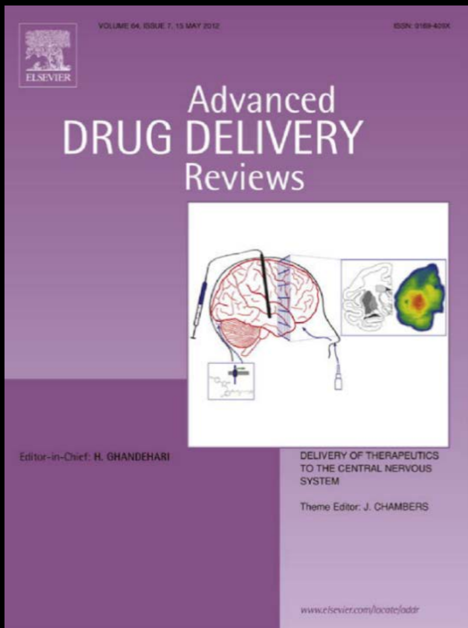
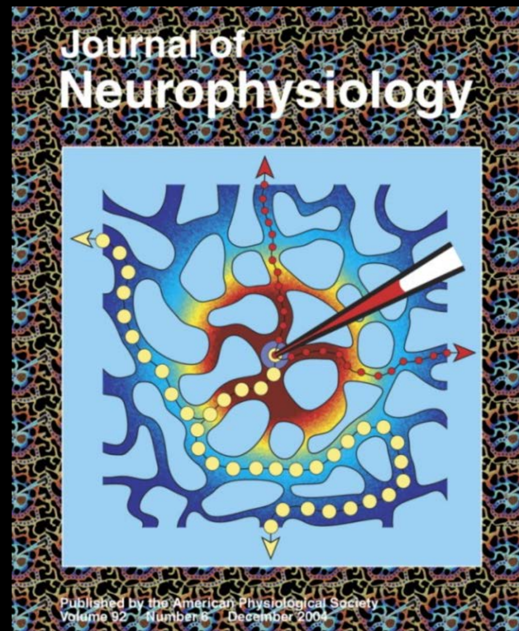
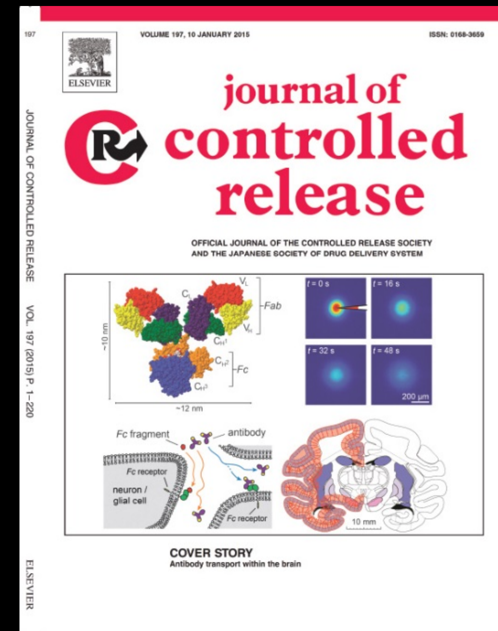
Bypassing the central nervous system barriers: *current state of the art*



Lochhead & Thorne (2012)



Thorne et al. (2004)



Wolak & Thorne (2015)

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THE UNIVERSITY of WISCONSIN MADISON

Disclosure

Robert Thorne acknowledges:

(i) periodically receiving honoraria for speaking on CNS drug delivery to organizations within academia, foundations, and the biotechnology and pharmaceutical industry

(ii) being listed as an inventor on patents and patent applications related to CNS drug delivery

(iii) occasional service as a consultant on CNS drug delivery to the biotechnology and pharmaceutical industry and to other entities

Thorne laboratory – Acknowledgements

Thorne Lab

Niyanta Kumar

Michelle Pizzo

Geetika Nehra

Brynna Wilken-Resman

Sam Boroumand

Khoua Vang

Gretchen Greene

Lab Alumni – Jeff Lochhead, Ph.D. (Univ. of Arizona); Dan Wolak, Ph.D. (Scientist, Amgen); Mohan Gautam, M.S.; Chris Viesselmann, Pharm.D.; Alina Sievers

Outside Collaborators – Joan Abbott, Ph.D. (King's College, London)

Charles Nicholson, Ph.D. (Dept. of Physiology and Neuroscience, NYU School of Medicine)

Lydia Sorokin, Ph.D. (Dept. of Physiol. Chem. & Pathobiochem., Muenster Univ., Germany)

Danica Stanimirovic, M.D., Ph.D. (Director, Translational Bioscience Dept., National Res. Council of Canada)

Funding Sources

UW-Madison School of Pharmacy & Graduate School

NIH – Clinical and Translational Science Award (CTSA) program – National Center for Advancing Translational Sciences (NCATS; grant UL1TR000427)

Michael J. Fox Foundation for Parkinson's Research

Wisconsin Alzheimer's Disease Research Center (NIH P50-AG033514)

WARF Accelerator Program & Discovery Challenge Awards

Wisconsin ICTR Novel Methods Pilot Award

Fellowships 2017 Fulbright scholar – G.G.

2014 National Science Foundation Graduate Research Fellowship Program (GRFP) – M.P. & B.W-R.

NIH – T32-EB011434 Fellowships (UW-Madison Clinical Neuroengineering Training Program) – D.W. & M.P.

PhRMA Foundation (pre-doctoral fellowship) – D.W.

University of Wisconsin-Madison Collaborators

Corinna Burger, Ph.D. (Neurology)

Aparna Lakkaraju, Ph.D. (Ophth. & Visual Sci.)

Lingjun Li, Ph.D. (Pharmaceutical Sciences)

Beth Meyerand, Ph.D. (Biomed. Engr. / Medical Physics)

Vince Cryns, M.D. (Medicine)

John Kuo, M.D., Ph.D. (Neurosurg.)

& Paul Clark, Ph.D.

