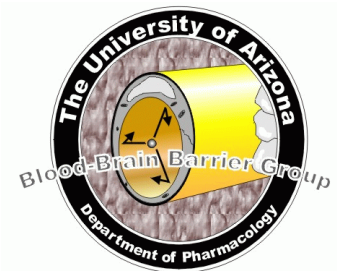


# Blood-Brain Barrier Transporters in Ischemic Stroke: Focus on Organic Anion Transporting Polypeptides (Oatps)



**Patrick T. Ronaldson, Ph.D.**  
**Associate Professor**  
**Department of Pharmacology**  
**University of Arizona College of Medicine**



*Invited Presentation:*  
*Solvo Biotechnology Meet the Experts Transporter Conference 2019*  
*September 4, 2019*

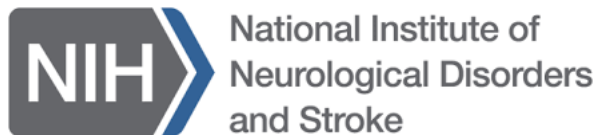


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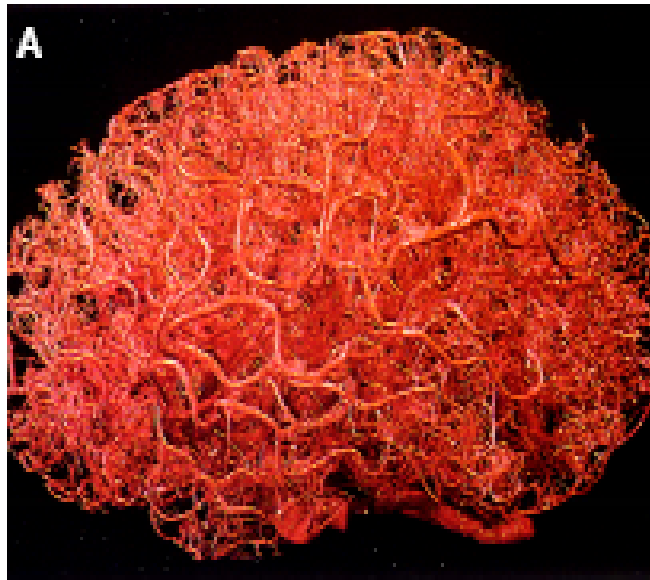
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# Disclosures

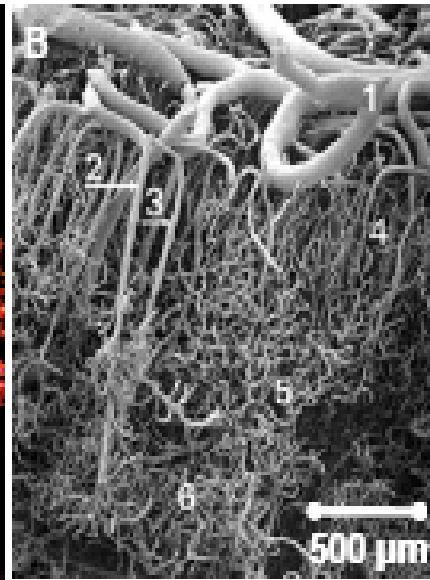
- Research Funding
  - NINDS/NIH R01-NS084941 (PT Ronaldson, PI)
  - American Heart Association 19TPA34910113 (PT Ronaldson, PI)
  - Arizona Biomedical Research Commission ADHS16-162406 (PT Ronaldson, PI)



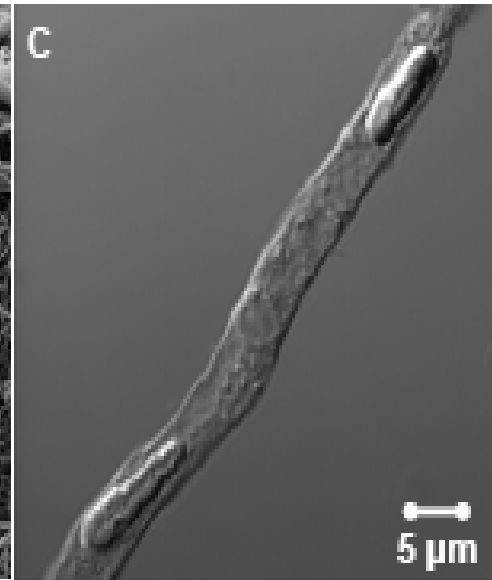
# The BBB – A Vast Microvascular Network



**400 miles of  
capillaries  
in the human brain**



**> 100 billion  
capillaries in the  
human brain  
comprising ~215  
ft<sup>2</sup> surface area**



**One capillary per  
neuron and  
average distance  
~8-20 μm**

A. Zlokovic and Apuzzo, 1998; B. Rodriguez-Baeza et al., 2003; C. Hartz et al., 2006

## Challenge: Neuroprotective Drugs for Stroke - Many Failures

- Preclinical success in neuroprotective drug development has not resulted in translation of new therapeutics to the clinic.
  - As noted by Jun Chen's group (University of Pittsburgh), 95% of published neuroprotective studies between 1990 and 2018 describing positive results in animal models - none have progressed to phase III trial success. (Shi et al., 2018. *J Cerebral Blood Flow Metabolism* 38,12. 2073-2091).
- Failures may be attributed, in part, to the fact that most preclinical stroke studies do not evaluate biological mechanisms that can deliver these drugs successfully to ischemic brain tissue.
  - Targeting uptake transporters such as Organic Anion Transporting Polypeptides (Oatps) may address this problem !

