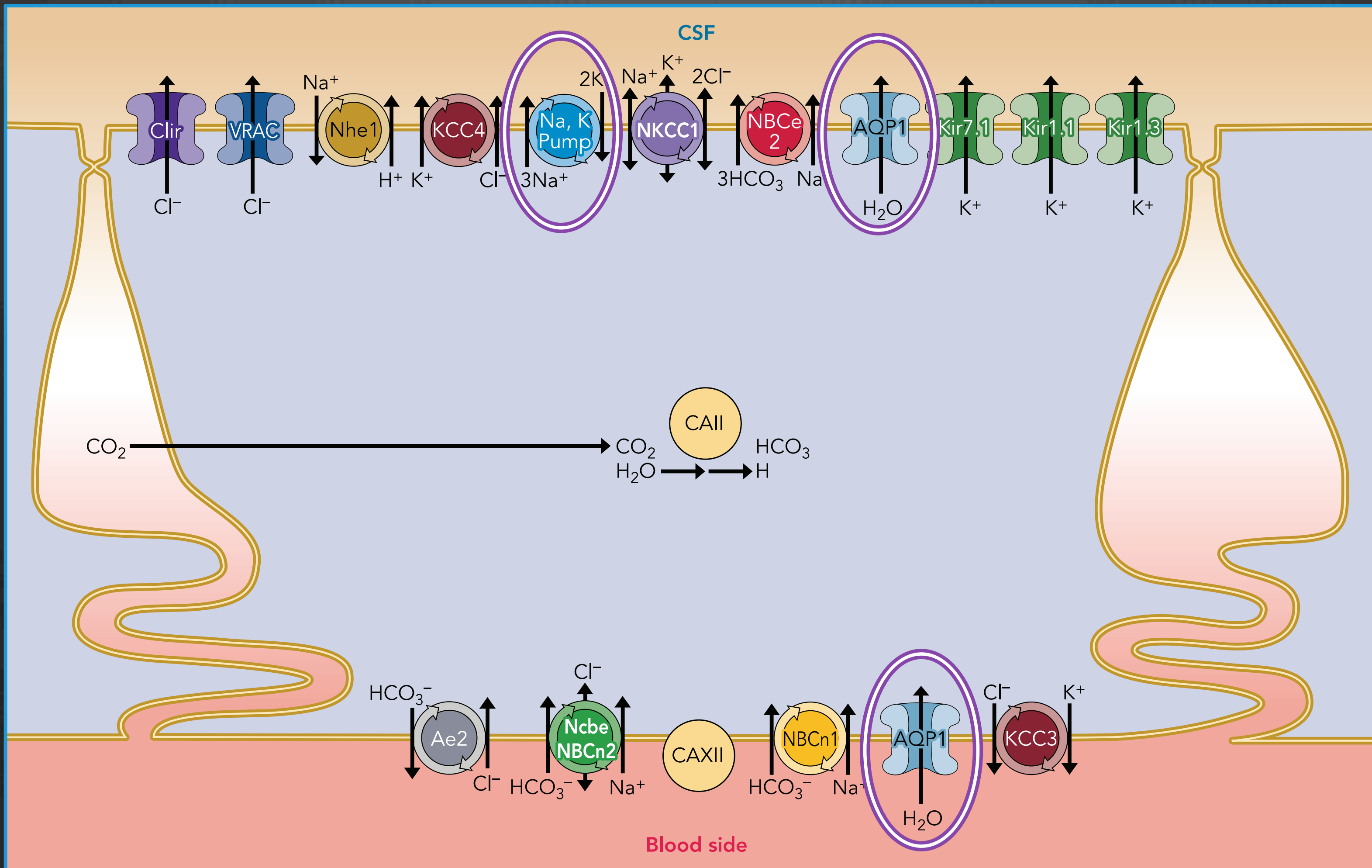




A biological membrane that lines a preformed cavity-space and regulates the turnover of the pleural fluid.

Pleura, Pericardium, Peritoneum are MESOTHELIAL tissues ***SO WHAT ABOUT CSAS ???***

choroid plexus: analogy with CSAS ?



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

Solute-coupled water transport: *the analogy*

Fluid movement, CFD

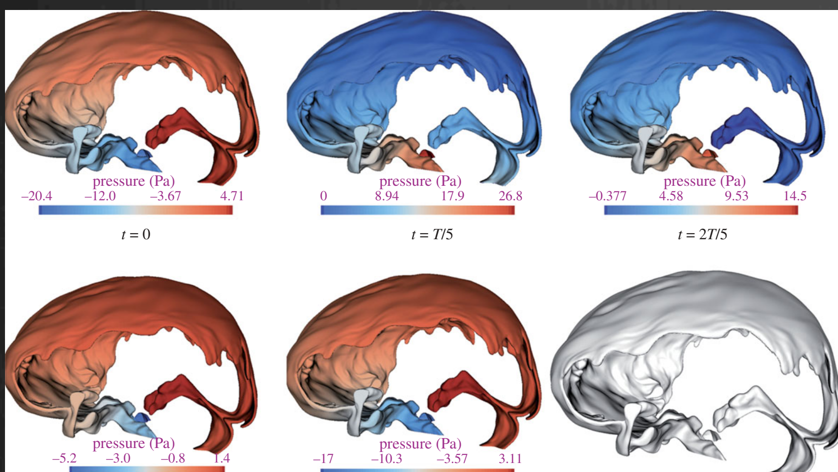
In Macro scale

Pressure gradients & pulse amplitude important

Water movement

In Cellular scale

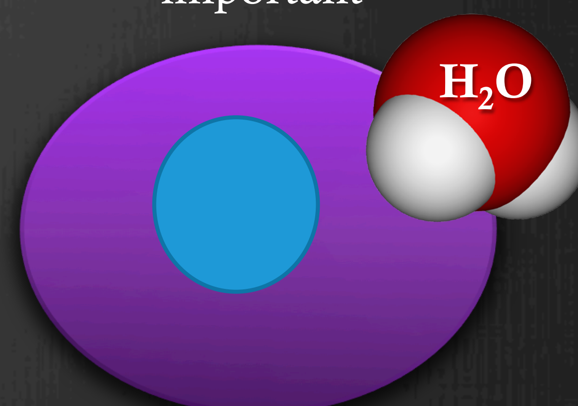
Osmotic gradient & cellular permeability important



Cerebrospinal fluid dynamics in the human cranial subarachnoid space: an overlooked mediator of cerebral disease. I. Computational model

Gupta et al.

J. R. Soc. Interface (2010) 7, 1195–1204

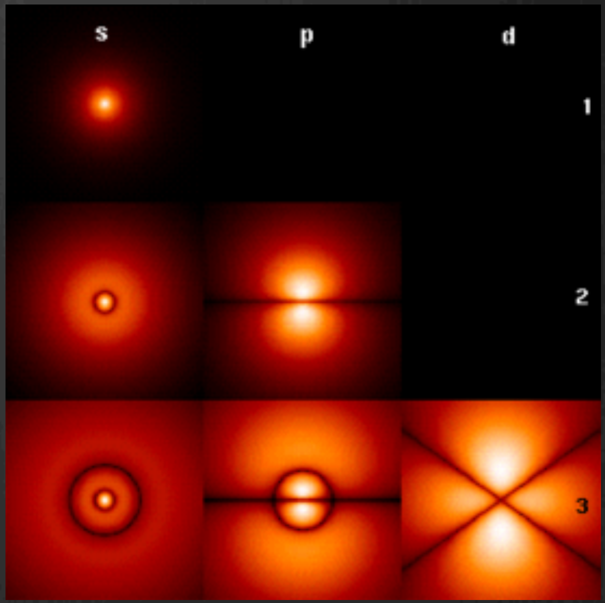


"Ion & Water Mechanics"

Solute-coupled water transport: *analogy*

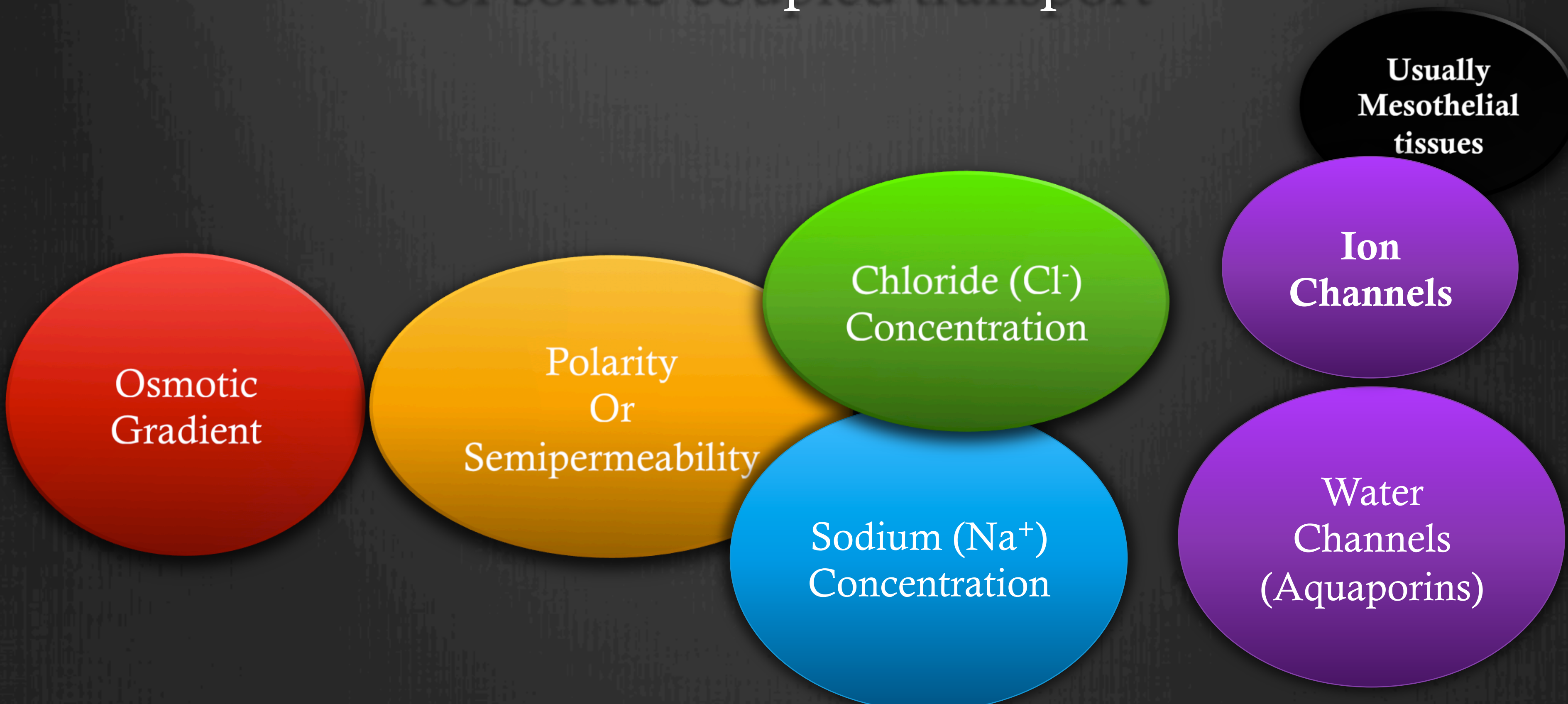
Newtonian Physics
Macro scale

Quantum physics
Subatomic scale



Thinking beyond structures → “Ion & Water mechanics”

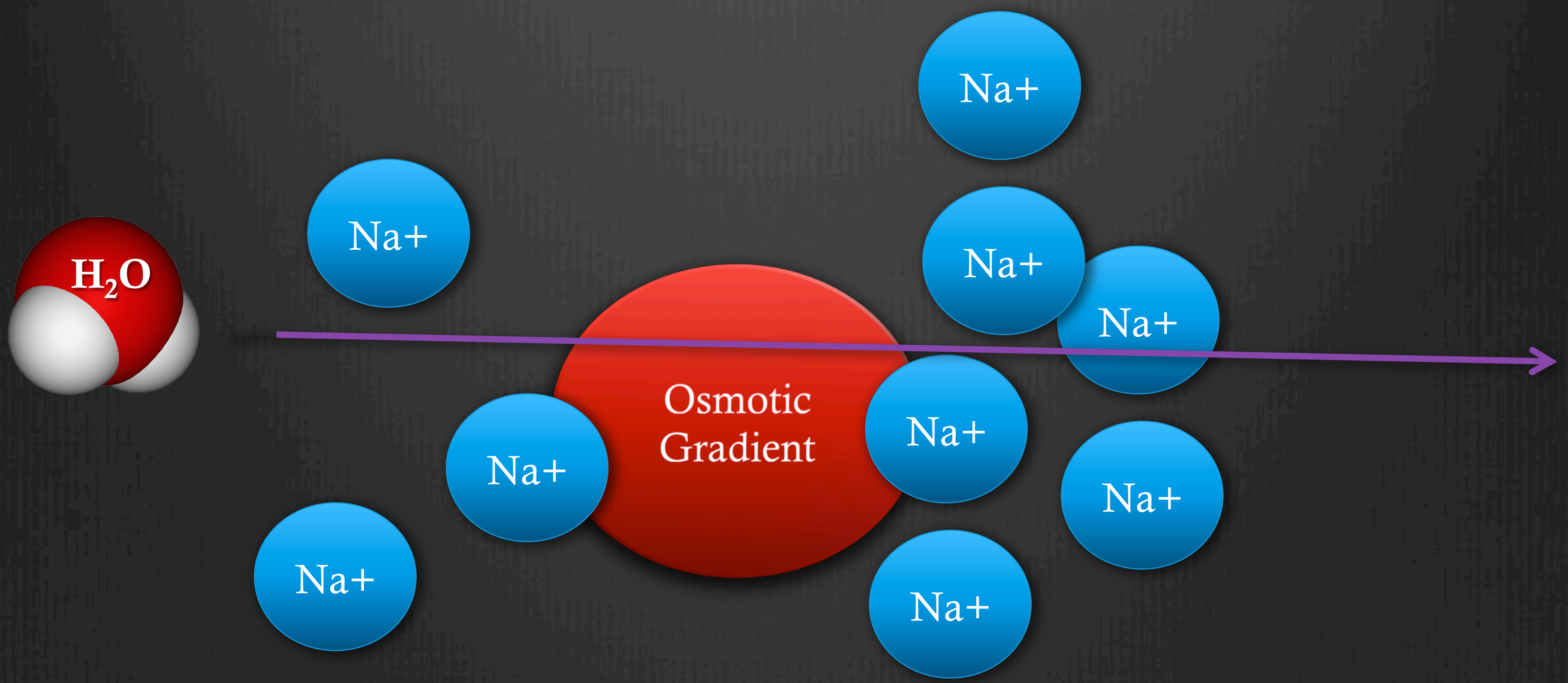
The BASIC PRINCIPLES for solute-coupled transport