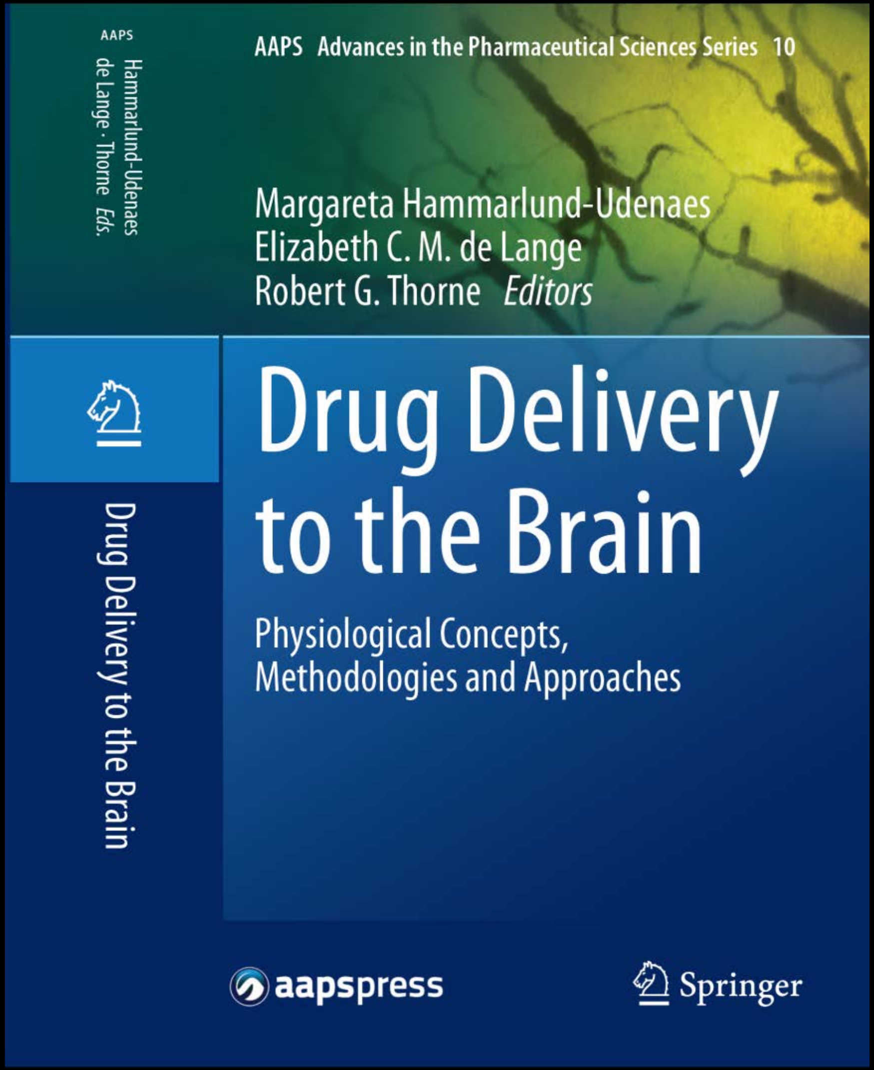
Utilizing biologics as CNS therapeutics – Where are we today?

- *Systemic approaches have yet to be translated fully to the clinic (no approvals) – the CNS barriers pose a unique challenge*

- *Only 3 FDA-approved CNS biologics are actually delivered into the brain* *all → CSF*
  – intrathecal ziconotide (2.6 kDa) / *chronic pain*
  – intrathecal nusinersen (~7 kDa) / *SMA*
  – intraventricular cerliponase alfa (59 kDa) / rhTripeptidyl peptidase (N-term) Late infantile neuronal ceroid lipo-fuscinosis type 2 (CLN2) – *Batten disease*

- *Many uncertainties remain*
  – *precise brain / spinal cord distribution*
  – *relative effectiveness of different routes*
  – *if limited delivery, how can we enhance?*

- *What is needed? We need to better understand the mechanisms governing drug delivery and distribution in the CNS*



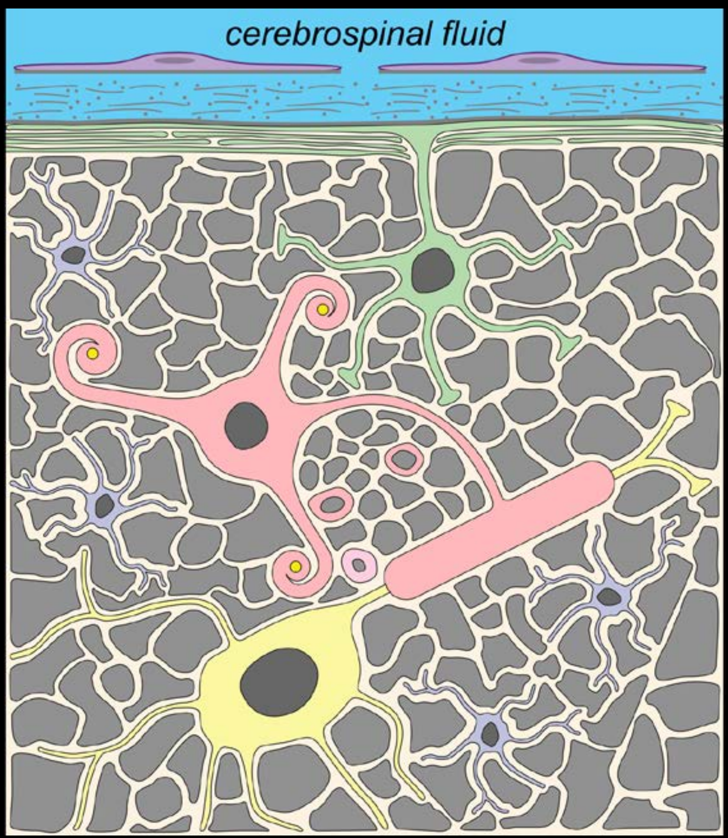
(2014)

