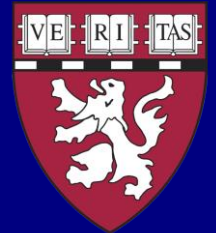


CEREBRAL VENOUS POTASSIUM EFFLUX DURING SPREADING DEPRESSION



JESSICA SEIDEL Ph.D.

FIELDS INSTITUTE: Workshop on CSD and Related Neurological Phenomena

JULY 7-11, 2014

OUTLINE

1. Introduction

- Hypothesis and Methods

2. Results

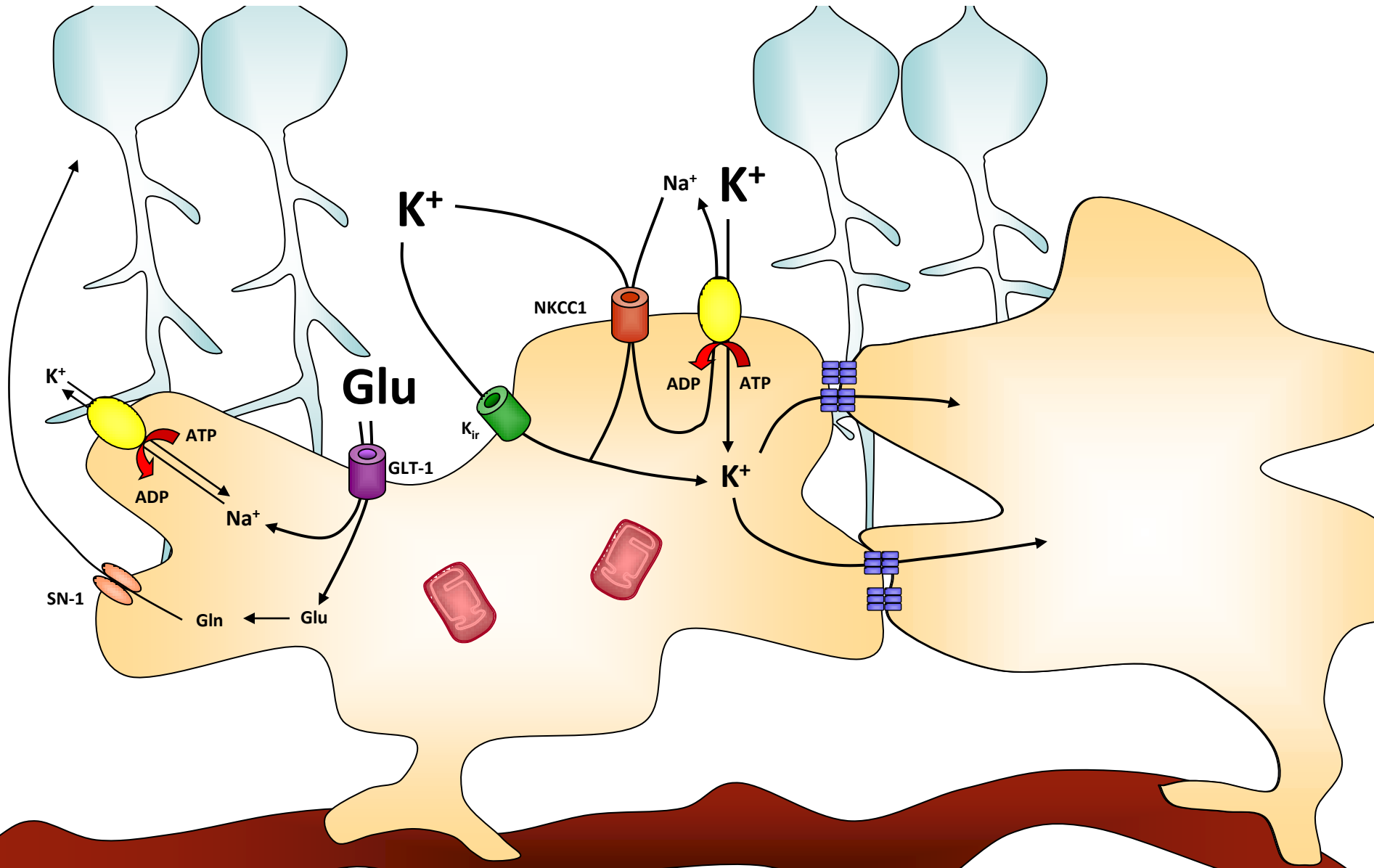
- Cortical and Venous K⁺ Recordings
- Models of Ischemia
 - Venous Thrombosis
 - dMCAo
- Role of Astrocytes in Vascular K⁺ Clearance
- Role of K⁺ Channels in Venous K⁺ Clearance