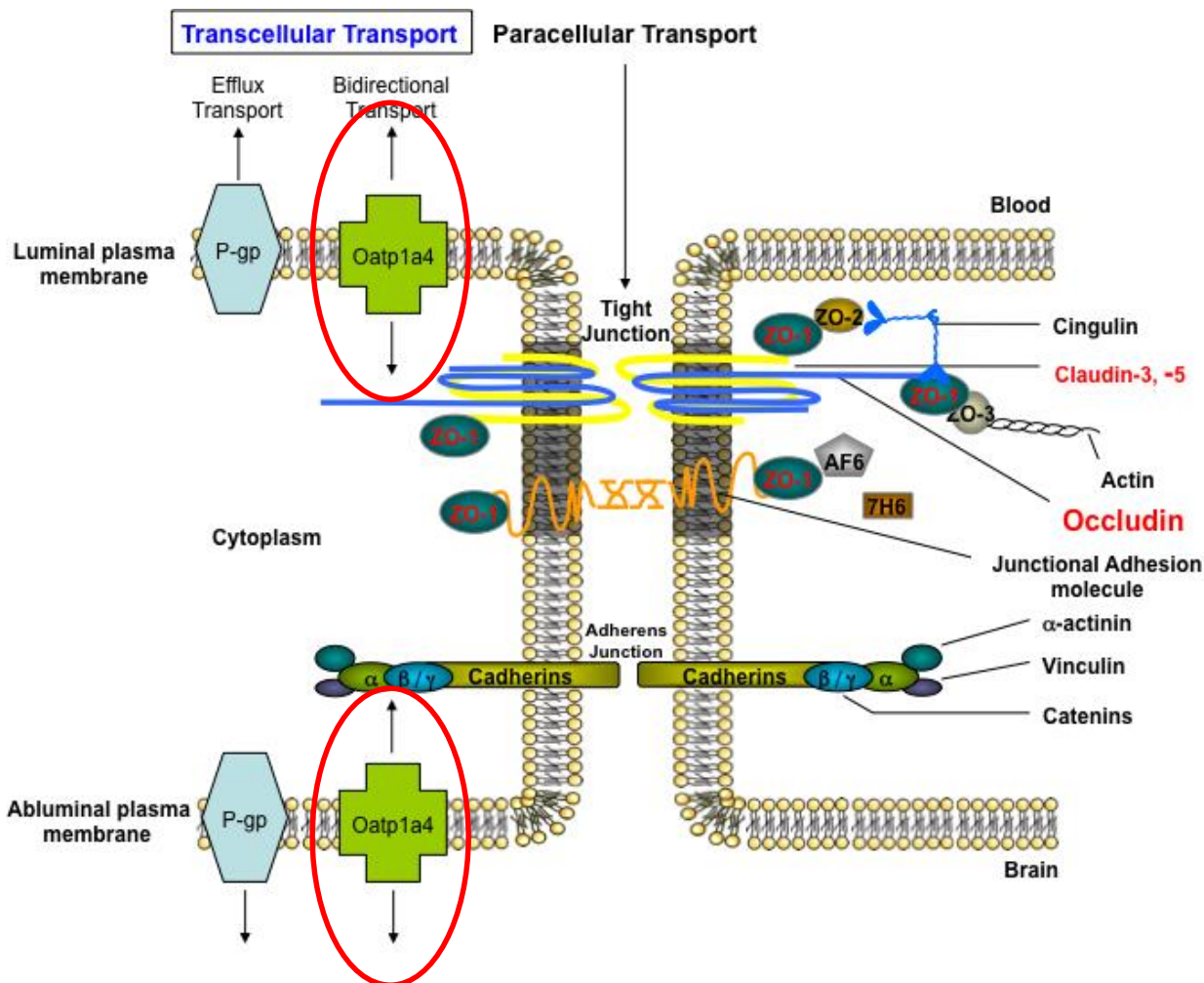


# Why study Oatps in Stroke?

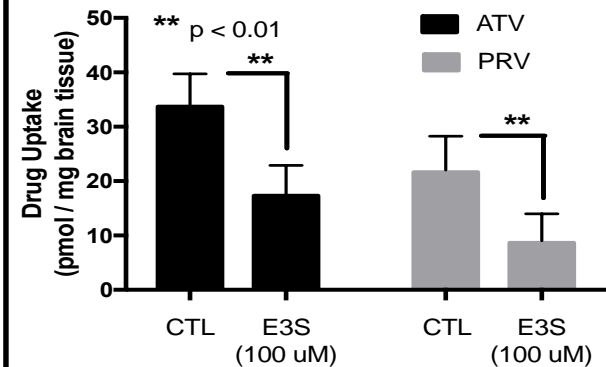
- Statins have been shown to improve functional outcomes in stroke patients.
  - Amarenco et al. 2006. *New Engl J Med*. **355**: 549-559.
  - Castilla-Guerra et al. 2006. *Stroke*. **37**: 1153.
  - Montaner et al. 2008. *Eur J Neurol*. **15**: 82-90.
  - Salat et al. 2009. *Expert Rev Cardiovasc Ther*. **7**: 1219-1230.
  - Huisa et al. 2010. *Vasc Health Risk Manag*. **6**: 229-236.
  - Montecucco et al. 2012. *Curr Pharm Biotechnol*. **13**: 68-76.
  - Montaner et al. 2016. *Stroke*. **47**: 2870-2873.
  - Lee et al. 2017. *J Am Heart Assoc*. **6**.
  - Zhang et al. 2017. *Int J Neurosci*. **127**: 92-97.
- Statins are transport substrates for Oatps
  - Work from our group has shown, for the first time, that Oatp1a4 enables these drugs to permeate the BBB and accumulate in the CNS (Thompson et al. 2014. *J Cereb Blood Flow Metab*. **34**: 699-707; Abdullahi et al. 2018. *Molecular Pharmacology*. **94**: 1321-1333).

***These papers  
provide CLINICAL  
evidence that statins  
are EFFECTIVE in  
providing  
neuroprotection to  
stroke patients.***





## Oatp-Mediated Brain Uptake of Statins

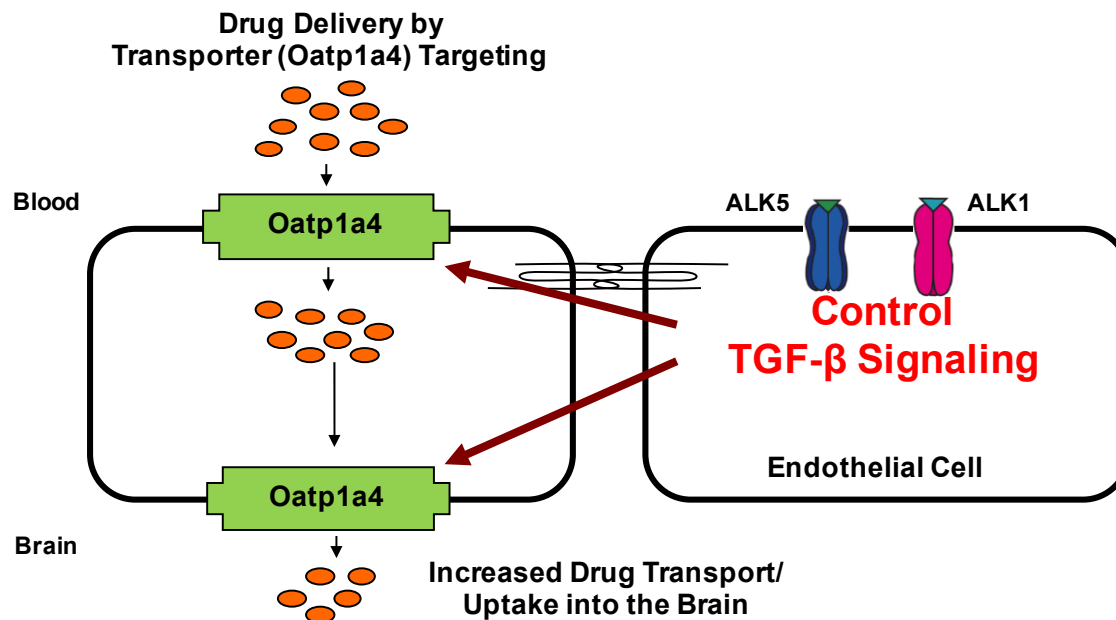


Adapted from: Abdullahi et al. 2018. *Mol Pharmacol.* **94**: 1321-1333. E3S=OATP inhibitor.