Diffusive transport of therapeutics in the neuropil

Brain extracellular spaces (ECS) are *40-60 nm wide*

(Thorne & Nicholson. *PNAS*, 2006) —

an environment favoring diffusion

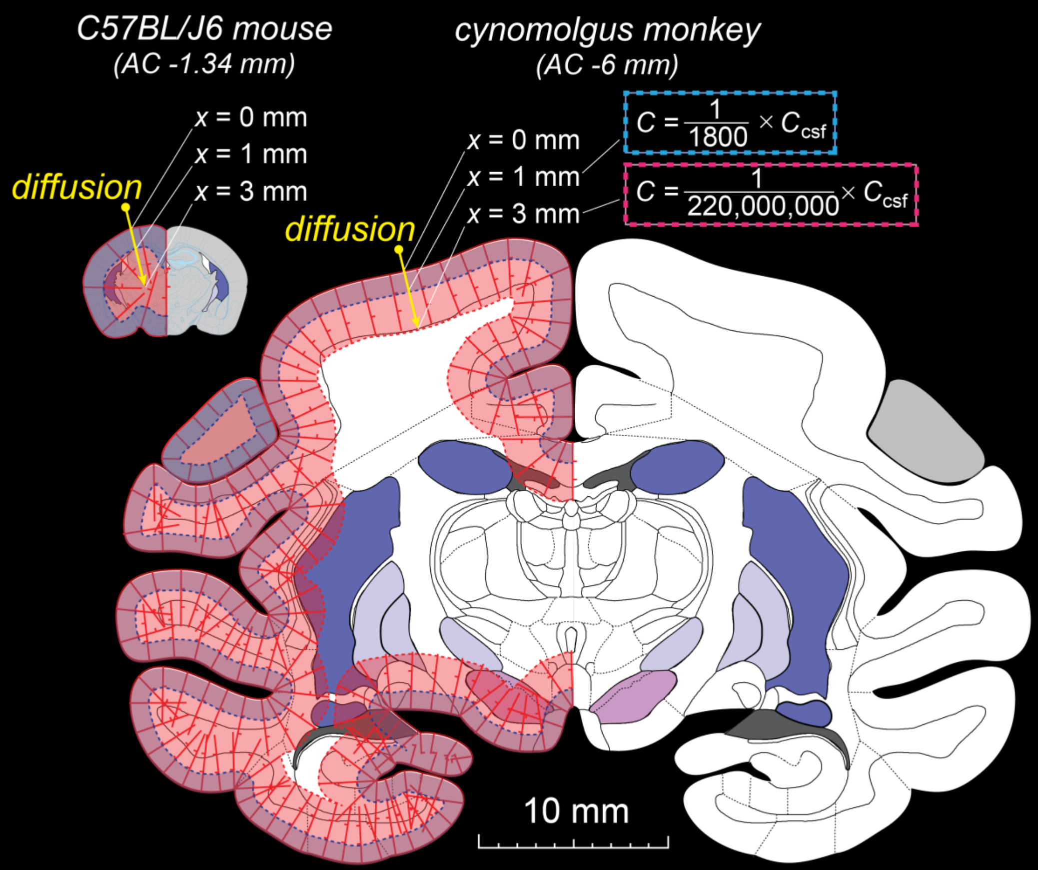


Adapted from: Wolak & Thorne. *Mol Pharm.* 2013. Abbott, Thorne et al. *In prep.*

RTI: Nicholson & Phillips. *J Physiol.* 1981; Cserr, DePasquale, Nicholson, Patlak, Pettigrew & Rice. *J Physiol.* 1991; **IOI**: Nicholson & Tao. *Biophysica J.* 1993; Thorne & Nicholson. *PNAS.* 2006; **VCP**: Rall, Oppelt & Patlak. *Life Sci*. 1962; Levin, Fenstermacher & Patlak. *Am J Physiol*. 1970

Predicted diffusion gradients from *in vivo* diffusion measurements (IgG, 150 kDa) —

limited penetration / NOT scalable

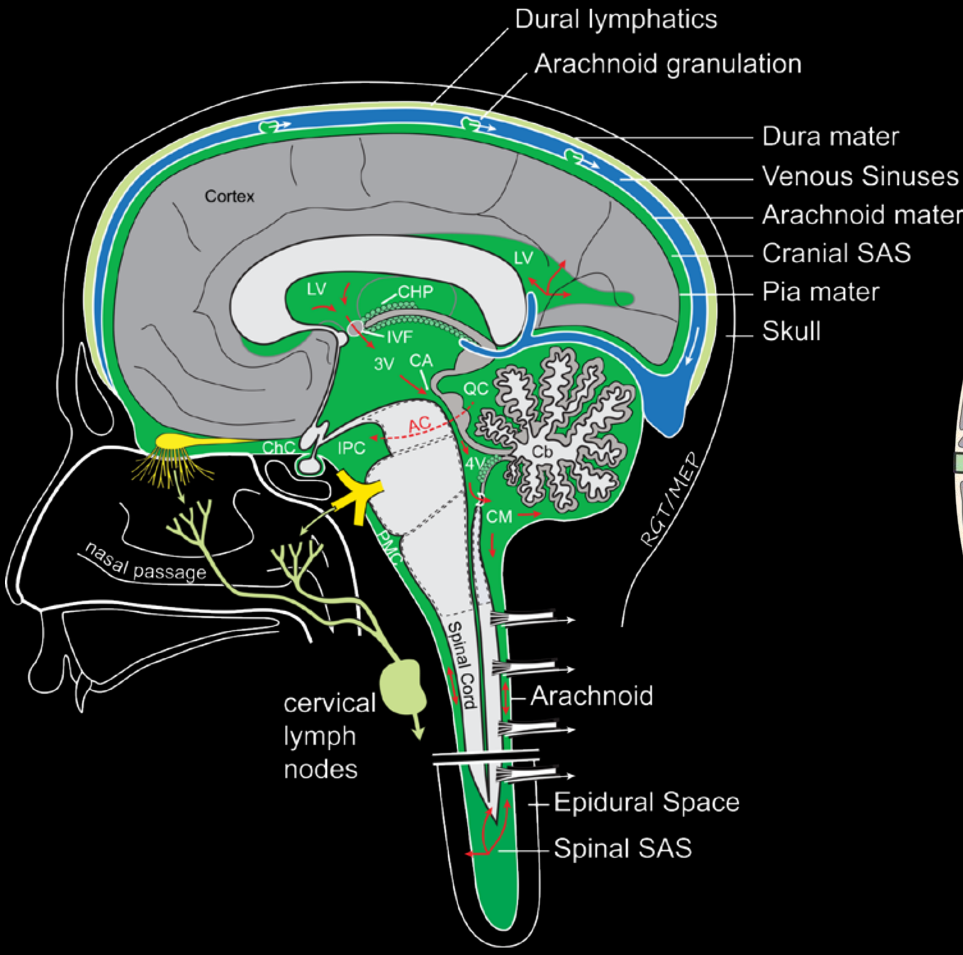


non-targeted IgG; based on in vivo brain diffusion coefficient (using integrative optical imaging point source method)