3. Future directions

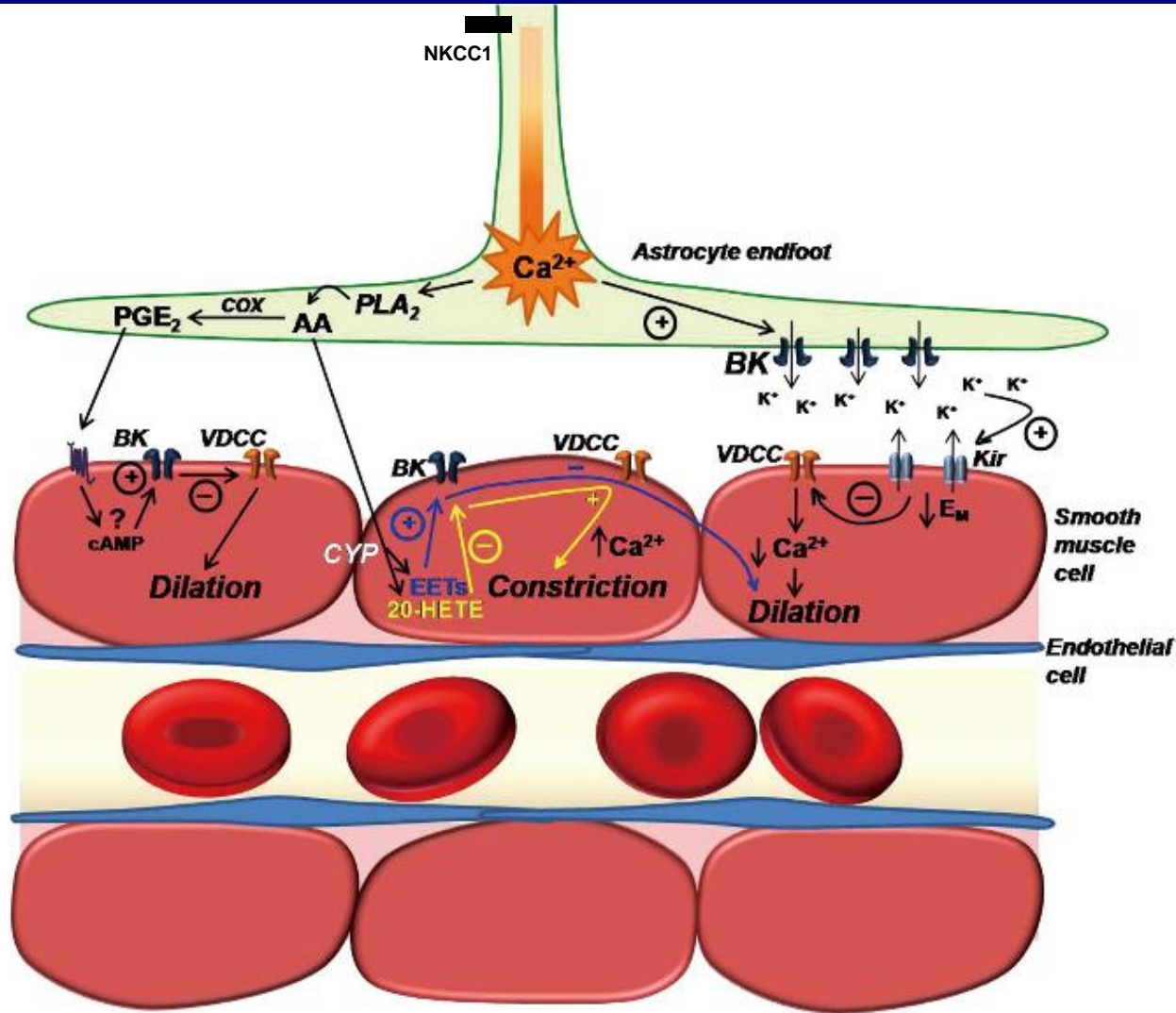
HYPOTHESIS

Clearance of extracellular K^+ post-SD is done by astrocytic siphoning into the vasculature.

ASTROCYTE CLEARANCE OF $[K^+]_e$



SIGNALLING BETWEEN ASTROCYTES AND VASCULATURE



OBJECTIVES

1. Measure $[K^+]_e$ in cerebral venous blood during SD under normoxic and ischemic conditions.
2. Determine the contribution of active K^+ uptake by astrocytes to the clearance of $[K^+]_e$ post-SD.

METHODS

ANIMALS: Mice (C57BL/6, male, 20-30 g)

SURGICAL PROCEDURE: Arterial Cannulation

NO Mechanical Ventilation

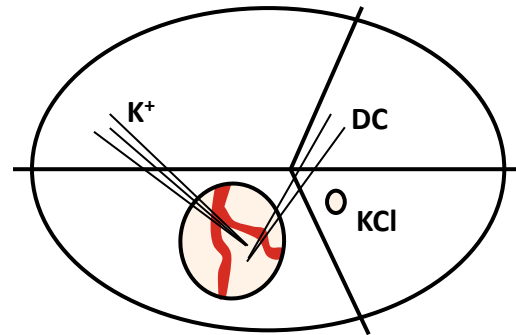