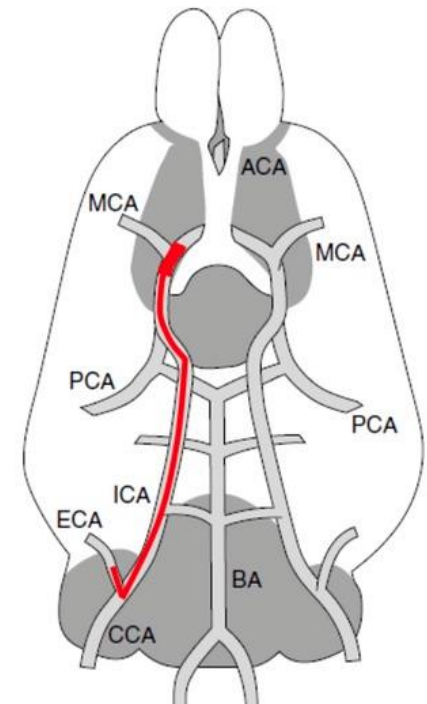
Modified from: Ronaldson & Davis. 2015. *Brain Res.* **1623**: 39-52.

# Targeting Oatp1a4 for CNS Drug Delivery

**Hypothesis:** Oatp1a4 expression and activity at the BBB is an absolute requirement for statins to exert neuroprotective effects in the brain following ischemic stroke.



## *tMCAO Model of Focal Cerebral Ischemia*



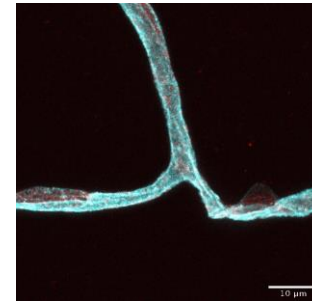
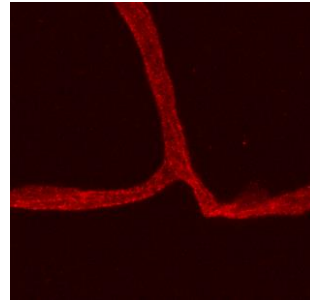
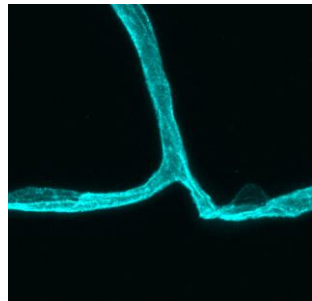
# Oatp1a4 Localization: Brain Microvascular Endothelium

Tomato Lectin

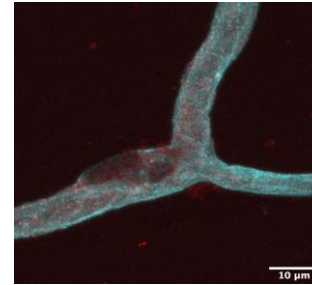
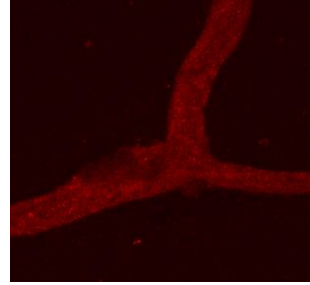
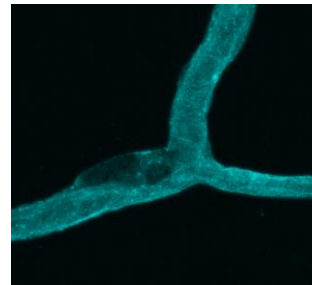
Oatp1a4

Merge

Control  
Microvessel



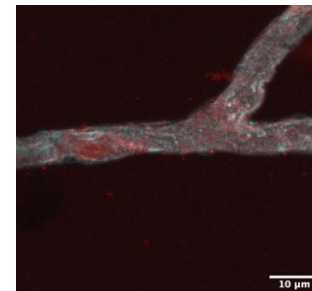
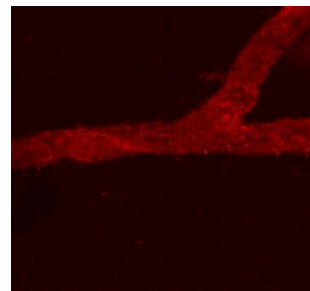
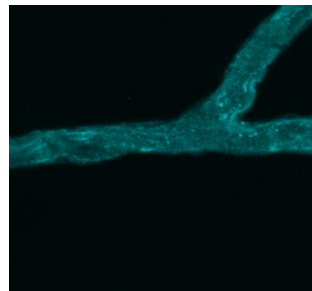
tMCAO  
(Ipsilateral CTX)



Dr. Jeff Lochhead



tMCAO  
(Contralateral CTX)



tMCAO:

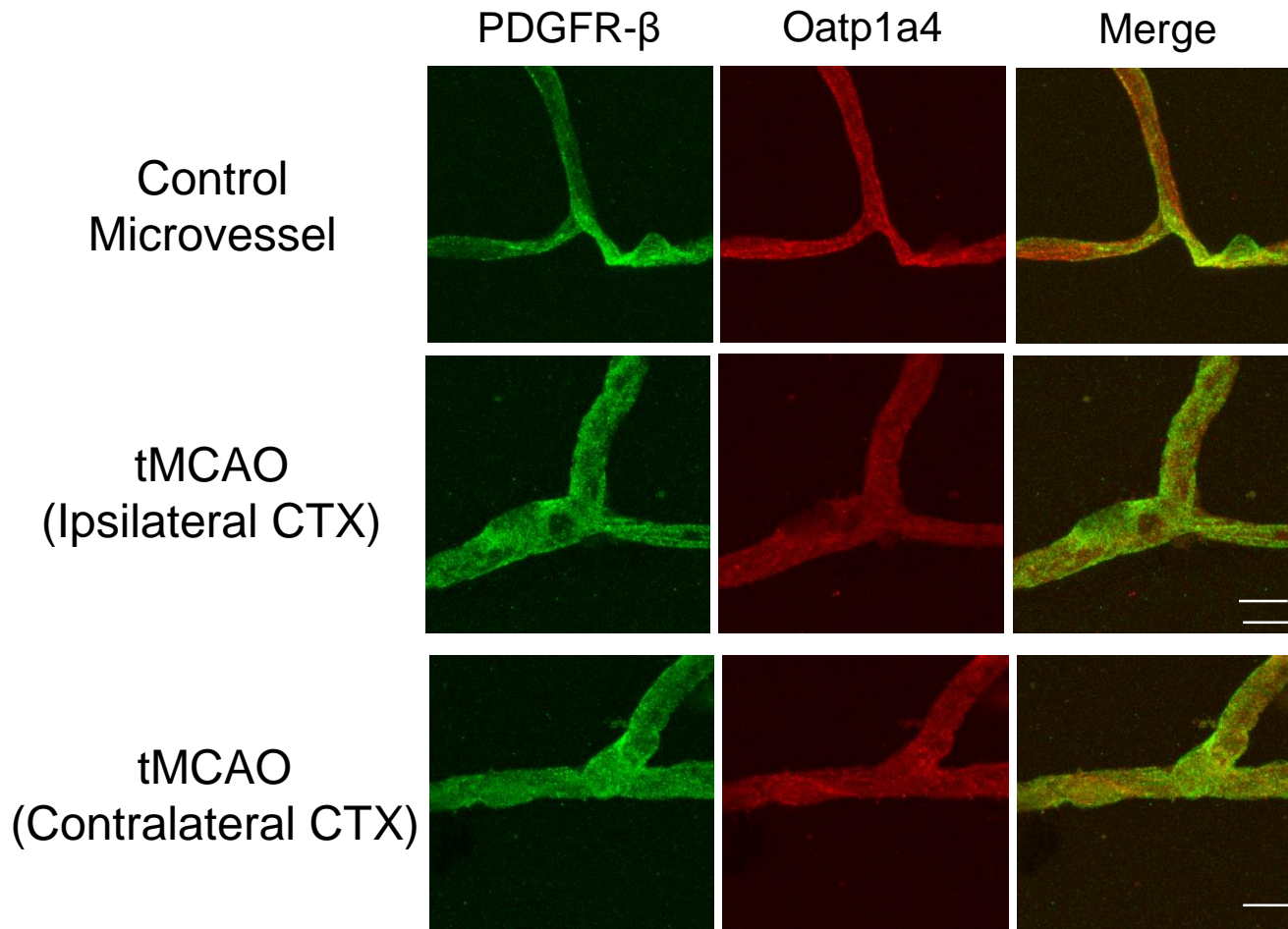
90 min MCAO  
22.5 hrs reperfusion



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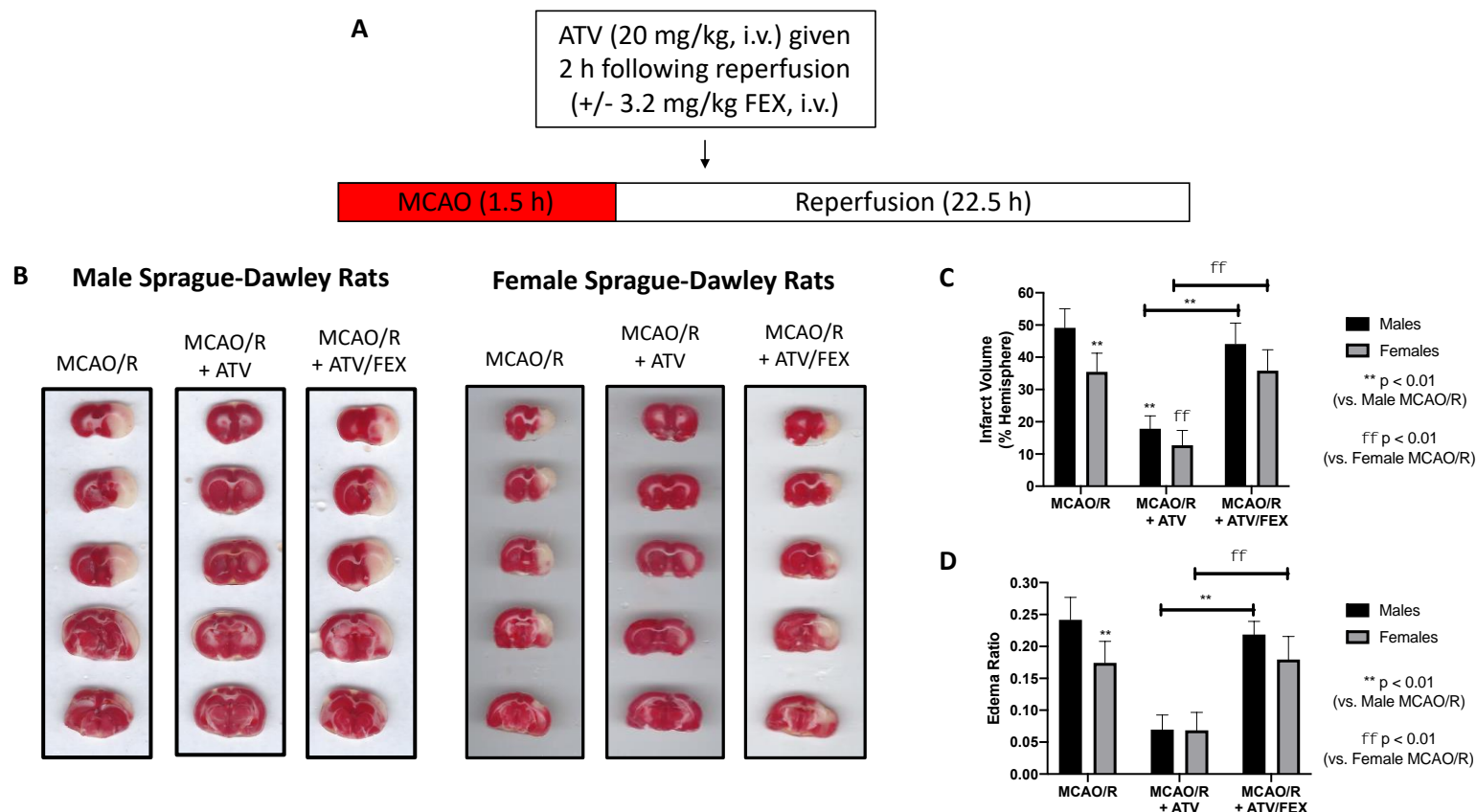
# Oatp1a4 Localization: Pericytes



tMCAO:

90 min MCAO  
22.5 hrs reperfusion

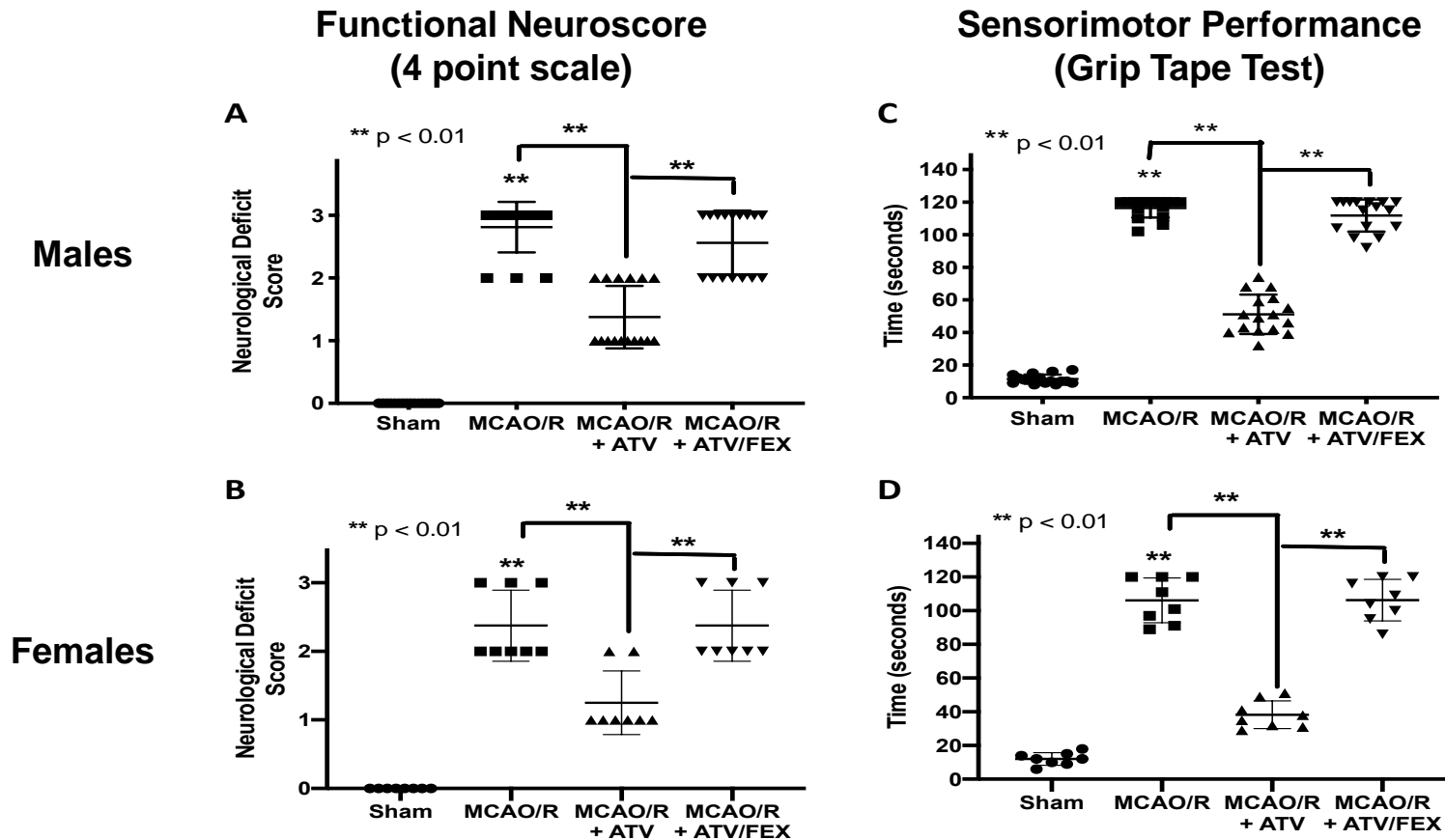
# Statins require Functional Expression of Oatp1a4 at the BBB to exert Neuroprotective Effects in Stroke