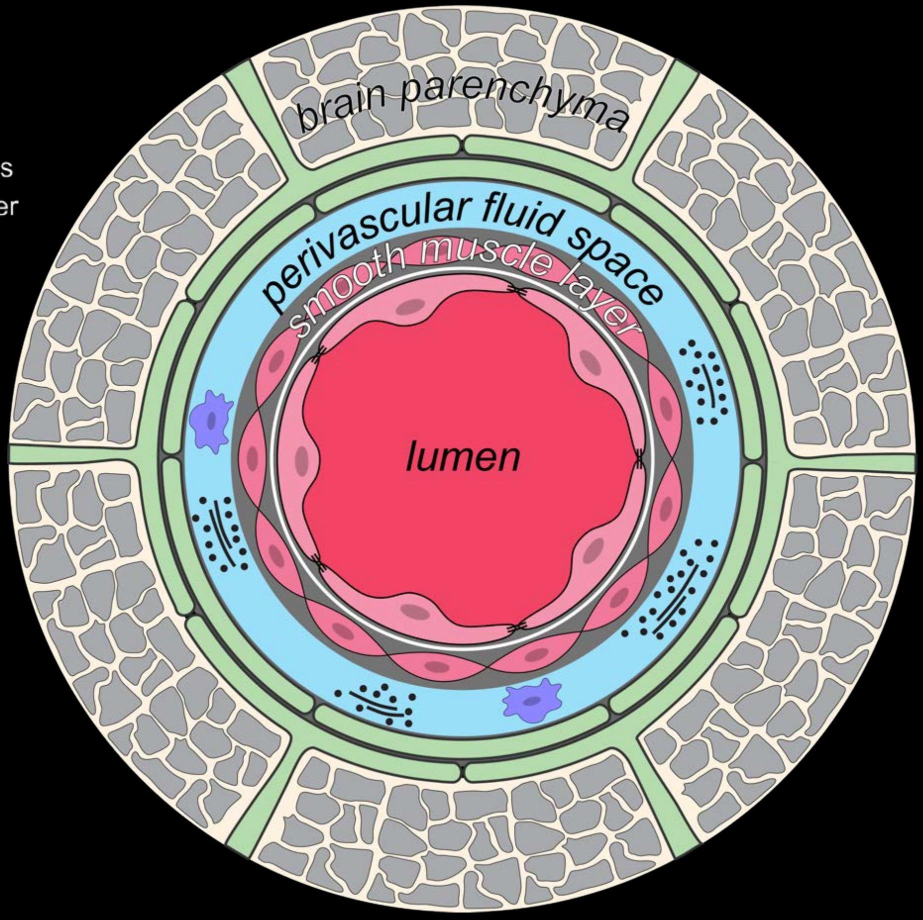
From: Wolak, Pizzo & Thorne. *Journal of Controlled Release* (2015)

Convective transport of therapeutics in the CSF & PVS

Cerebrospinal fluid (CSF) circulation pathways – *scalable across species*



Perivascular space (PVS) fluid compartments – *may also allow for some circulation / potentially scalable*



Adapted from: Thorne. IN: *Drug Delivery to the Brain.* Springer (2014)

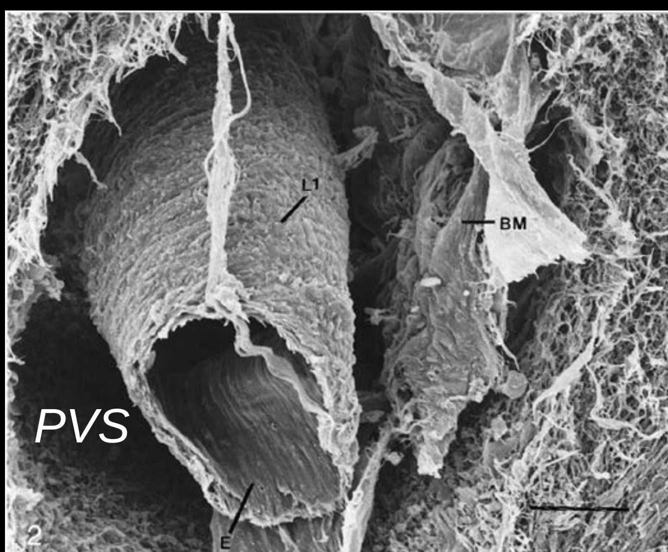
Pizzo & Thorne. IN: Brain Edema. Academic Press (2017)