Thinking beyond structures → "Ion & Water mechanics"

Water FOLLOWS Sodium in polarized epithelia

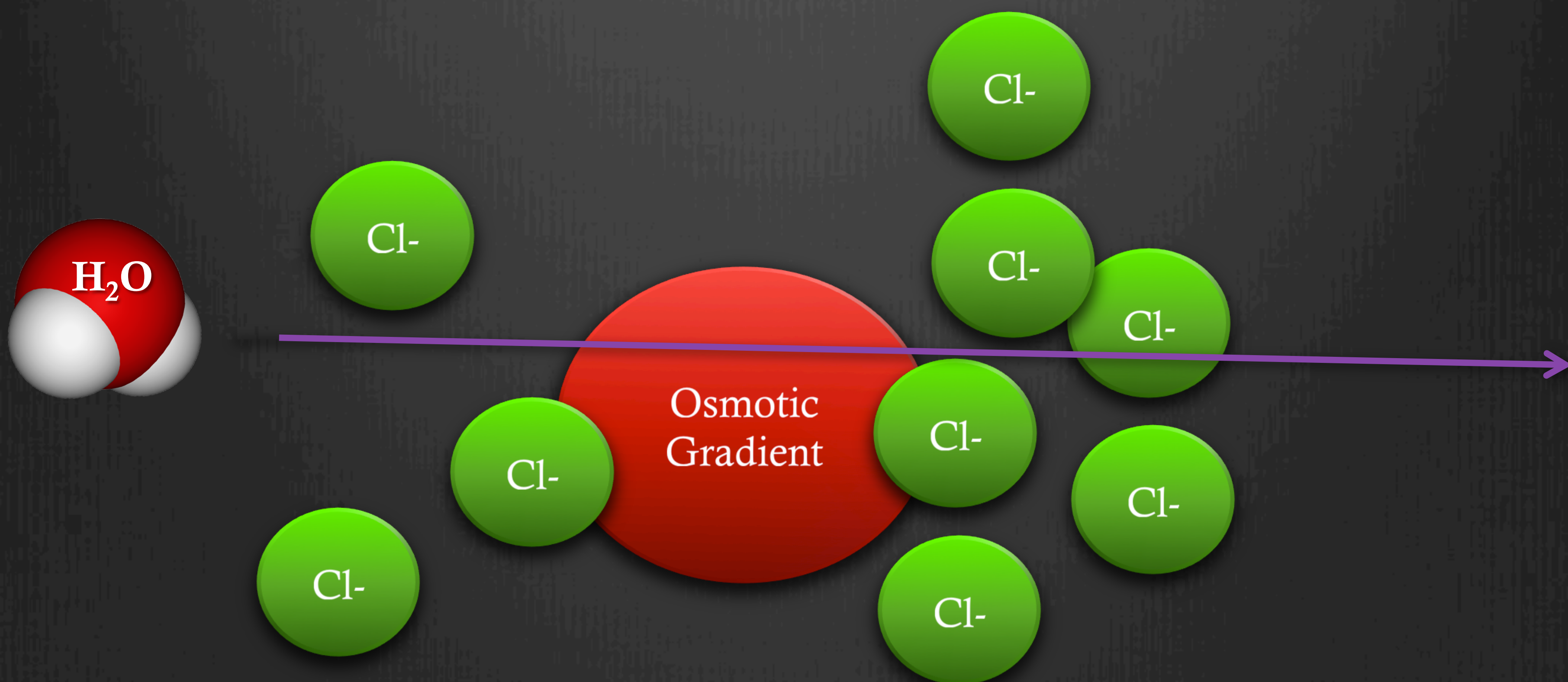
(e.g. choroid plexus, pleura, pericardium, omentum, nephron)



Thinking beyond structures → "Ion & Water mechanics"

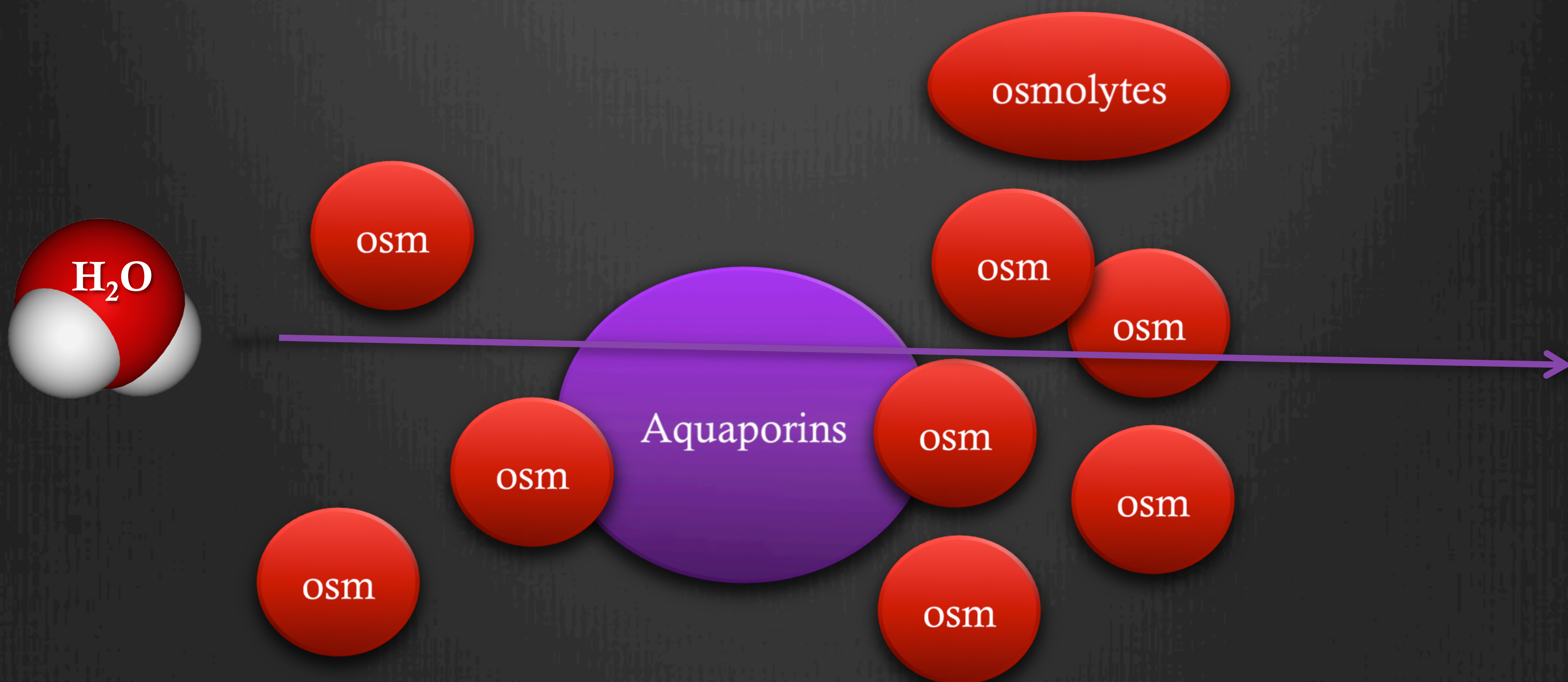
Water FOLLOWS Chloride in polarized epithelia

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



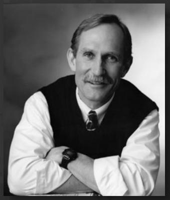
Thinking beyond structures → "Ion & Water mechanics"

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

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

AQP4 LOCALIZATION

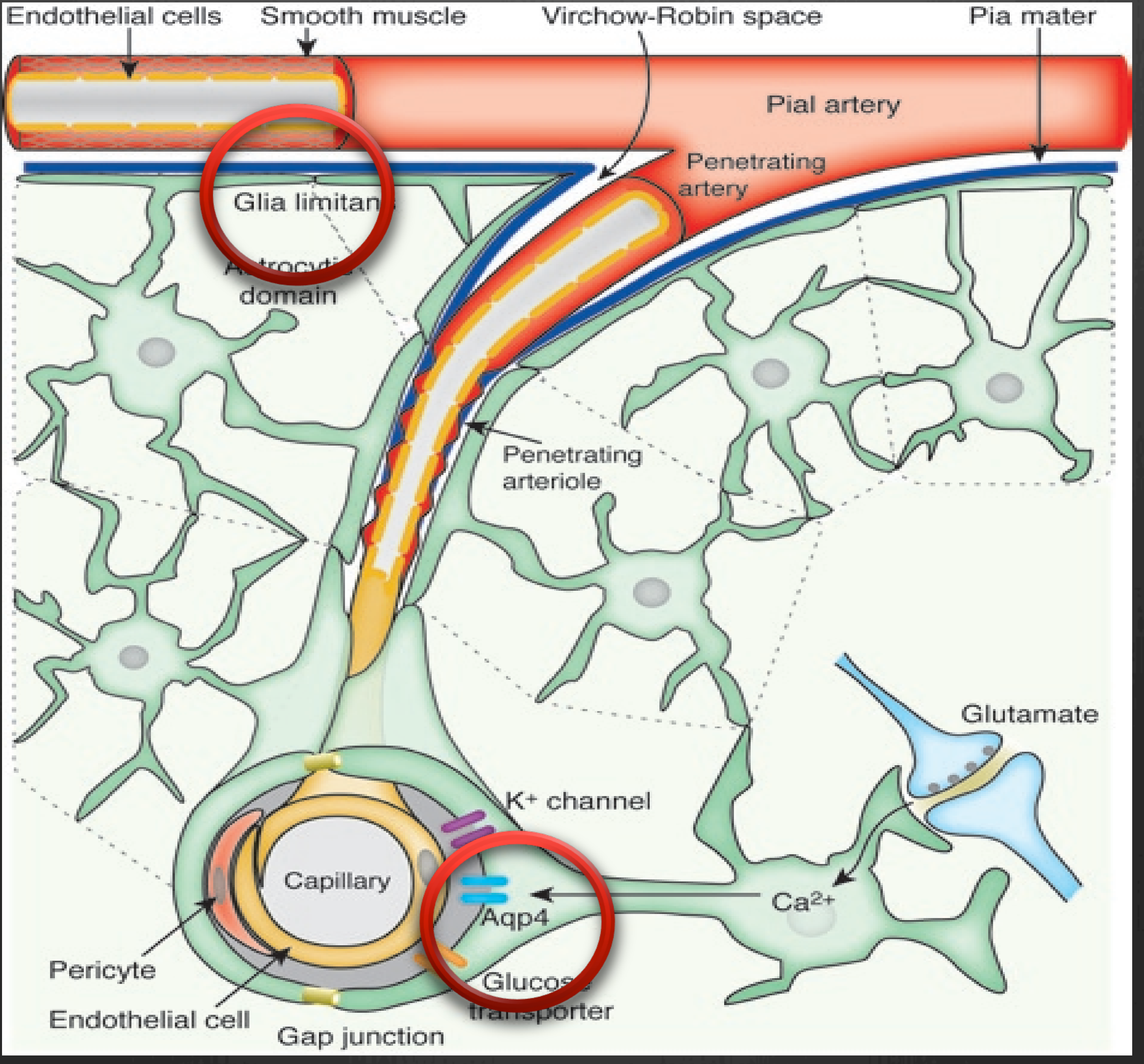
- **Glia Limitans**
- **Astrocyte foot processes around capillaries that form the Blood-Brain-Barrier (BBB)**
- Ependymal cells
- Supraoptic and suprachiasmatic nuclei of hypothalamus
- Cerebellum
- Hippocampal dentate gyrus,
- Hippocampal areas CA1-CA2
- Neocortex
- Nucleus of stria terminalis
- Medial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