Arterial BP, pH, pO₂ and pCO₂ were monitored.

| n | Weight (g) | pH | pCO ₂ (mmHg) | pO ₂ (mmHg) | BP (mmHg) |
|----|------------|-------------|-------------------------|------------------------|-----------|
| 93 | 25 ± 2 | 7.35 ± 0.03 | 37 ± 4 | 108 ± 14 | 82 ± 10 |

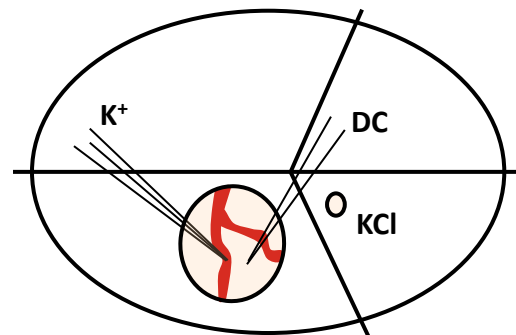
SD INDUCTION: Topical 300 mM KCl Application

METHODS

CORTICAL RECORDINGS: K^+ -sensitive and DC electrodes were $\sim 300 \mu\text{m}$ deep, equidistant to the KCl stimulation site.



VENOUS RECORDINGS: Made from pial veins $\sim 20 \mu\text{m}$ in diameter. DC electrode was $\sim 300 \mu\text{m}$ deep in the cortex, adjacent to the K^+ electrode.