Perivascular spaces

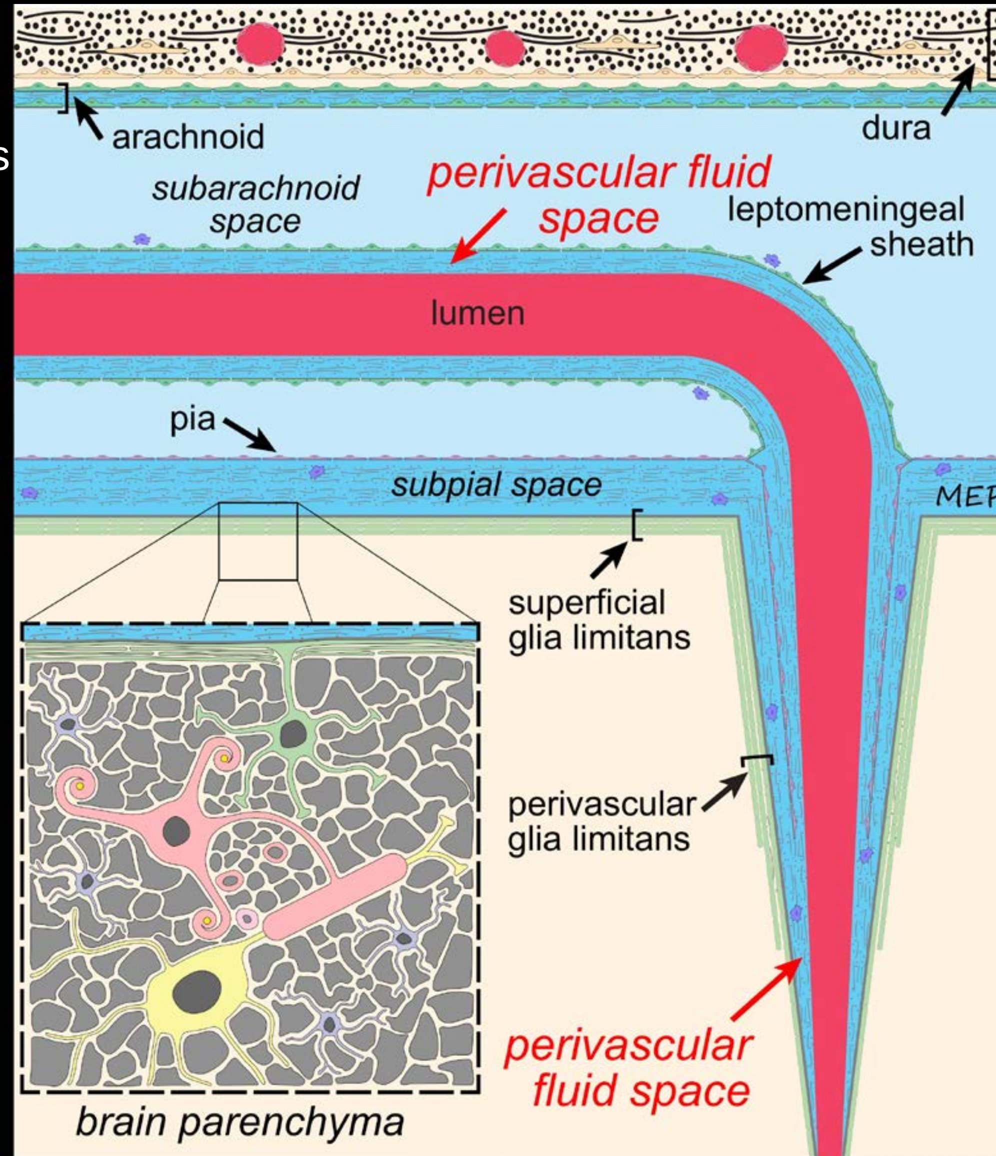
- fluid and connective tissue compartments surrounding subarachnoid & cerebral vessels

- large enough (5-10 μm) to allow for flow (convection); arterial pulsations may serve as a driving force