THEY NEED !!! an Osmotic Gradient

They are important for Hydrocephalus

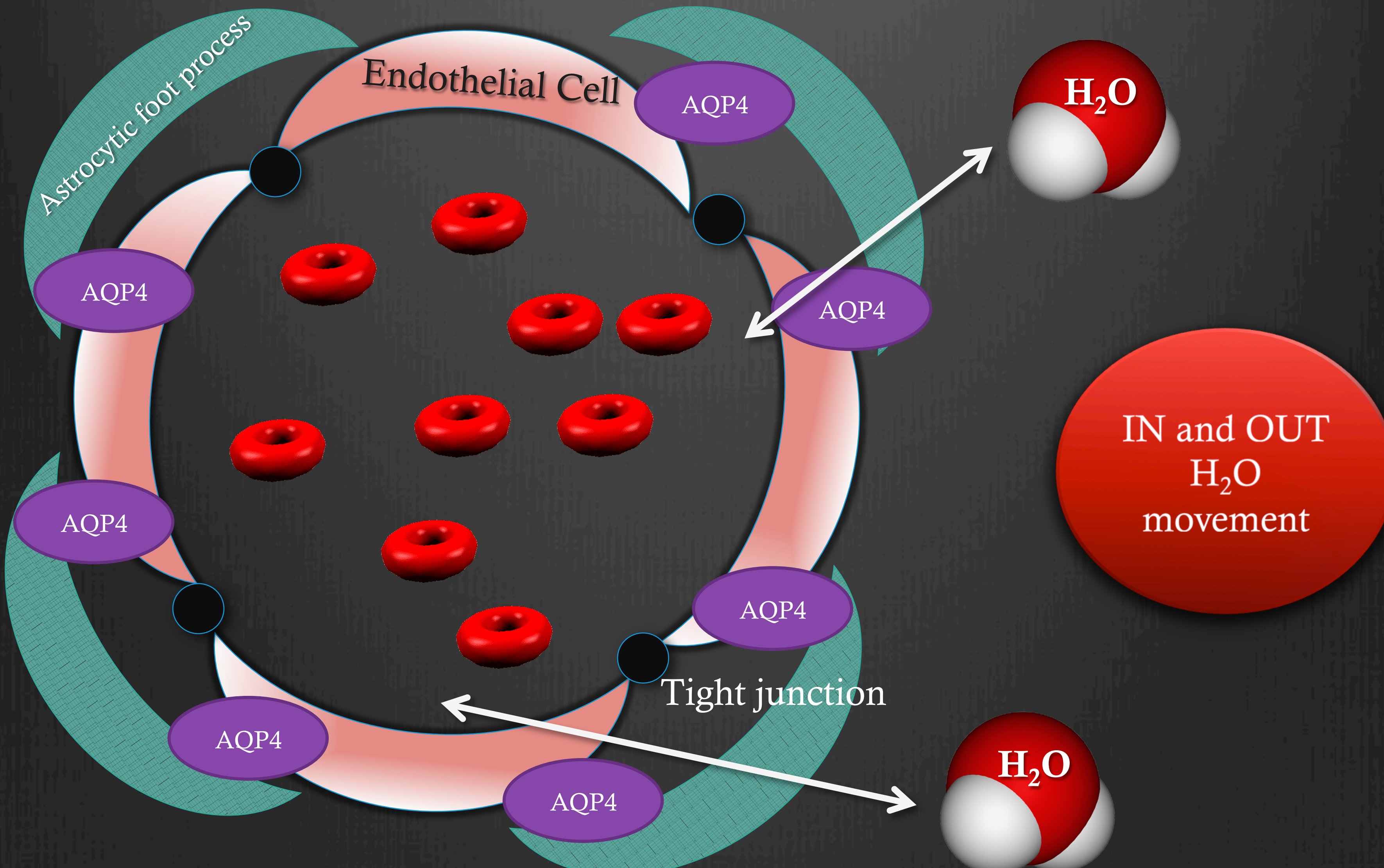
Badaut et al.
Aquaporins in brain: distribution, physiology, and pathophysiology.
J Cereb Blood Flow Metab (2002) vol. 22 (4) pp. 367-78

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



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

AQP4 and the Blood-Brain-Barrier and Cerebral vessels



Could this be also the case for CSAS ?

Is Solute-coupled transport of water present ?

Skimming for Evidence…

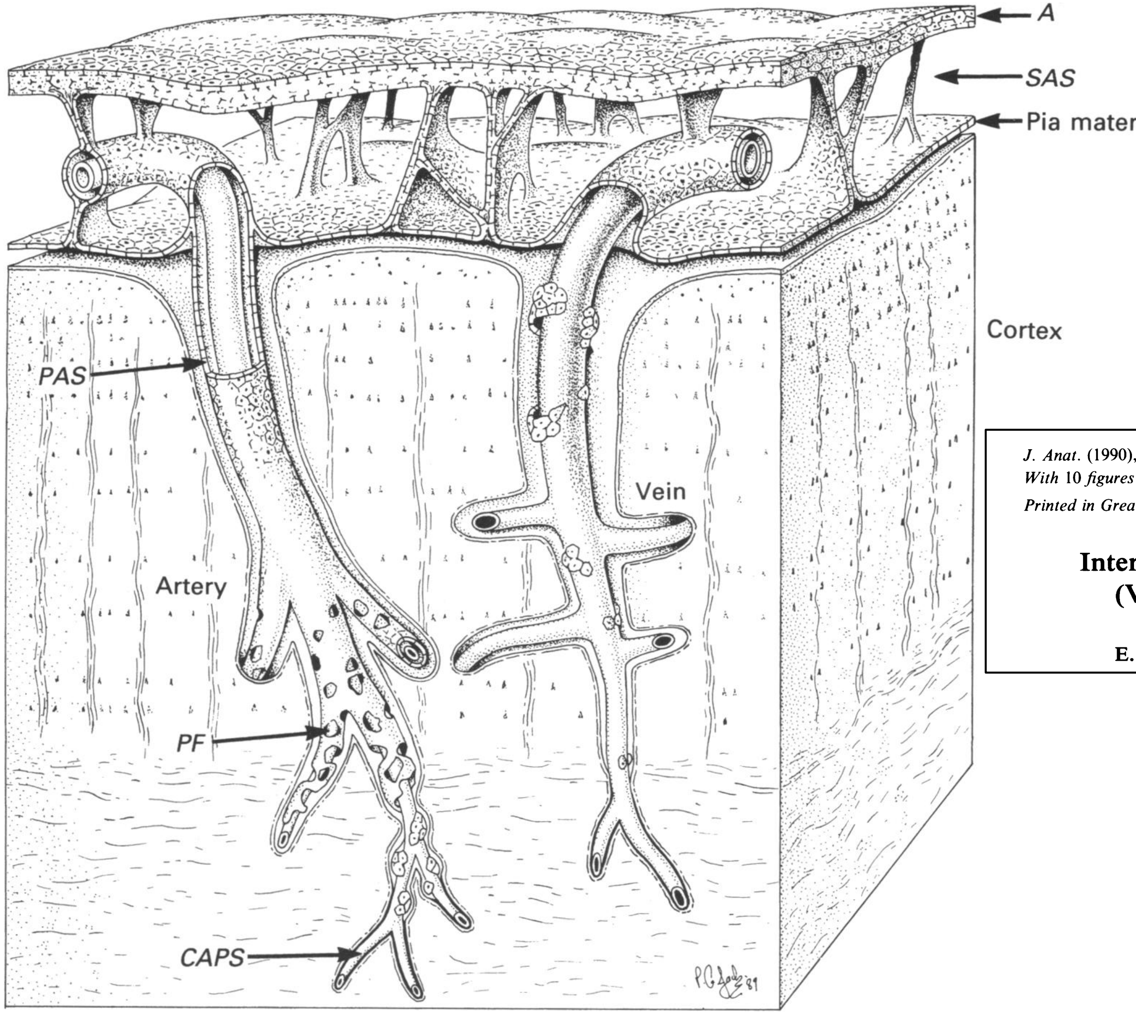
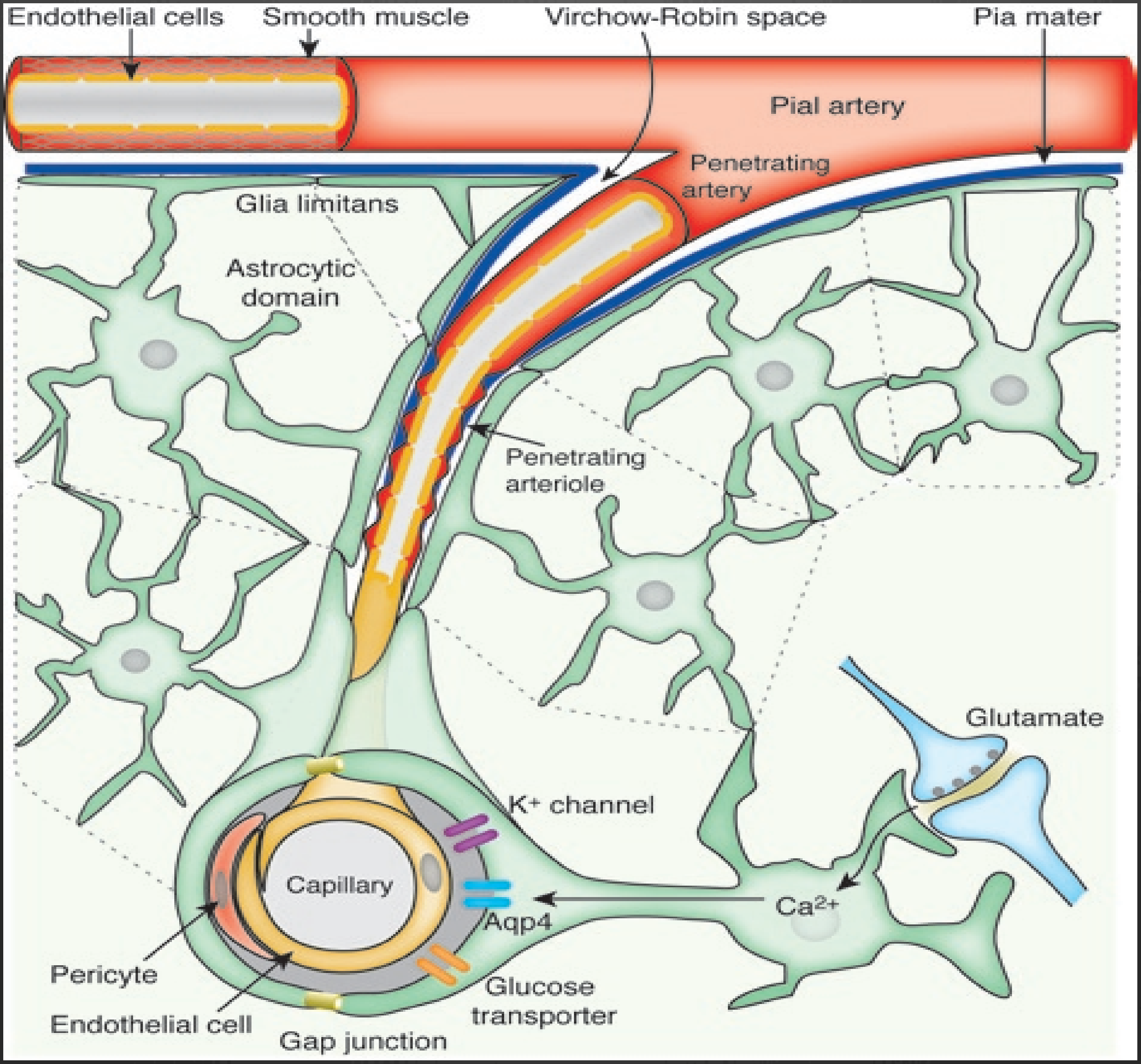


Fig. 10. Diagram demonstrating the relationships of the pia mater and intracerebral blood vessels. Subarachnoid space (*SAS*) separates the arachnoid (*A*) from the pia mater overlying the cerebral cortex. An artery on the left of the picture is coated by a sheath of cells derived from the pia mater;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