n = 8 animals per group

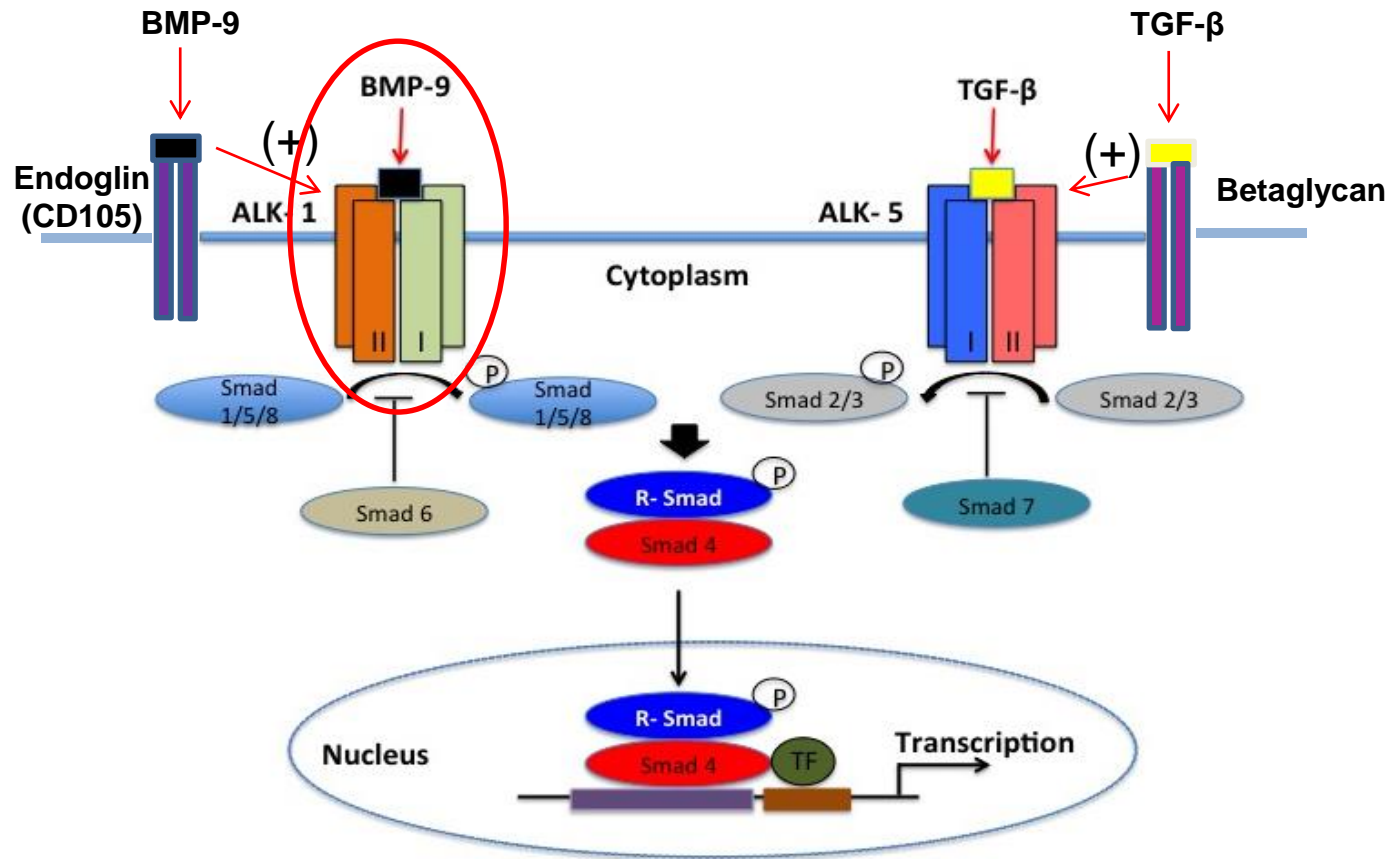
From: Lochhead et al. 2019. *Stroke*. Submitted

# Statins require Functional Expression of Oatp1a4 at the BBB to exert Neuroprotective Effects in Stroke



n = 16 animals per group (males); n = 8 animals per group (females)

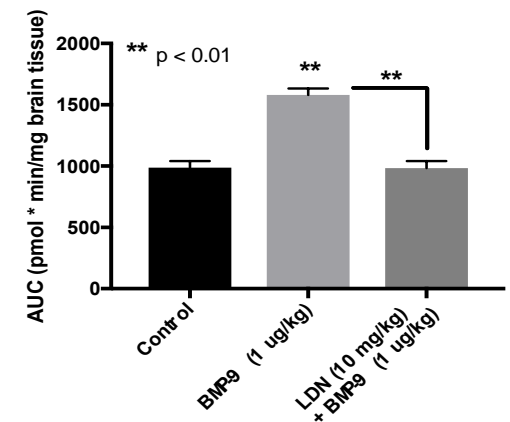
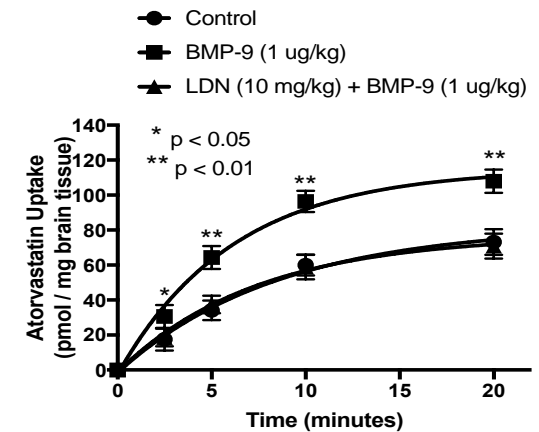
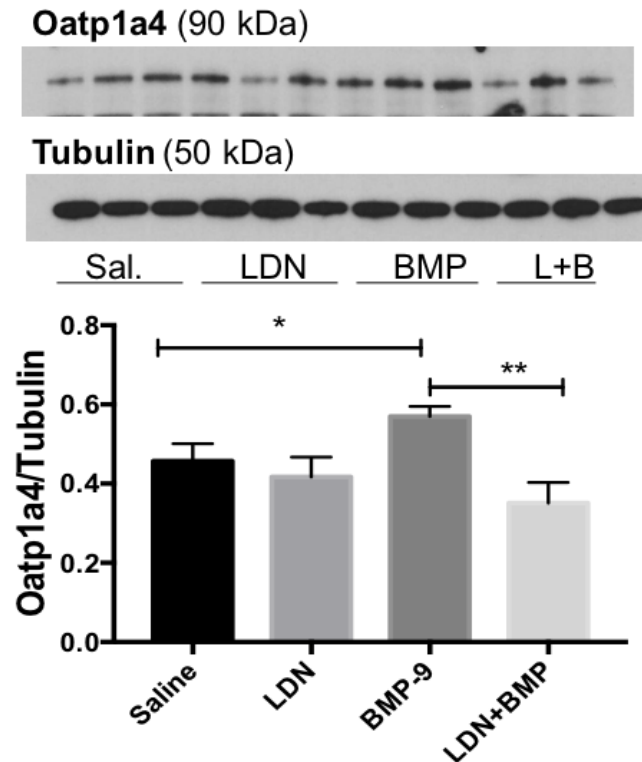
# TGF- $\beta$ Signaling Pathway



Adapted from: Abdullahi et al. 2017. *AAPS J.* **19**: 931-937

# Regulation of Oatp1a4 Expression by Transforming Growth Factor- $\beta$ Signaling

Dr. Wazir Abdullahi



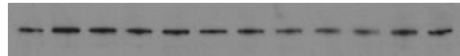
From: Abdullahi et al. 2017. *J Cereb Blood Flow Metab.* **37**: 2340-2345.

Abdullahi et al. 2018. *Mol Pharmacol.* **94**: 1321-1333.

Abdullahi et al. 2019. *Drug Metab Dispos.* Submitted.