- serve a possible lymphatic function



Pollock et al. *J Anat.* 1997

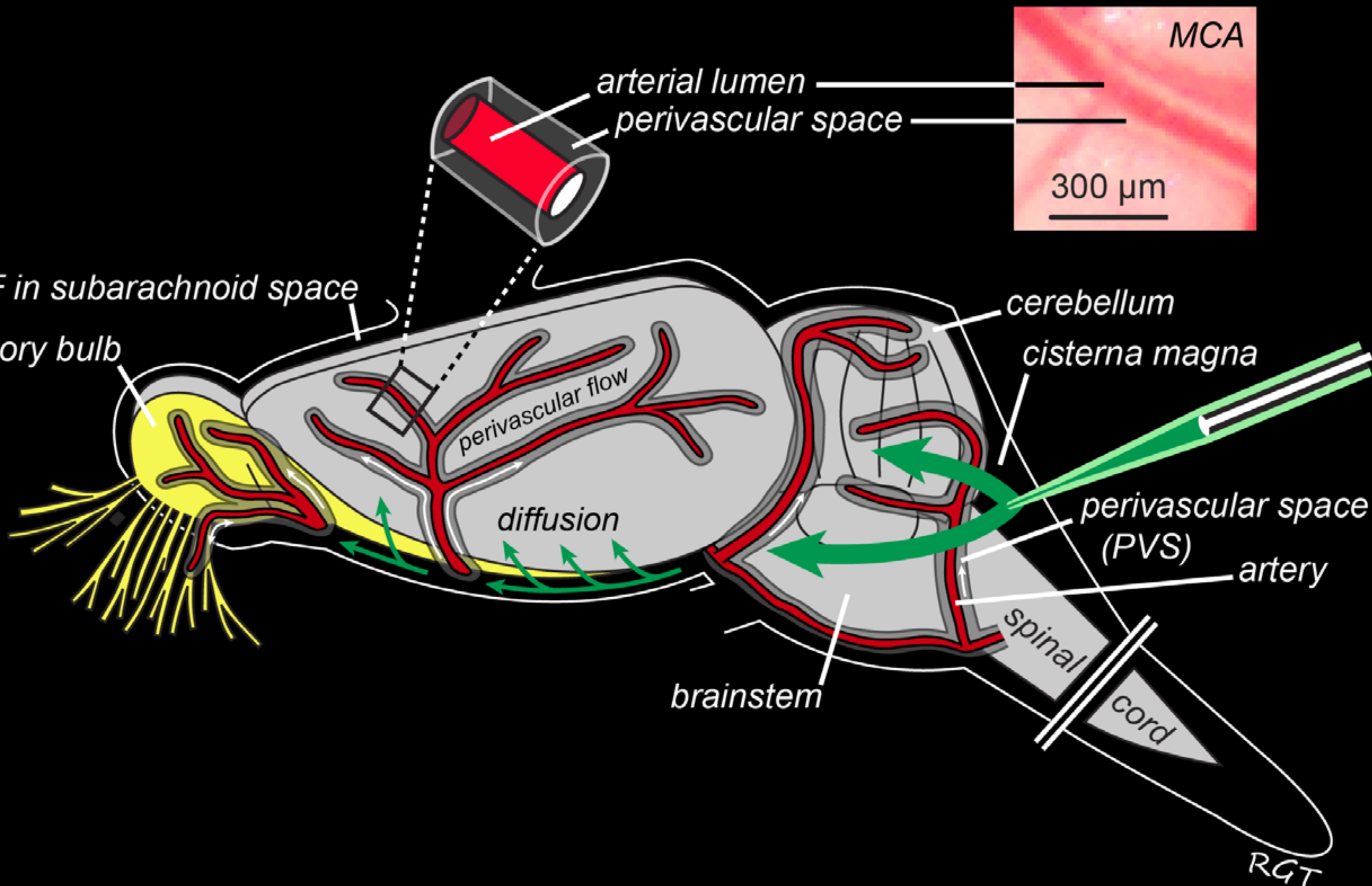


Pizzo, … & Thorne. *Journal of Physiology, in press* (2017)

Sources: Zhang et al. *J Anat.* 1990; Frederickson & Low. *Am J Anat* .1969; Ichimura et al. *Brain Res.* 1991; Iliff et al. *Sci Transl Med.* 2012; Foley et al. *Ann Biomed Eng.* 2012; Abbott. *Neurochem Int.* 2004; Cserr et al. *Exp Eye Res.* 1977; Cserr et al. *Am J Physiol.* 1981; Rennels et al. *Brain Res.* 1985; Hadaczek et al. *Mol Ther.* 2006; Iliff et al. *J Neurosci.* 2013; Carare et al. *Neuropath Appl Neuro.* 2008; Bilston et al. *Comput Methods Biomech Biomed Engin.* 2003; Schley et al. *J Theor Biol.* 2006; Morris et al. *Acta Neuropathol.* 2016; Wang & Olbricht. *J Theor Biol.* 2011

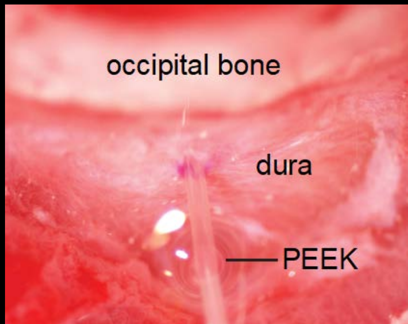
CNS distribution resulting from intrathecal infusions

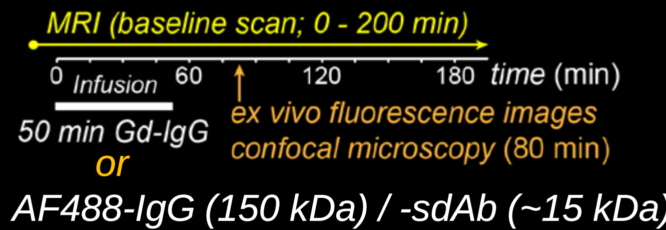
How widespread can it be? What determines the distribution?



Transport at the brain – CSF interface: A delicate mix of diffusion within brain extracellular spaces & convection within perivascular spaces

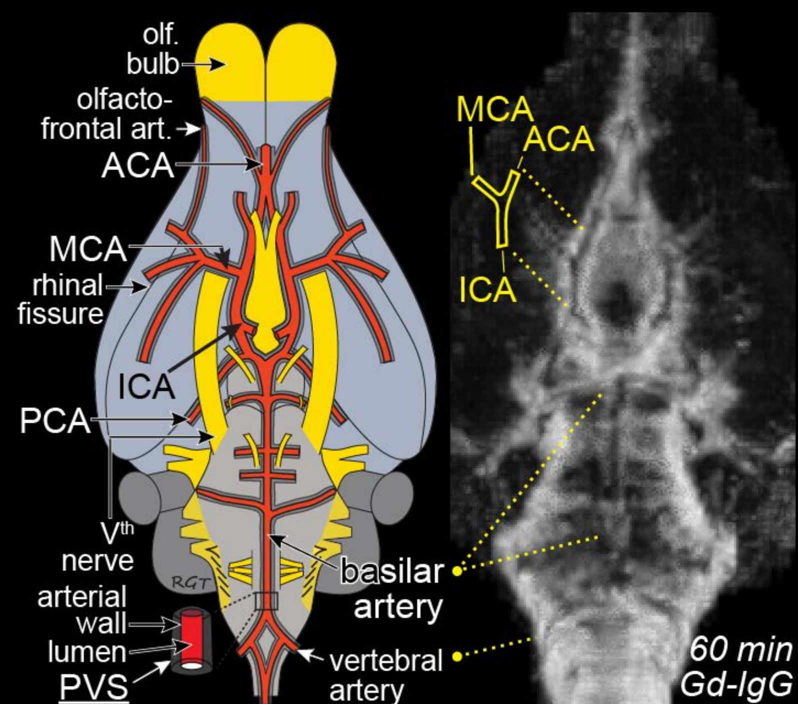
<u>*Intrathecal infusions*</u> – Imaging I.T. IgG distribution in rats reveals the critical role of perivascular flow





Michelle Pizzo

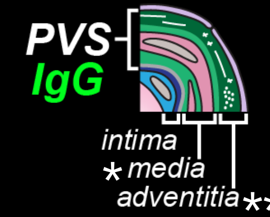
<u>*MRI of I.T. Gd-IgG*</u> <u>*Ex vivo fluorescence imaging of I.T. AF488-IgG*</u>