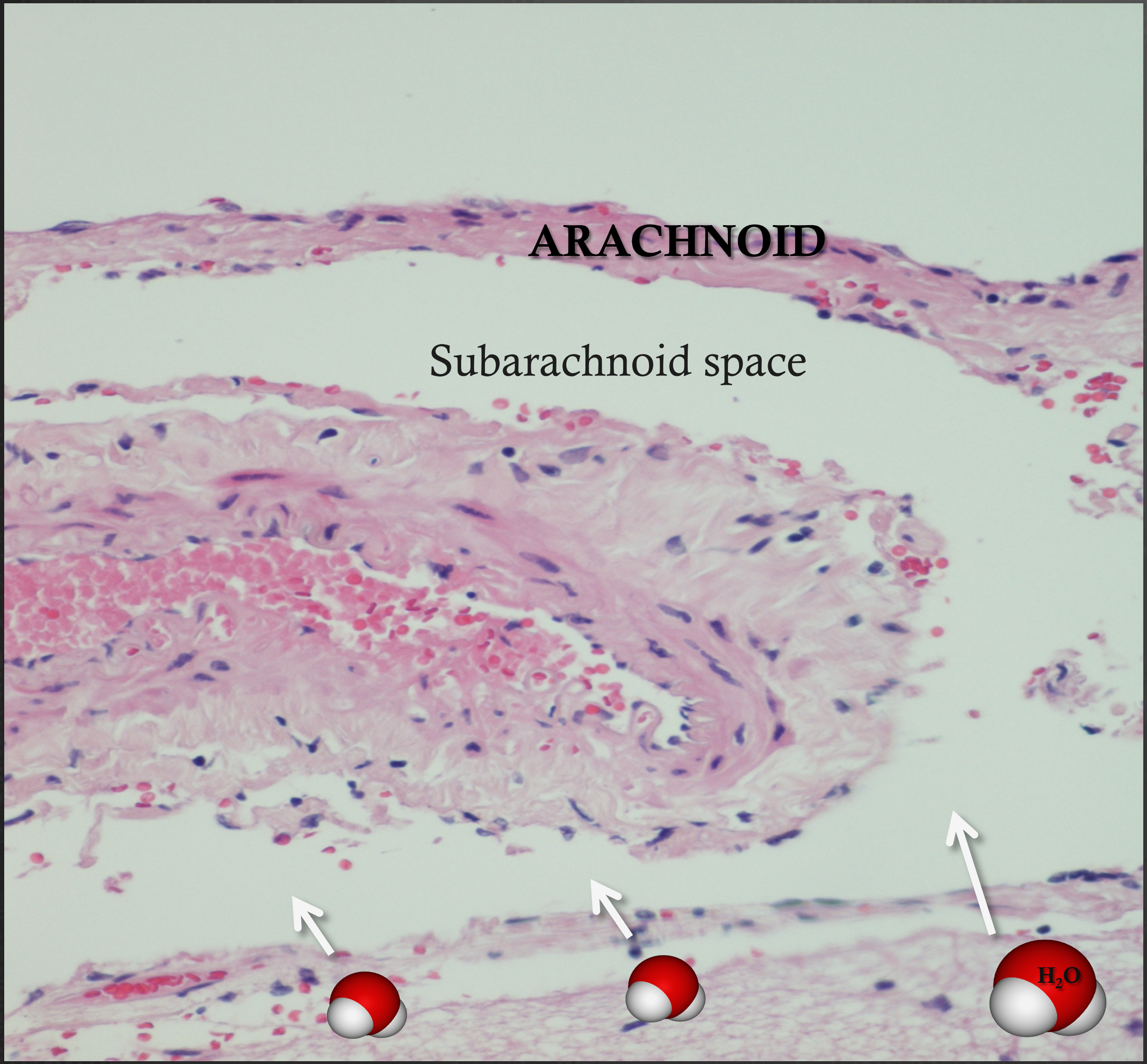
E. T. ZHANG,†‡ C. B. E. INMAN† AND R. O. WELLER†



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

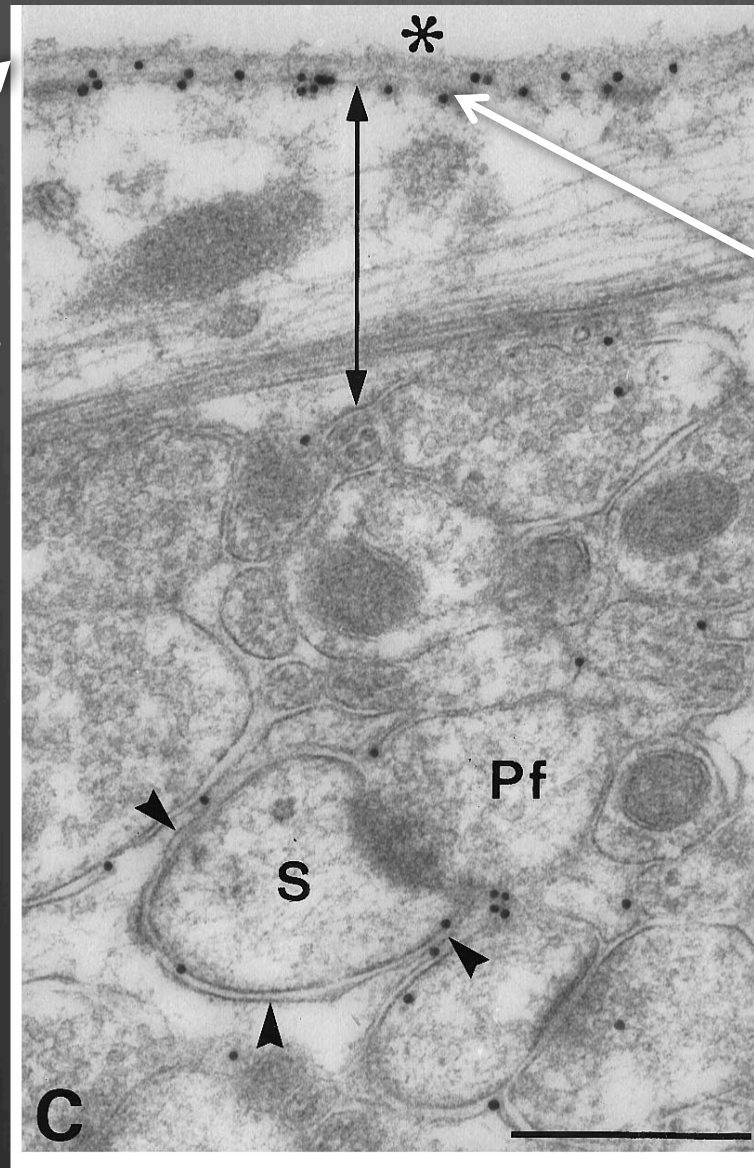
Cerebrospinal Fluid Production by the Choroid Plexus and Brain

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

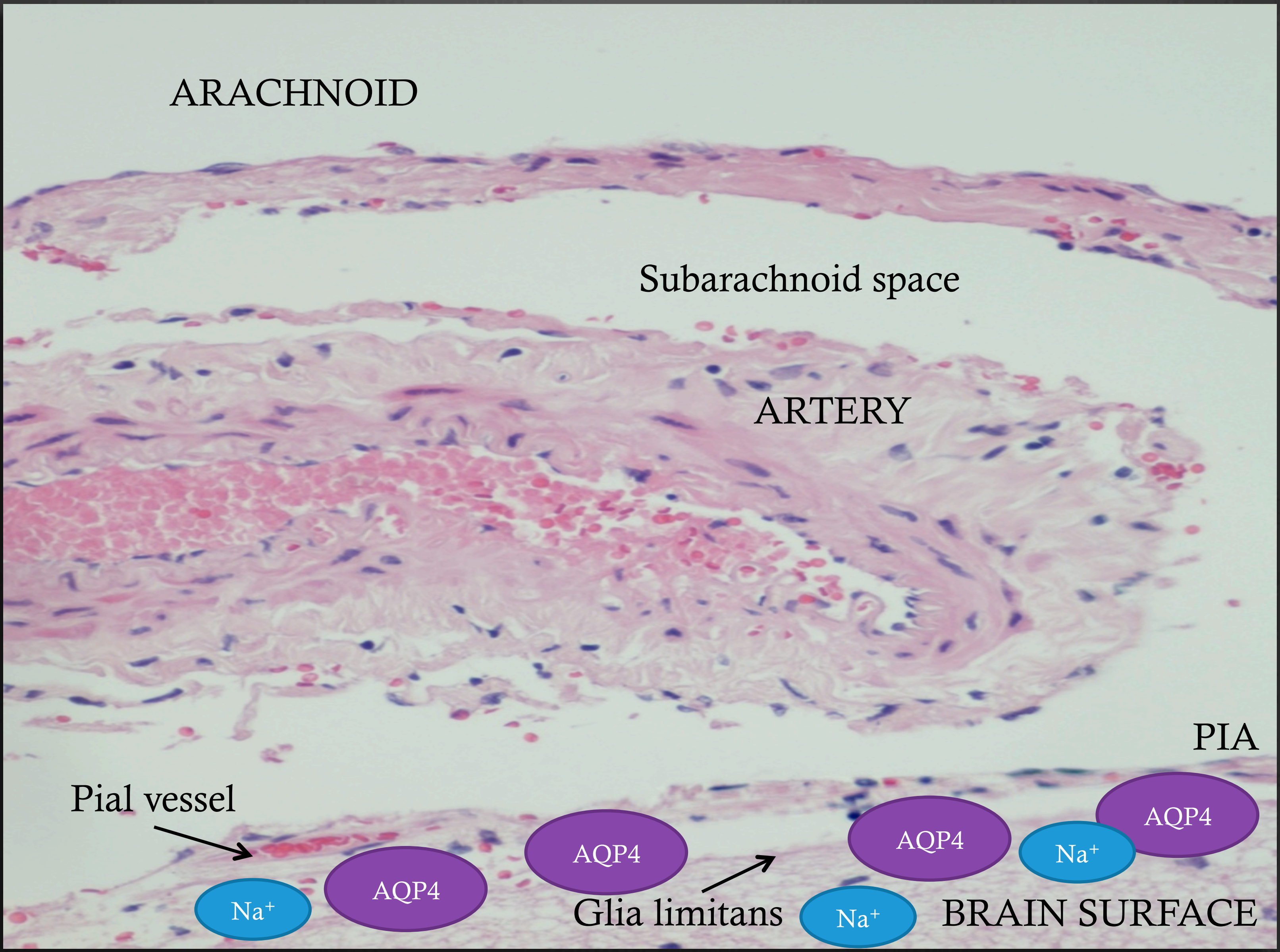
PIA mater - Glia Limitans
lower CSAS interface



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 !



What is missing ?

Water & Ion channels !