# Regulation of Oatp1a4 Expression by Transforming Growth Factor- $\beta$ Signaling

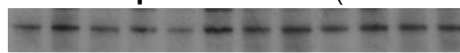
Cytoplasmic pSMAD1/5/8 (52-56 kDa)



Tubulin (50 kDa)



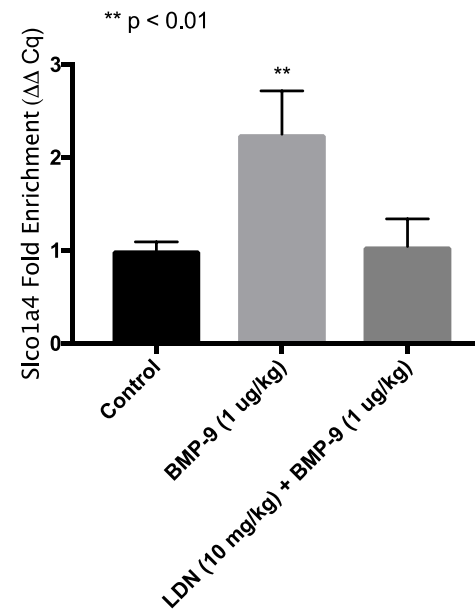
Nuclear pSMAD1/5/8 (52-56 kDa)



Lamin B (67 kDa)

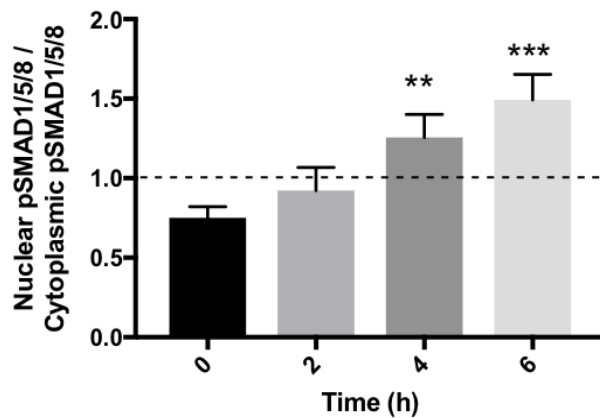


## ChIP Analysis



Smad Binding Element:

GC-rich sequences flanking CAGA boxes



From: Abdullahi et al. 2018. *Mol Pharmacol.* **94**: 1321-1333.



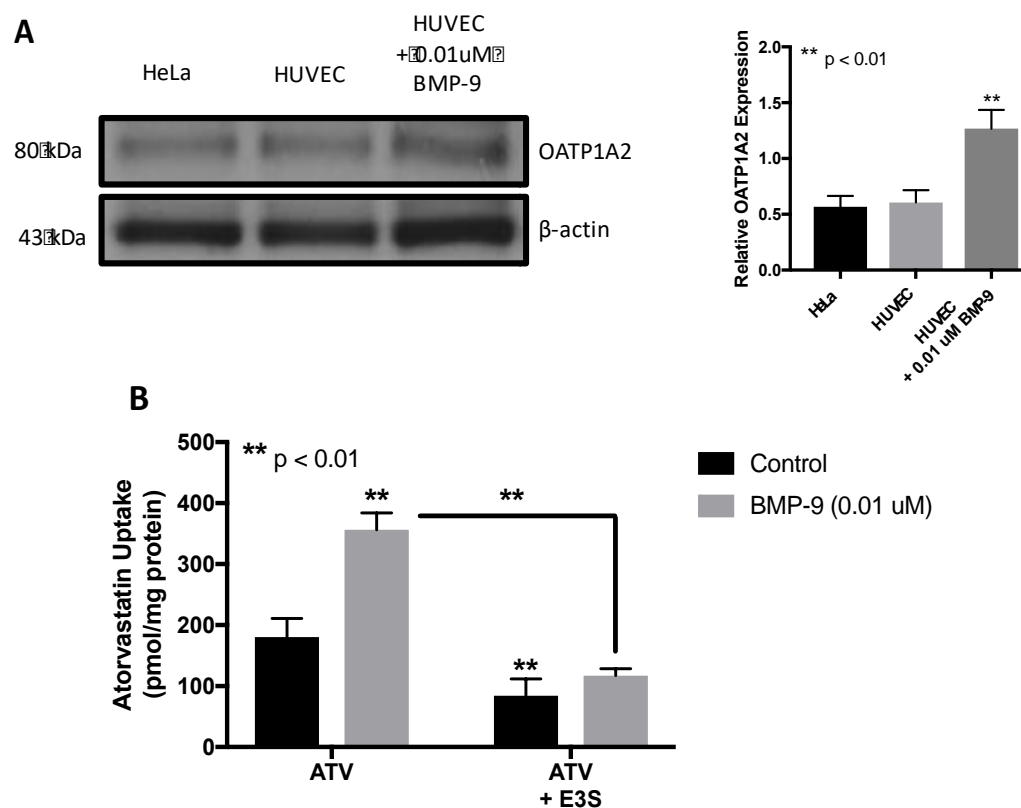
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# Functional Expression of OATP1A2 in HUVECs

(HUVEC cells kindly provided by Dr. Gregory Bix at the University of Kentucky)