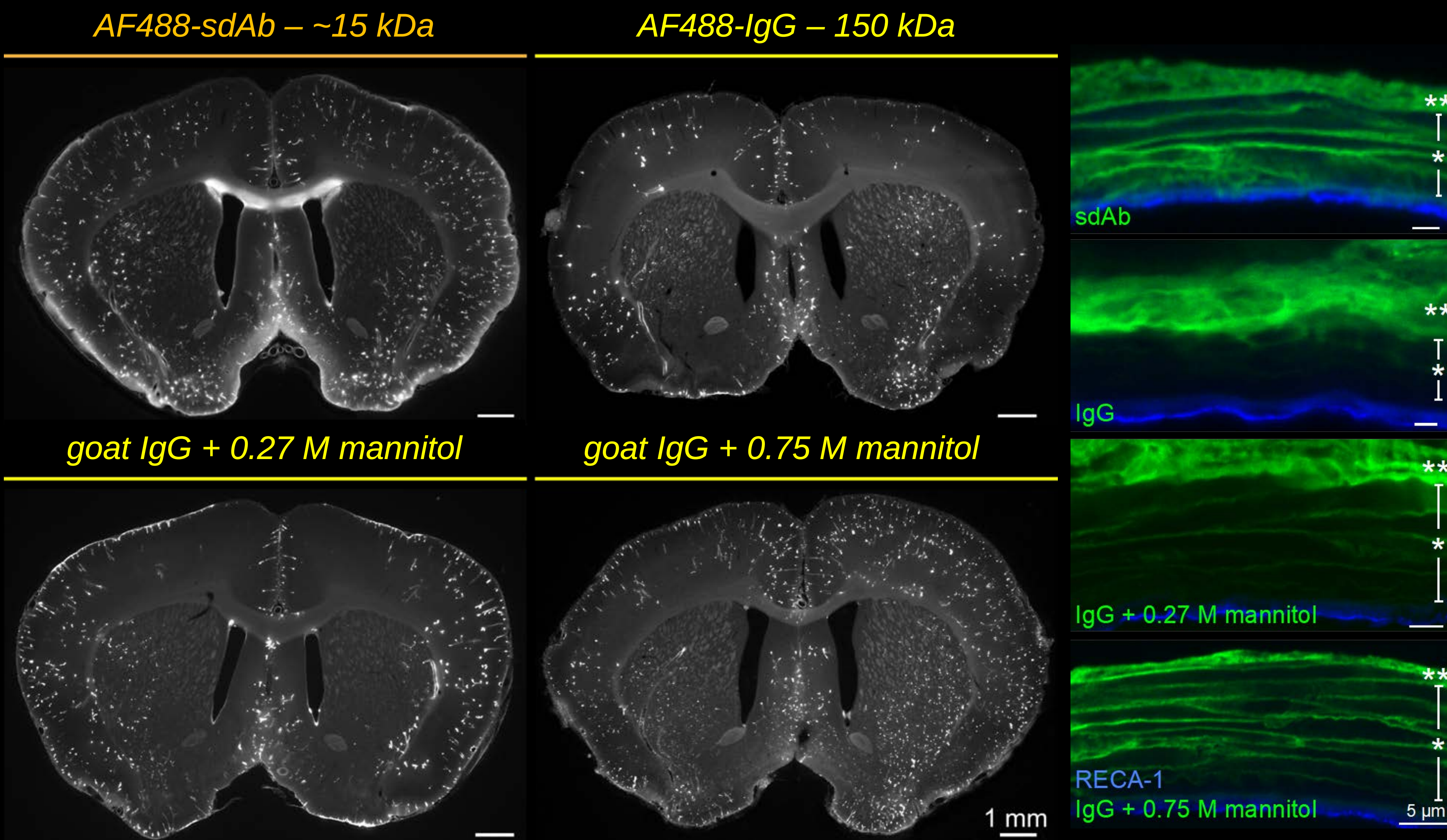


Left panel: Lochhead et al. *JCBFM* (2015); *Right panel:* T1 MRI – baseline subtracted (visualization using ImageJ); 50 min infusion + post-infusion imaging

Pizzo, … & Thorne. *Journal of Physiology, in press* (2017)

<u>*Intrathecal infusions*</u> – 1. antibody size-dependence
2. the balance between diffusion & perivascular flow
3. distribution enhancement by co-infusion of mannitol





Pizzo, … & Thorne. *Journal of Physiology, in press* (2017); AF488-labeled A20.1 VHH, llama sdAb provided by Dana Stanimirovic (NRC, Canada)

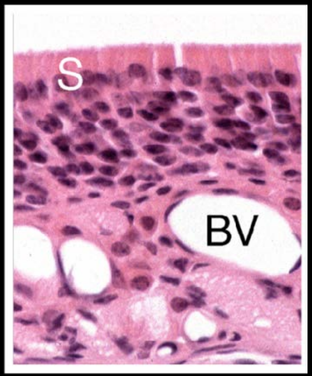
Intranasal targeting to the brain

What determines the distribution?

Jeff Lochhead

1 *Transport across nasal epithelia to reach brain entry pathways or blood vessels (BV) for systemic absorption*

Olfactory Mucosa (H&E)



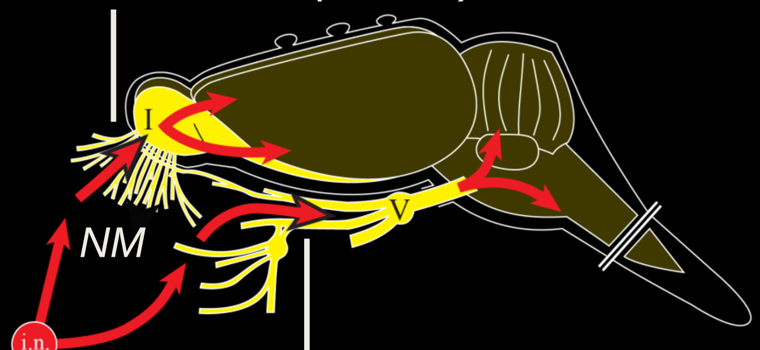
External environment

Epithelium

Lamina Propria (brain entry pathways)