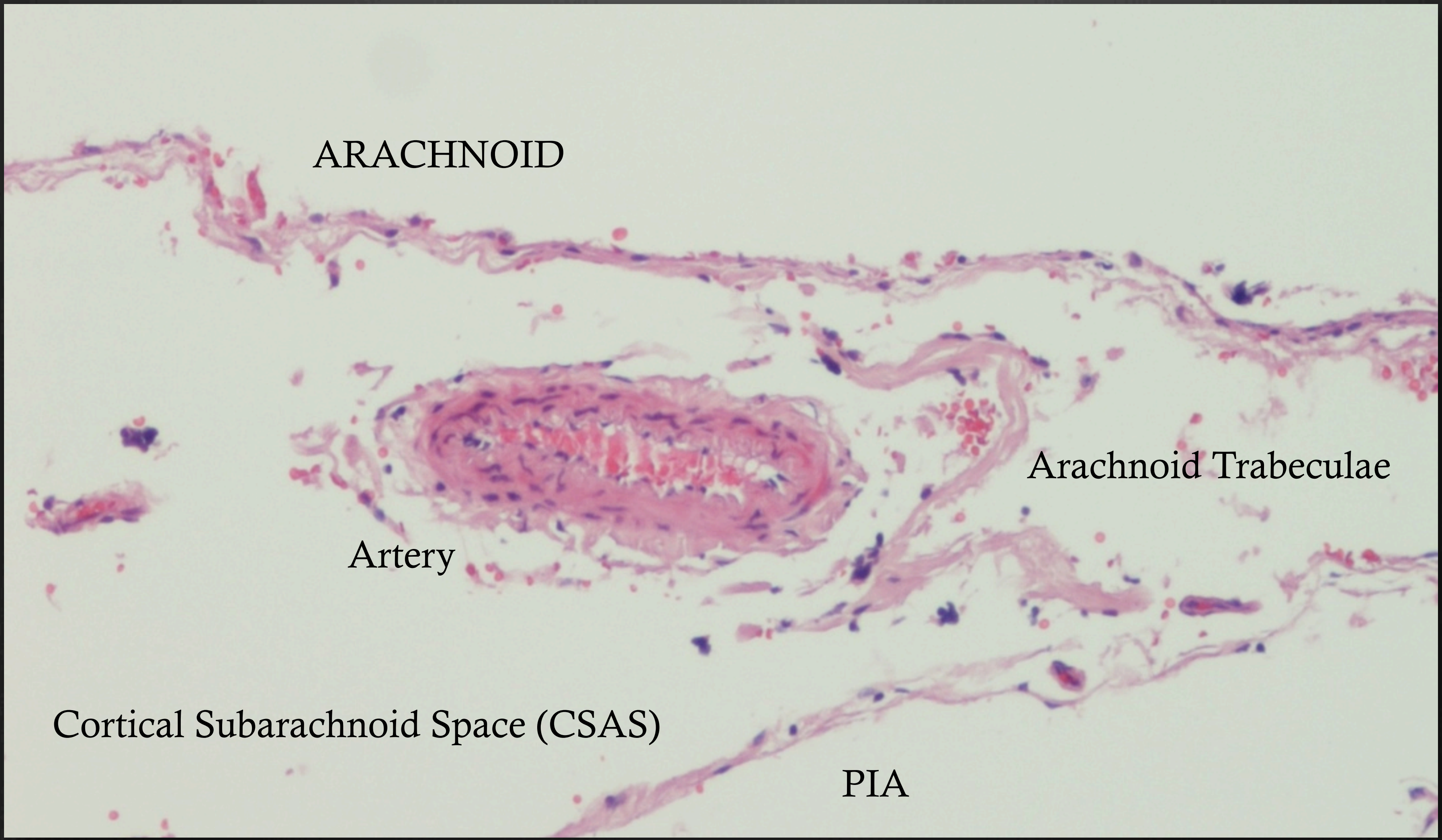
Solute-coupled transport

Experimental data

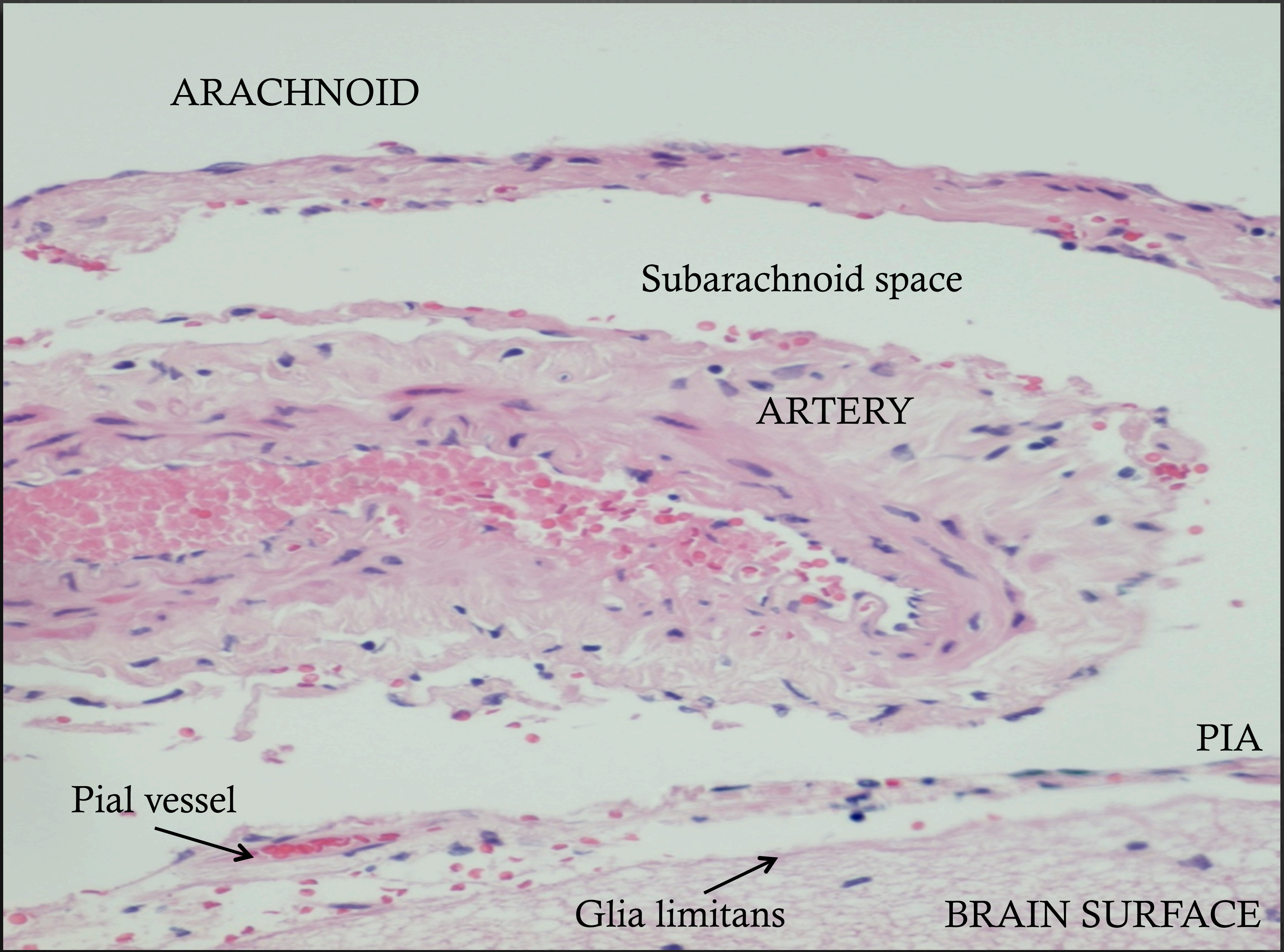
to identify key players of
solute-coupled transport at the CSAS

PIAL SURFACE

ARACHNOID SURFACE

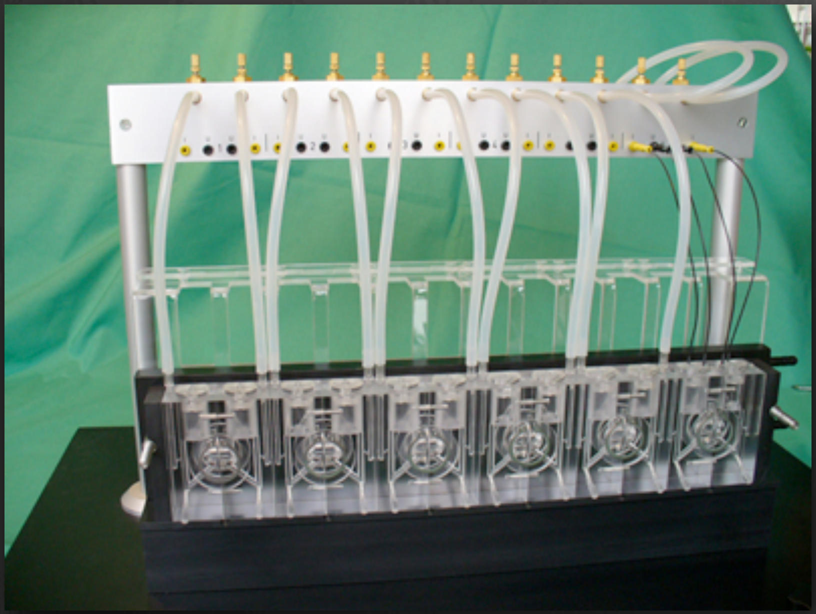


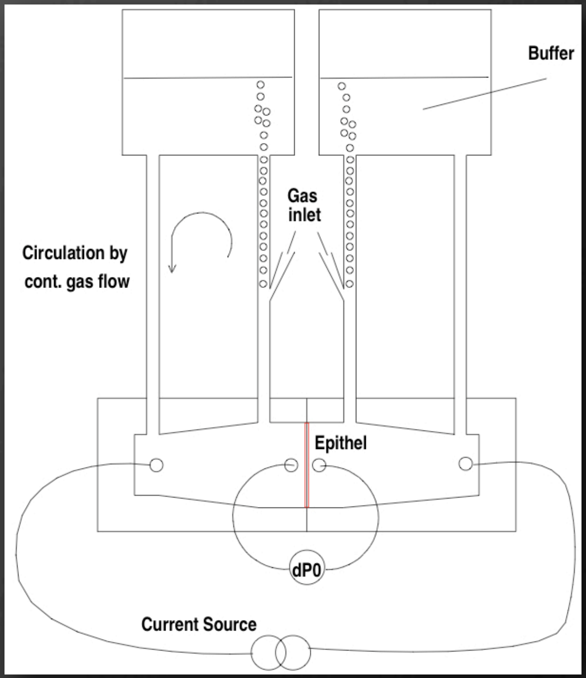
Filippidis et al.
Transmembrane resistance and histology of isolated sheep leptomeninges
Neurological Research (2010) vol. 32 (2) pp. 205



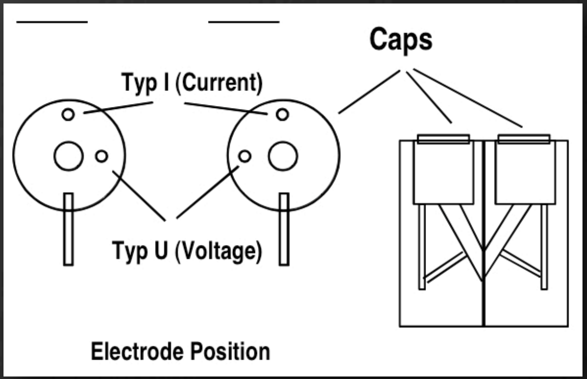
Filippidis et al.
Transmembrane resistance and histology of isolated sheep leptomeninges
Neurological Research (2010) vol. 32 (2) pp. 205

Membrane Electrophysiology
"Hans Ussing" chambers



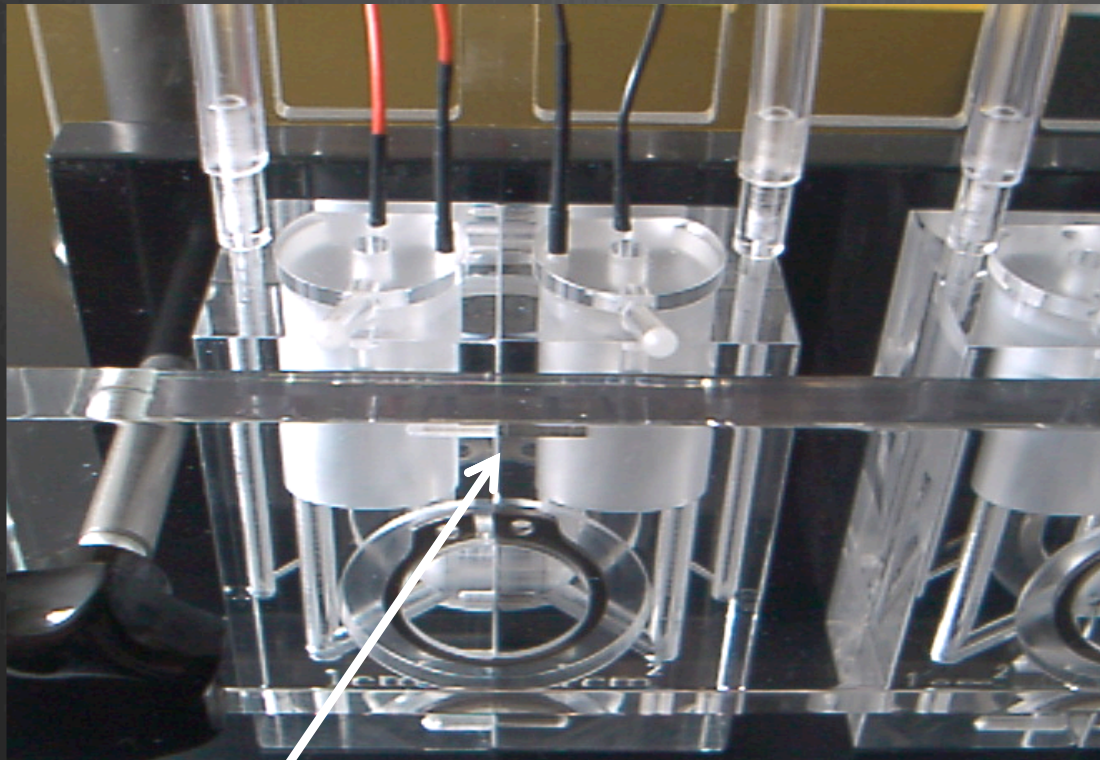


ESTABLISHED METHOD
FOR SOLUTE-COUPLED TRANSPORT STUDIES



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



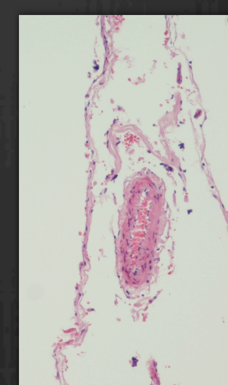
We get the:

Transmembrane Resistance

$\boldsymbol{R}$ **(Ω*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.