***“Emphasizes Translational Potential of our Oatp1a4 studies”***



From: Ronaldson et al. 2019. *J Pharm Sci.* Submitted

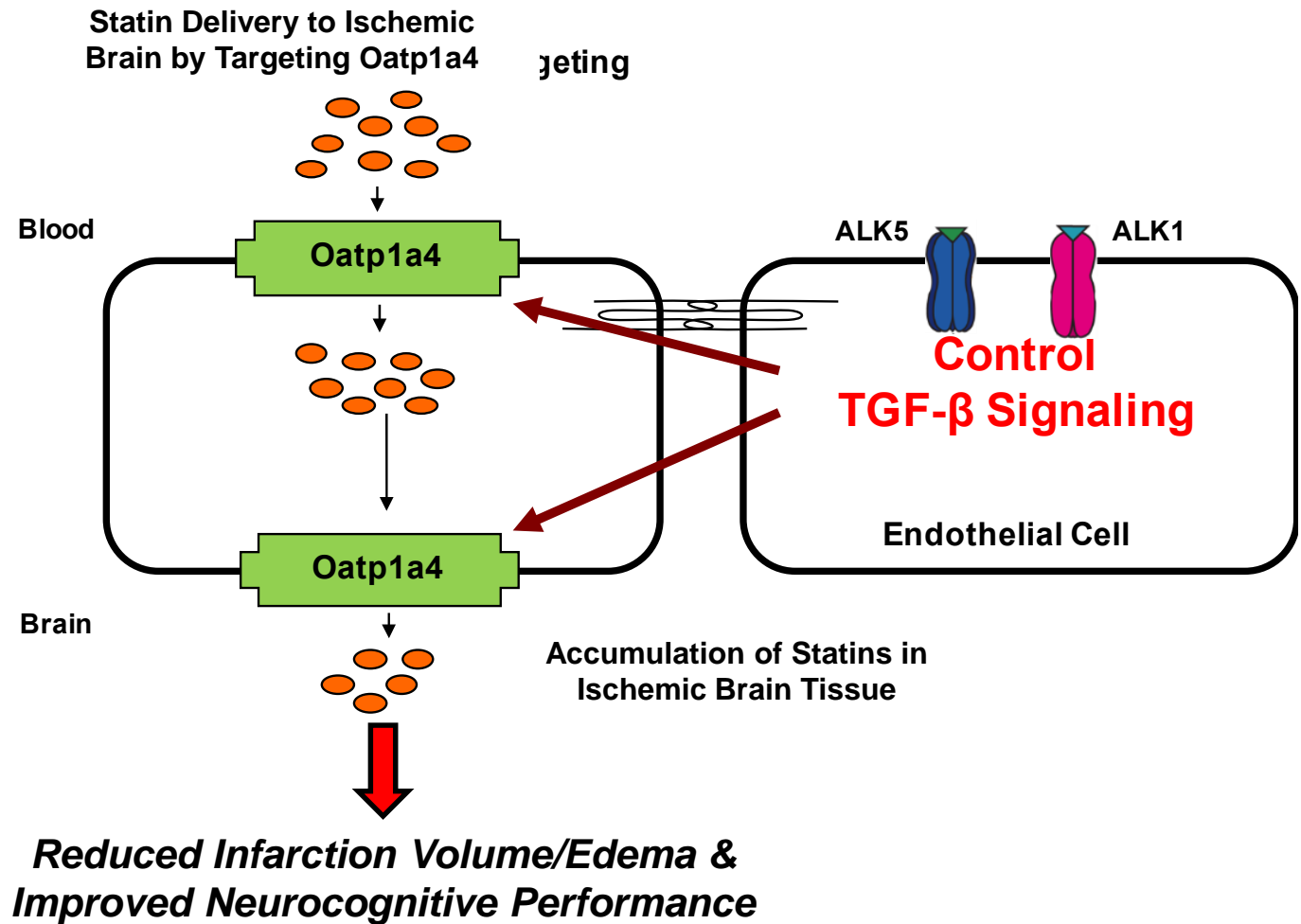
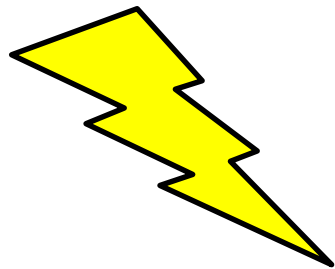
# Conclusions

- Our data shows, for the first time, that an uptake transporter (i.e., Oatp1a4) at the BBB is a *CRITICAL DETERMINANT* of atorvastatin neuroprotection in ischemic stroke.
  - Data are clinically relevant because they demonstrate that an endogenous BBB transporter is required for statins to be effective in stroke treatment.
- Results from this study emphasize the need to consider transport mechanisms in the development of neuroprotective treatment strategies for stroke.
- We have also identified a molecular pathway (i.e., TGF- $\beta$ /ALK1 signaling) that can be targeted to control Oatp-mediated delivery of statins to the brain
  - Opportunity to improve neuroprotective therapy with statins for stroke.



# Summary

**ISCHEMIC  
STROKE**



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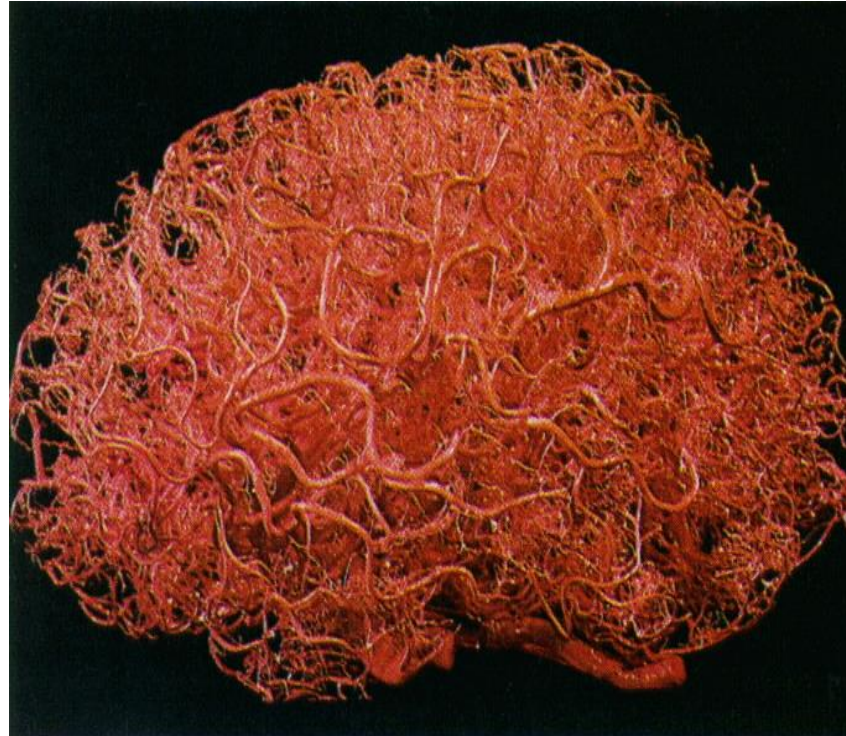
# Acknowledgements

## Arizona Blood-Brain Barrier Research Group

- Dr. Thomas P. Davis, Ph.D. (Professor & Collaborator)
- Dr. Tally Largent-Milnes, Ph.D. (Assistant Professor)
- **Dr. Jeffrey Lochhead, Ph.D. (Res. Asst. Professor)**
- Dr. Hrvoje Brzica, Ph.D. (Postdoctoral Fellow)
- **Dr. Wazir Abdullahi, Ph.D. (Ph.D. Student)**
- Qianying He, B.S. (Ph.D. Student)
- **Erica Williams, B.S. (Ph.D. Student)**
- Junzhi Yang, B.S. (Ph.D. Student)
- Tianhong Fu, B.S. (M.S. Student, Perfusion Sciences)
- Nicholas Hirsch, B.S. (M.S. Student, Perfusion Sciences)
- Raul Nava, B.S. (M.S. Student)
- Ayman Sami, B.S. (M.S. Student)
- Robert Betterton (Undergrad Res. Associate)
- Bianca Reilly (Undergrad Res. Associate)
- Samantha Serna (Undergrad Res. Associate)
- Joshua Stanton (Undergrad Res. Associate)



# Questions?



Zlokovic: Neurosurgery, Volume 43(4).October 1998.877-878