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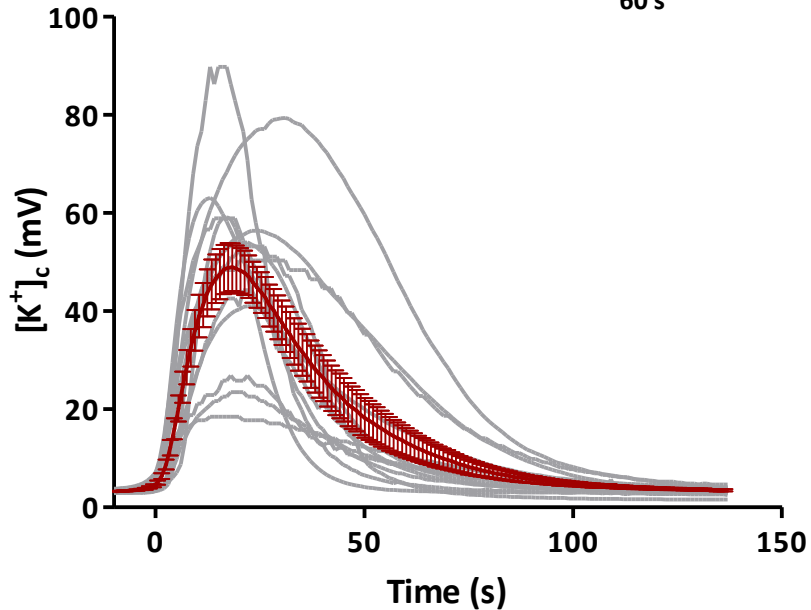
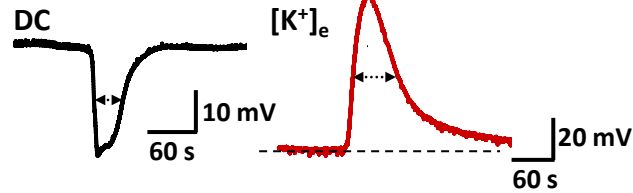
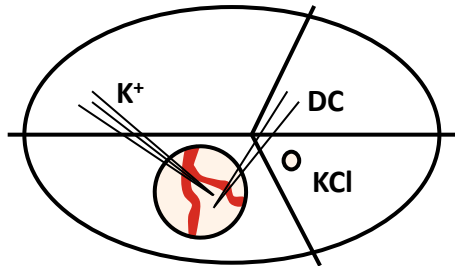
2. Results

- Cortical and Venous K⁺ Recordings
- Models of Ischemia
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- Role of Astrocytes in Vascular K⁺ Clearance
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3. Future directions

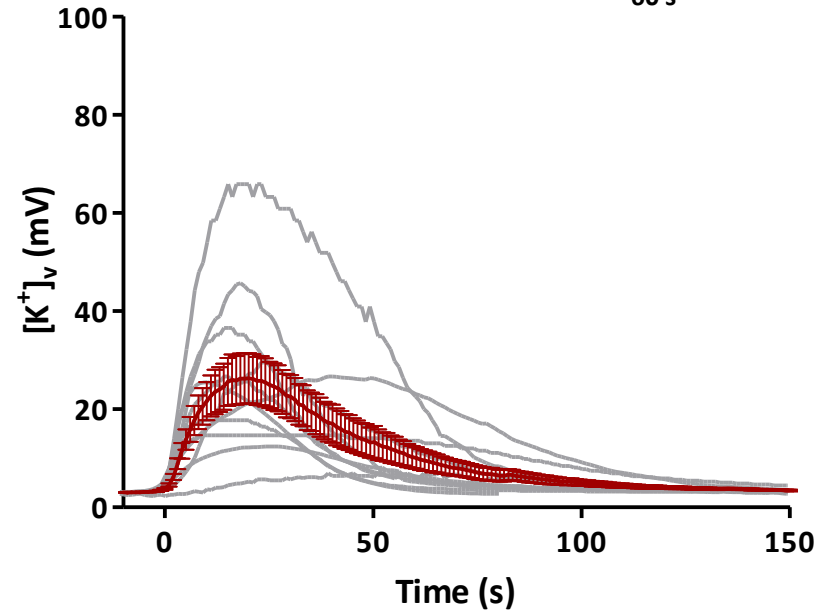
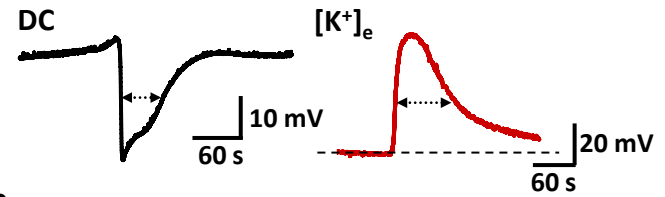
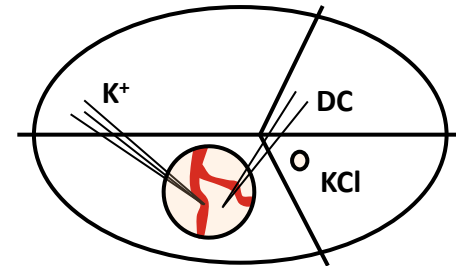
POTASSIUM RECORDINGS DURING SD