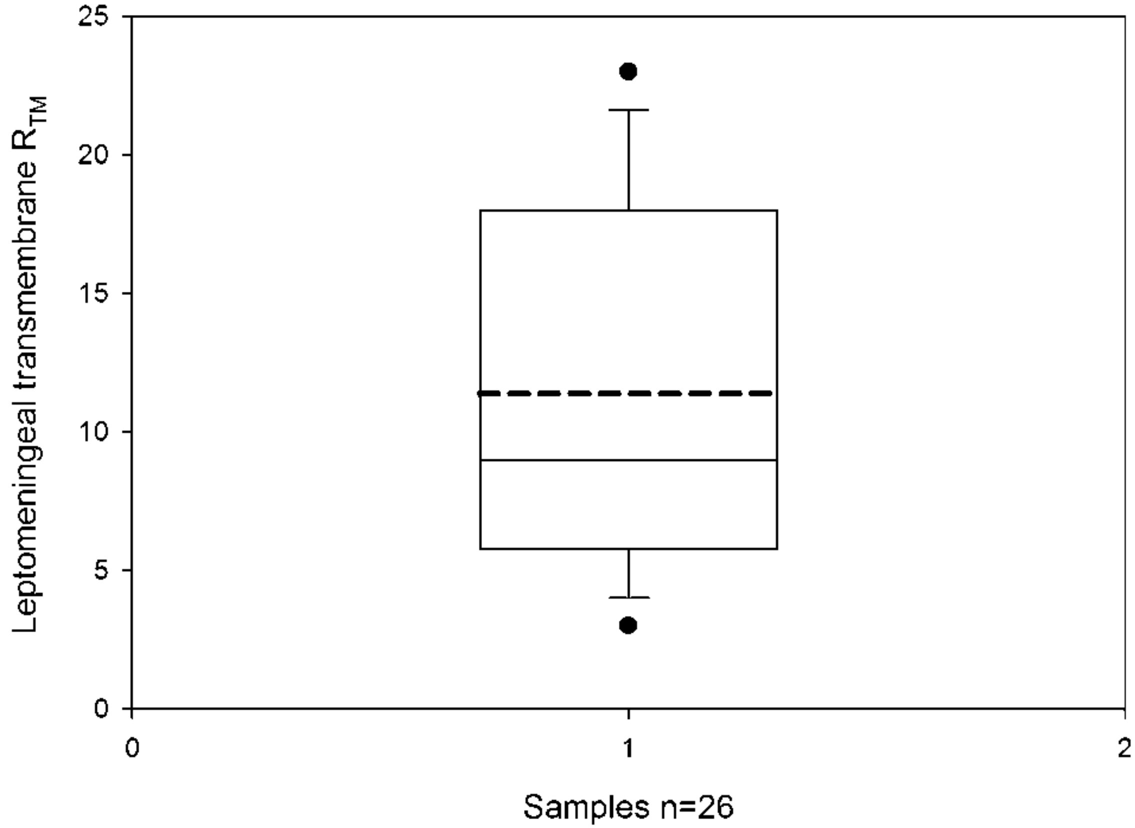


Figure 1 Boxplot diagram describing the distribution of measured values of leptomeningeal transmembrane resistance in sheep along with mean value and outliers. Dotted line in the box represents the mean value of 11·38 Ω cm^2 obtained from 26 experiments

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

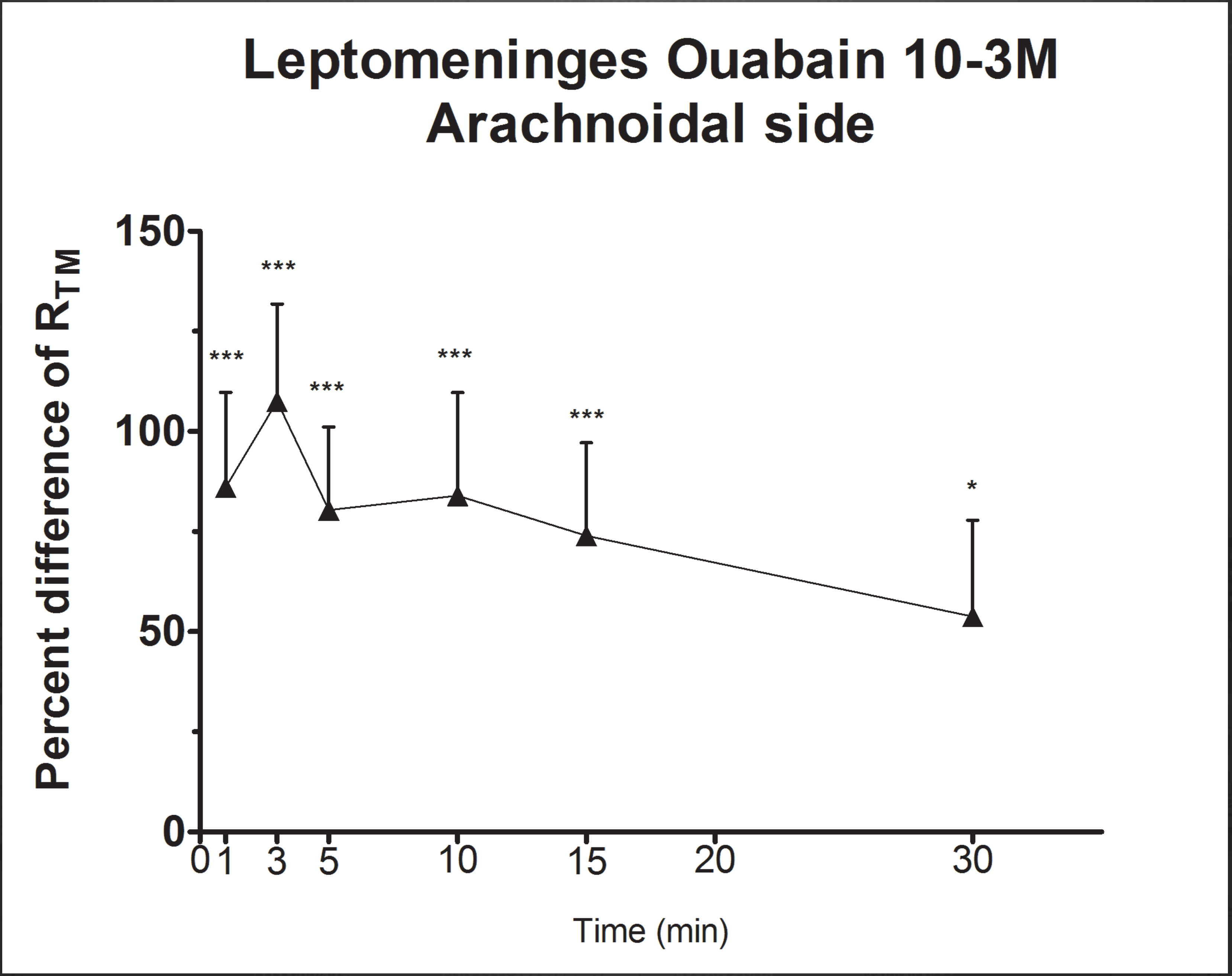
Permeability of the arachnoid and pia mater. The role of ion channels in the leptomeningeal physiology

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

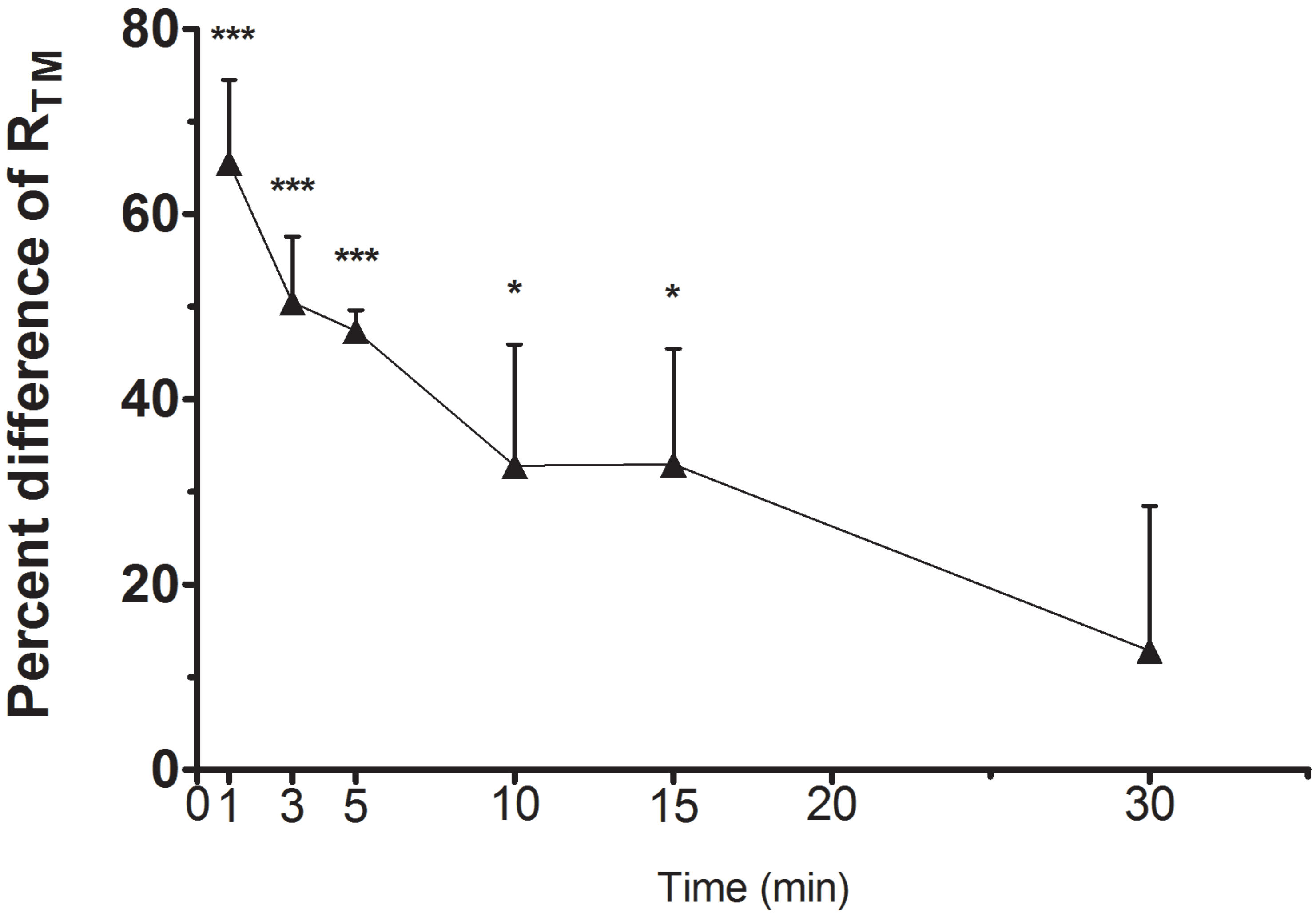
Sodium-Potassium-ATPase

Main source of extracellular Sodium

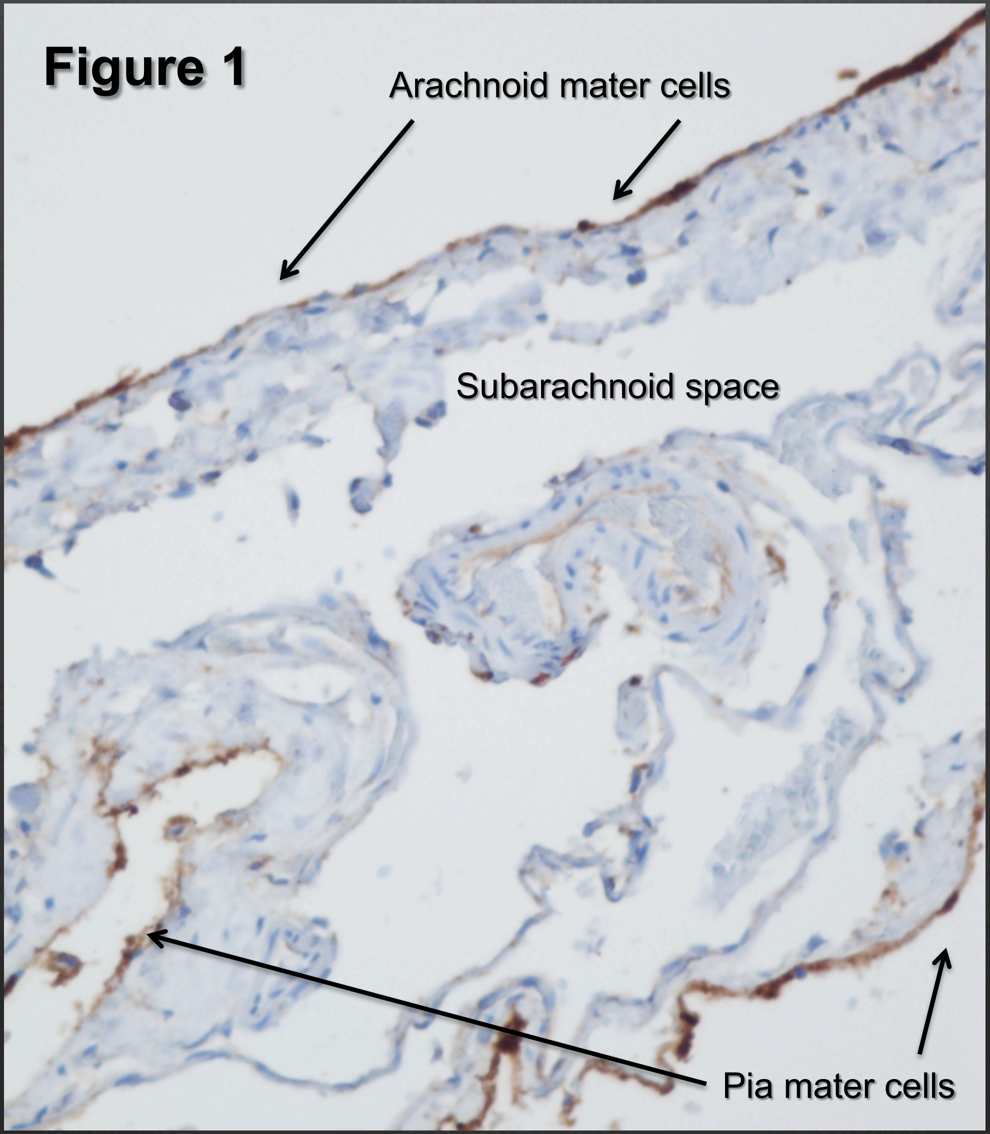
We tested inhibition with OUABAIN


Leptomeninges Ouabain 10-3M
Arachnoidal side
Percent difference of R_{TM}
150
100
50
0
***
***
***
***
***
*
0
1
3
5
10
15
20
30
Time (min)