2 *Transport along olfactory & trigeminal pathways from the nasal mucosa (NM)*

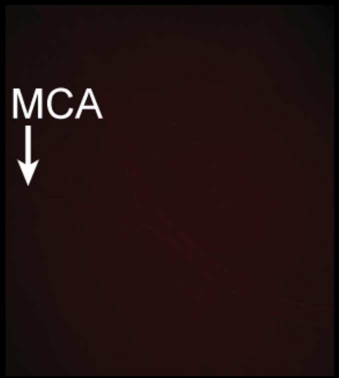
Olfactory nerve-associated pathway



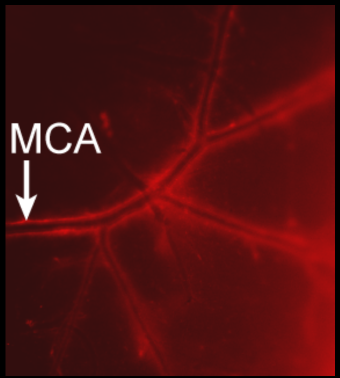
Trigeminal nerve-associated pathway

3 *Widespread distribution in the brain via flow within perivascular spaces associated with major cerebral arteries*

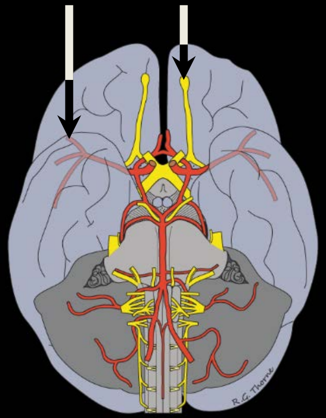
Intranasal PBS (control)



Intranasal TR-dextran (3 kDa)

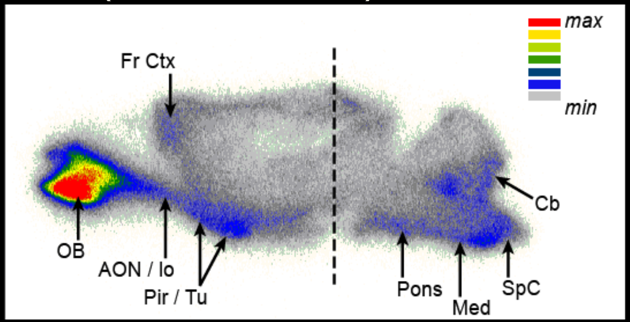


MCA *olfactory bulb*



From: Lochhead et al. *JCBFM* (2015); Reviewed in: Lochhead & Thorne. *ADDR* (2012)

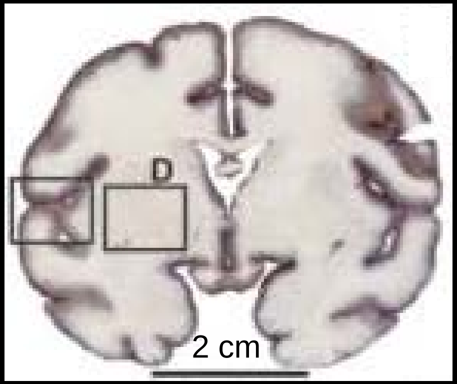
IGF-I (7649 Da; 70 aa)



Adapted from: Thorne et al. *Neuroscience* (2004)

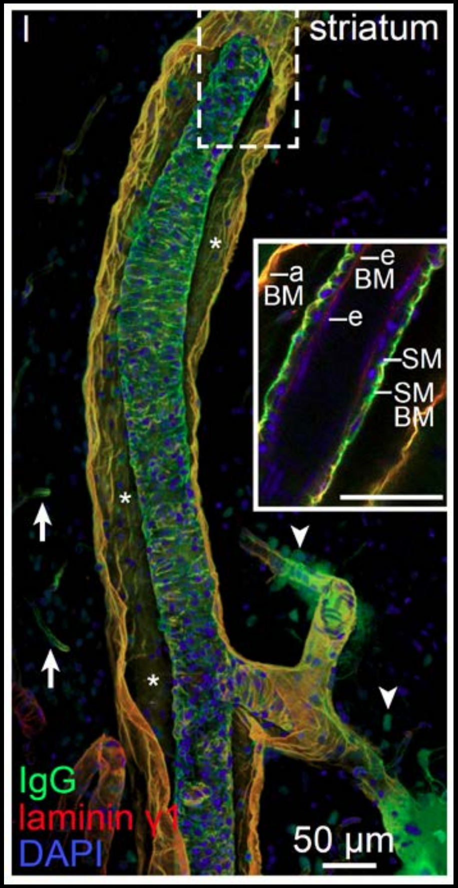
Conclusions

I.C.V. acid sphingomyelinase (70 kDa) in the rhesus monkey



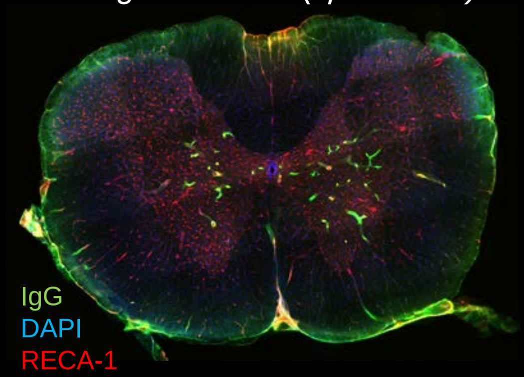
- Diffusive transport of large macromolecules (e.g. enzymes) into the brain from the CSF will be quite limited (several mm)

I.T. IgG in the rat

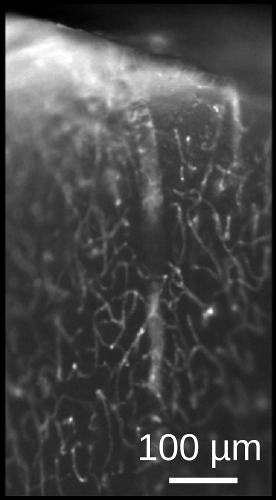


- Access to & distribution within the perivascular spaces will likely be critical for widespread distribution

I.T. IgG in the rat (spinal cord)



Intranasal IgG in the rat (frontal pole)



- There is an urgent need to understand all key variables
  - body position
  - disease / storage effects
  - intracranial pressure
  - individual variation
  - co-applied excipients (e.g. osmotic methods)

Top right: Ziegler et al. *Exp Neurol* (2011); Other images – Pizzo, Kumar & Thorne. *Submitted / Unpub.* + collab. w/ Lydia Sorokin (Univ. of Muenster, Germany)