CORTEX



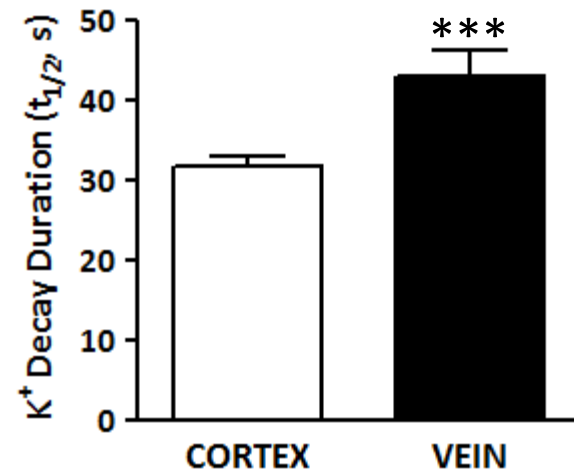
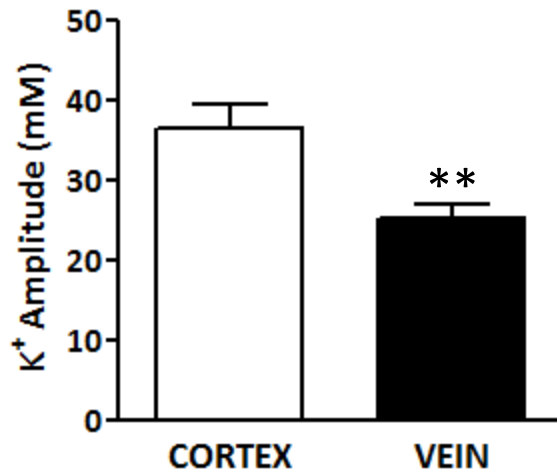
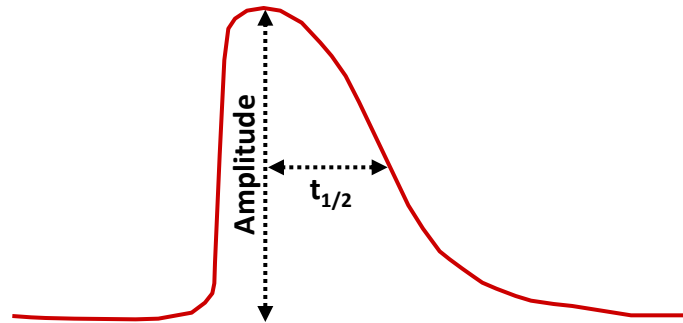
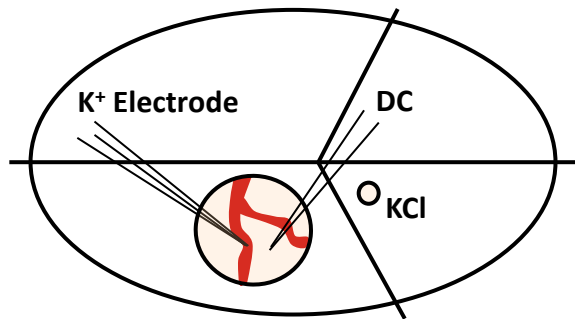
n=14