Leptomeninges Ouabain 10^{-3}M
Pial side



a1 subunit
Sodium-Potassium-ATPase

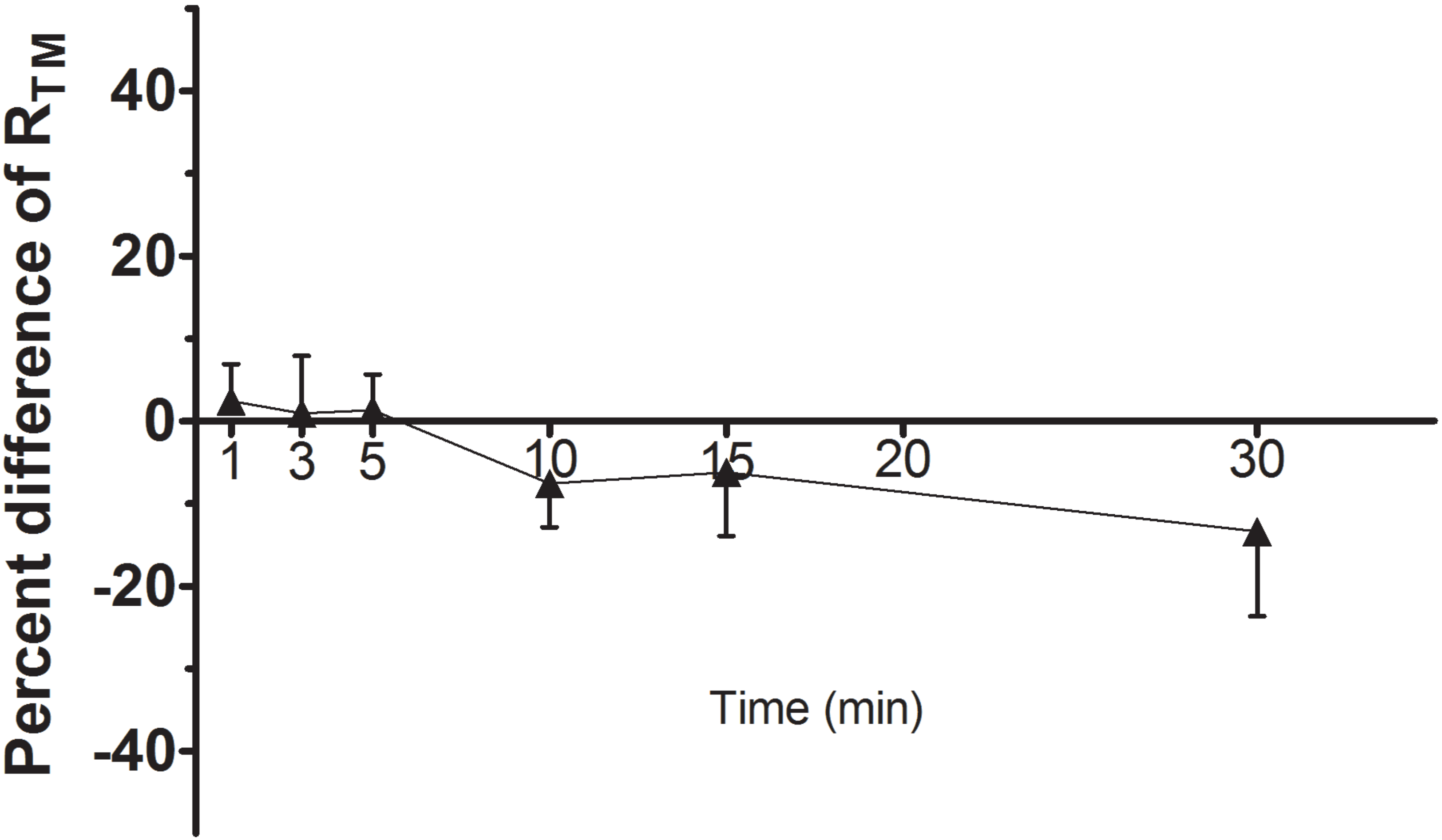


ENaC

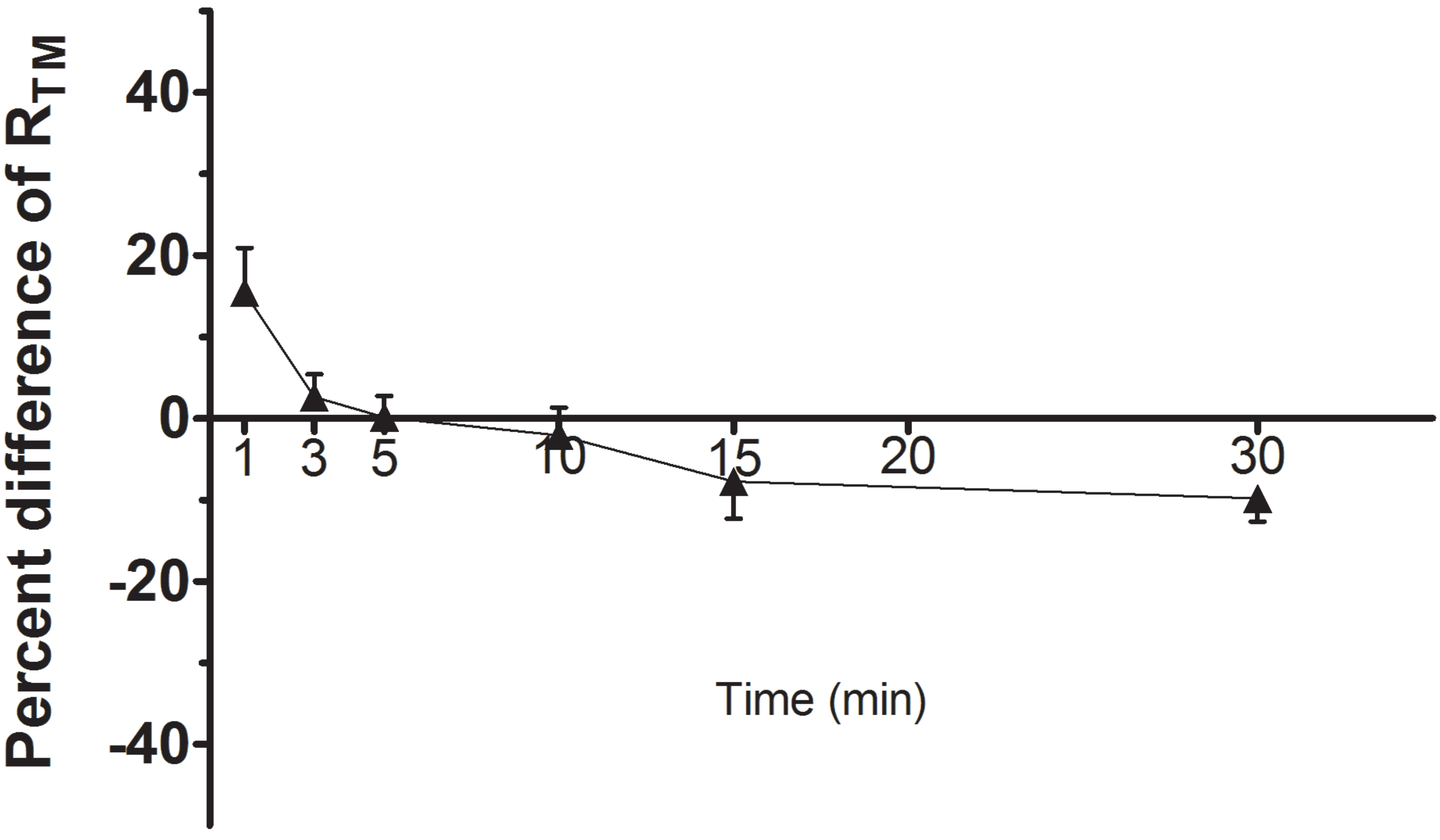
Epithelial Sodium Channel

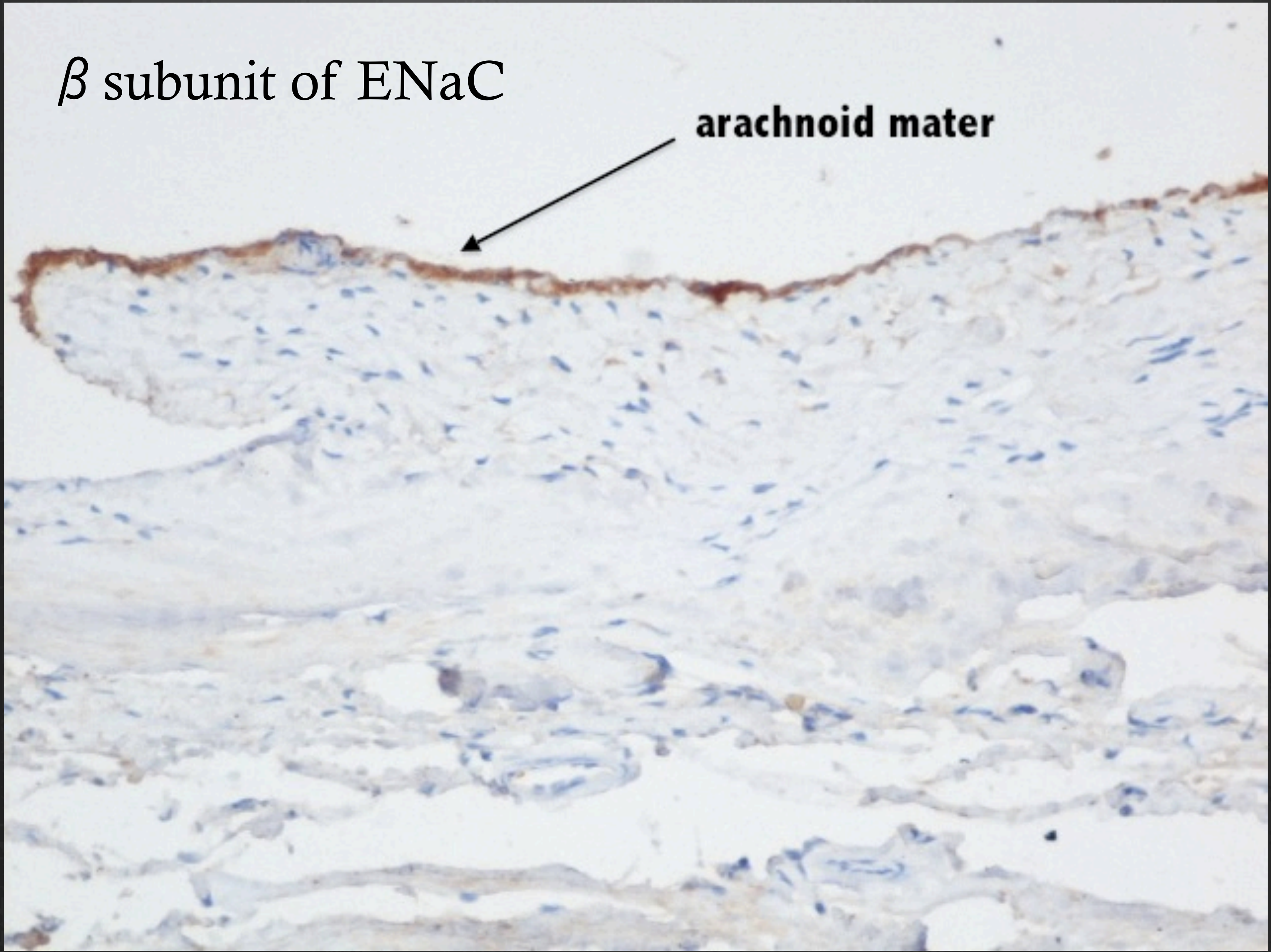
We tested inhibition with AMILORIDE

Leptomeninges Amiloride 10^{-5}M
Arachnoidal side

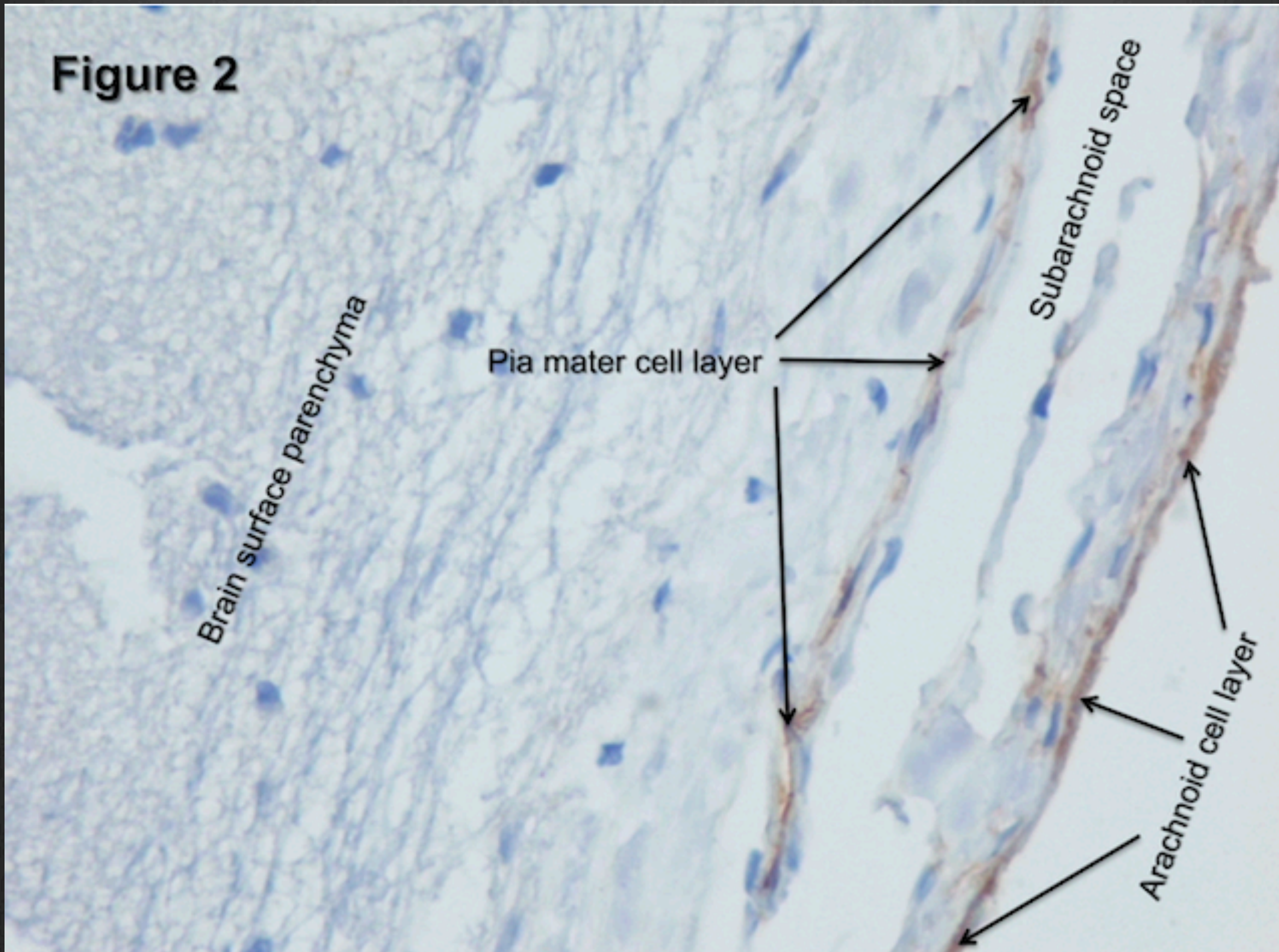


Leptomeninges Amiloride 10^{-5}M
Pial side




β subunit of ENaC
arachnoid mater

δ subunit - ENaC



Can ionic disturbances affect the CSF and ISF turnover ?

Pseudotumor Cerebri Secondary to Lithium Carbonate

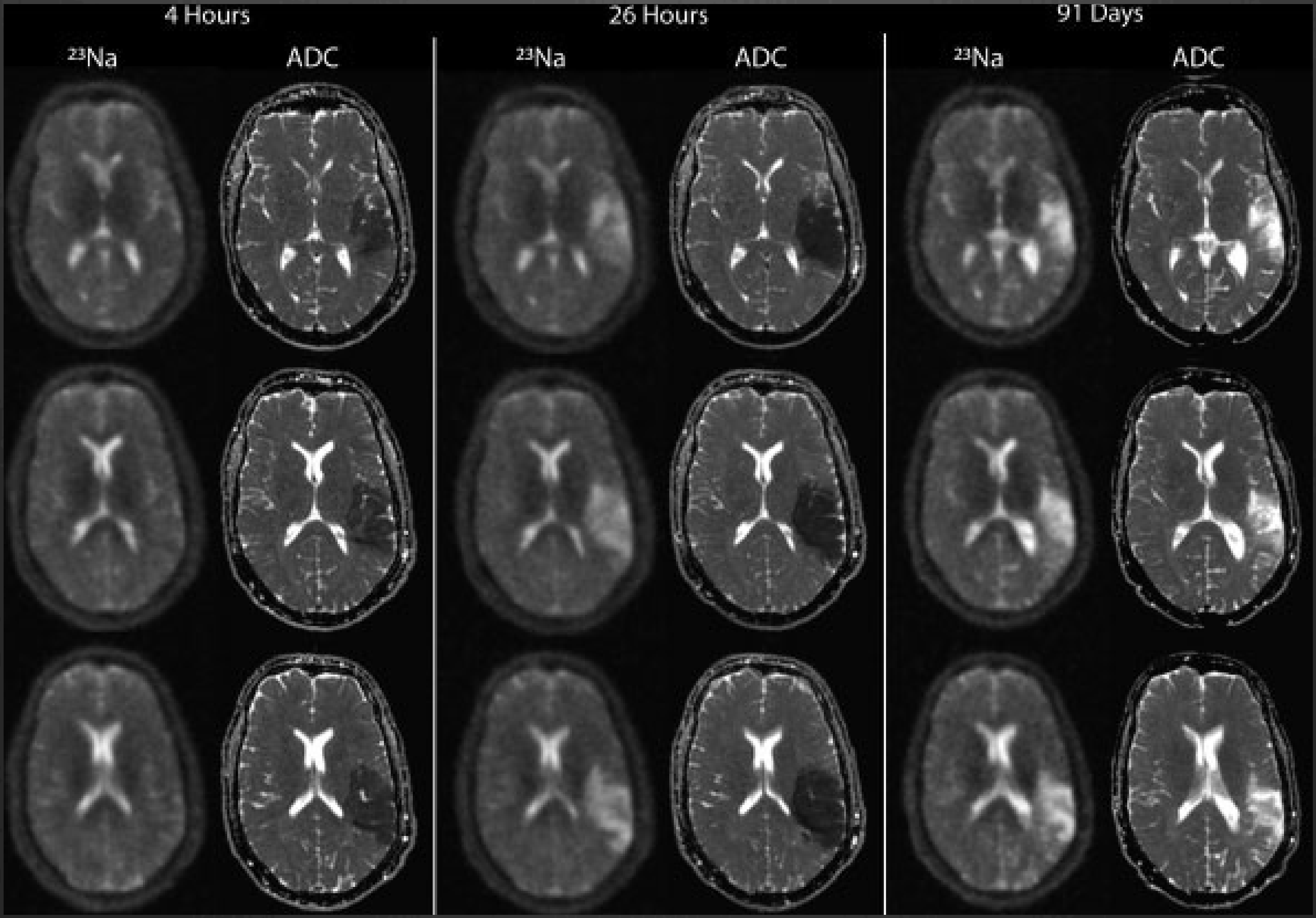
Robert F. Saul, MD; Harry A. Hamburger, MD; John B. Selhorst, MD

• **Three patients were initially seen with headache, blurred vision, and papilledema while taking lithium carbonate for their respective bipolar affective disorder. A diagnosis of pseudotumor cerebri was made in each case when a thorough evaluation revealed only elevated intracranial pressure. Two of the patients had complete resolution of their symptoms and papilledema after discontinuing use of the drug. Increased intracranial pressure with papilledema persisted in the third patient when she failed to adjust psychiatrically, necessitating continuance of the lithium carbonate therapy. A history of lithium carbonate ingestion should be sought in patients with the syndrome of pseudotumor cerebri. All patients receiving this drug should have a regular funduscopic examination.**
(*JAMA* 1985;253:2869-2870)

The mechanism by which lithium carbonate causes increased intracranial pressure is not known. No theories were given in the two previously cited reports. Lithium carbonate affects the sodium-potassium exchange pump, and has been shown to replace intracellular sodium ions.[5] Intracellular sodium concentration has also been shown to be higher in depressed patients *v* normal controls.[6] Sodium-potassium adenosine triphosphatase levels are found to be lower in successfully treated manic depressive patients and higher in nonresponders.[5] Possibly a combination of higher intracellular sodium and inhibition of the sodium-potassium pump leads to intracellular edema.

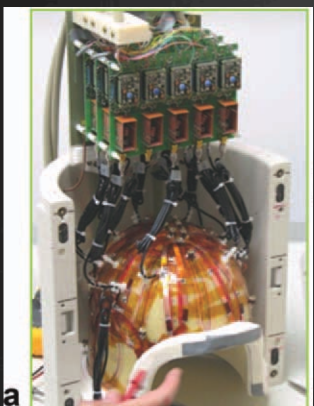
The function and structure of the cerebrospinal fluid outflow system.
Pollay M.
Cerebrospinal Fluid Res. 2010 Jun 21;7:9.

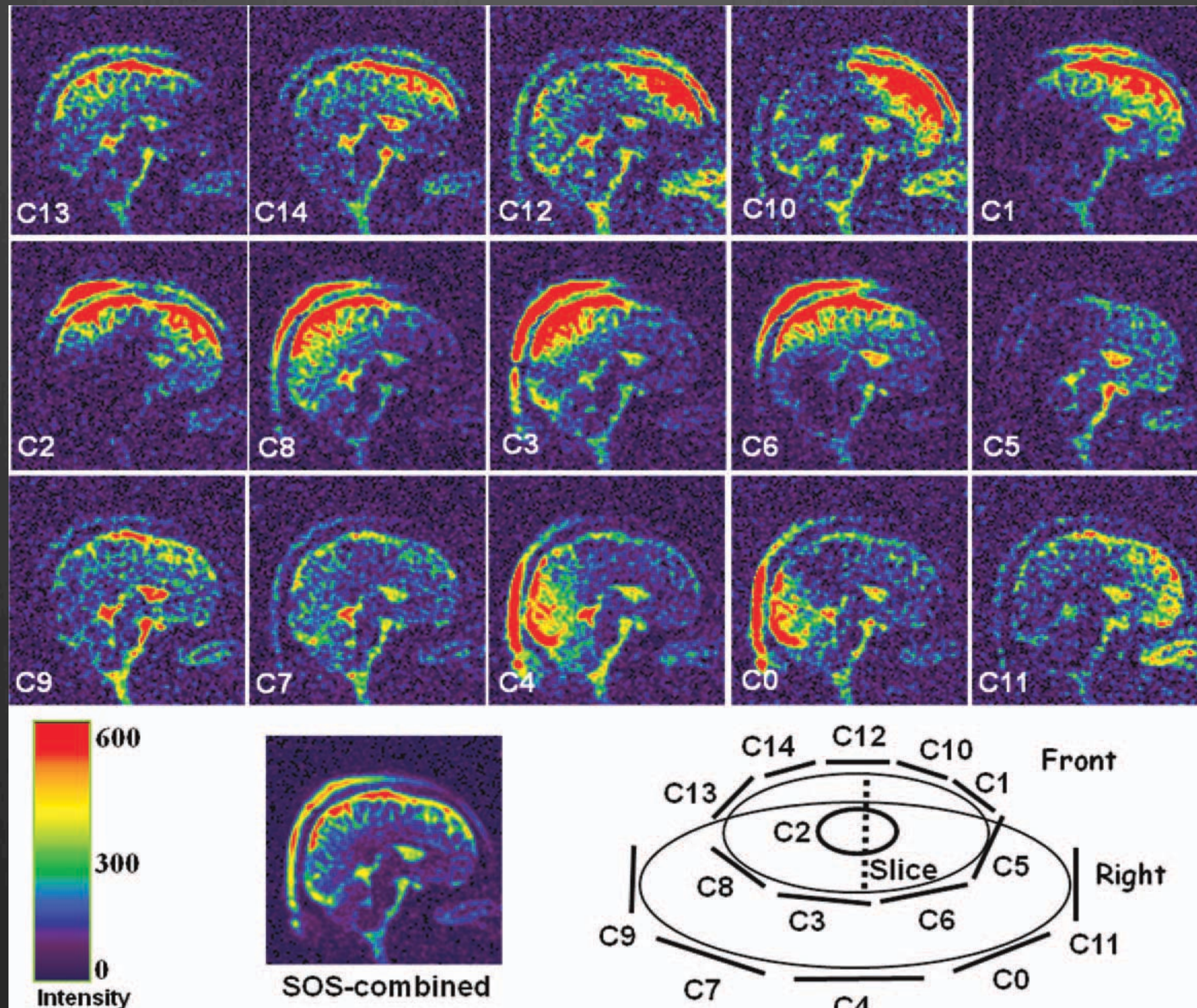
Can we see that ?



Sodium imaging intensity increases with time after human ischemic stroke.
Hussain MS, Stobbe RW, Bhagat YA, Emery D, Butcher KS, Manawadu D, Rizvi N, Maheshwari P, Scozzafava J, Shuaib A, Beaulieu C.
Ann Neurol. 2009 Jul;66(1):55-62.

MRI and ^{23}Na imaging





Magn Reson Med. 2012 Feb 29. doi: 10.1002/mrm.24192. [Epub ahead of print]
Sodium imaging of human brain at 7 T with 15-channel array coil.
Qian Y, Zhao T, Wiggins GC, Wald LL, Zheng H, Weimer J, Boada FE.

Conclusions

- CSAS absorption should be studied under a developmental, macroscale and cellular scale prism
- Currently there is no direct MRI imaging modality for CSAS outflow systems
- CSAS has a vibrant functional anatomy which becomes intriguing at the cellular level
- CSAS bears properties of mesothelial tissues
- CSAS it is a "leaky epithelium"
- Solute-coupled transport can potentially occur at this interface since key structures exist
- It shows polarity of ion channels
- We do not know if this property is related solely to CSF production or absorption.
- More studies needed to explore this new field. Different animal model, different results.

"We believe that the CSAS-brain surface interface is an active member of the CSF turnover involved structures…and not just an inactive "bag of fluid". It's role needs to be defined and re-explored under a new prism."

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

“Ἡ 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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