VEIN

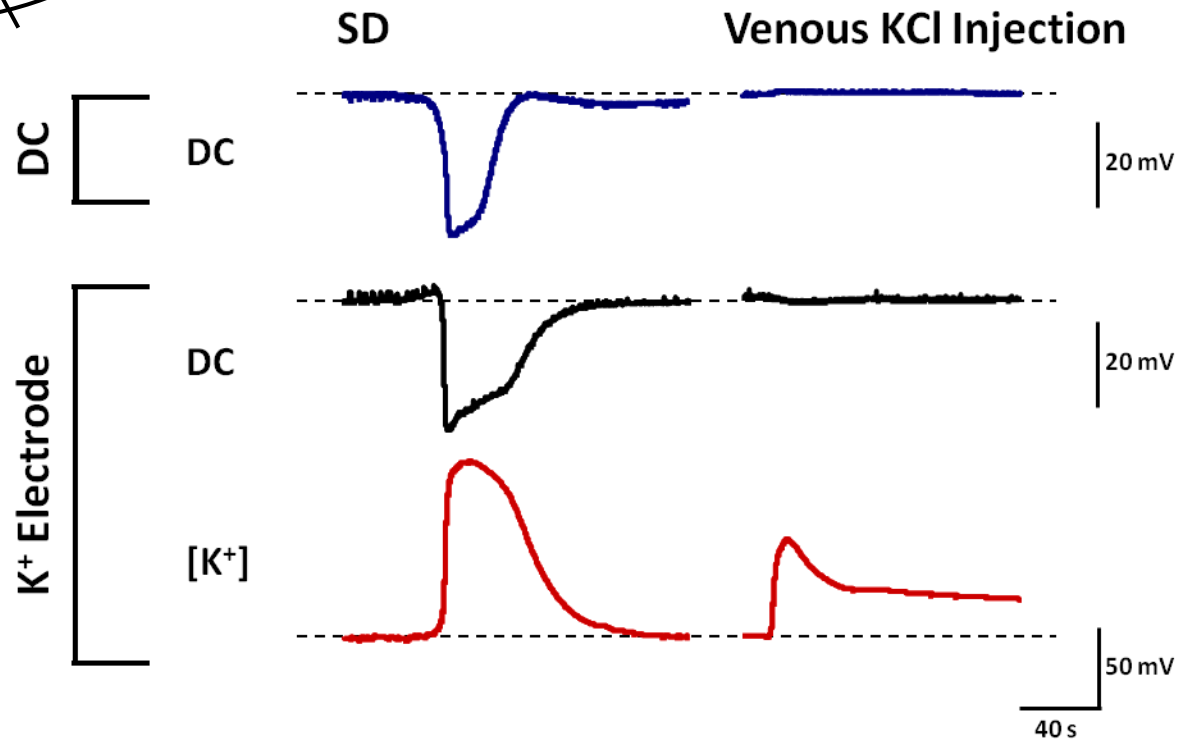
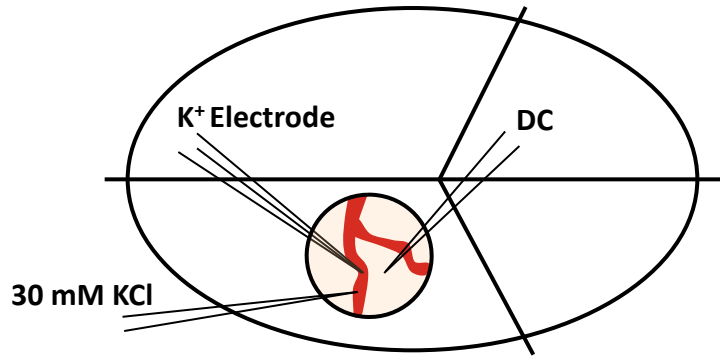


n=11

VENOUS RECORDINGS DURING SD

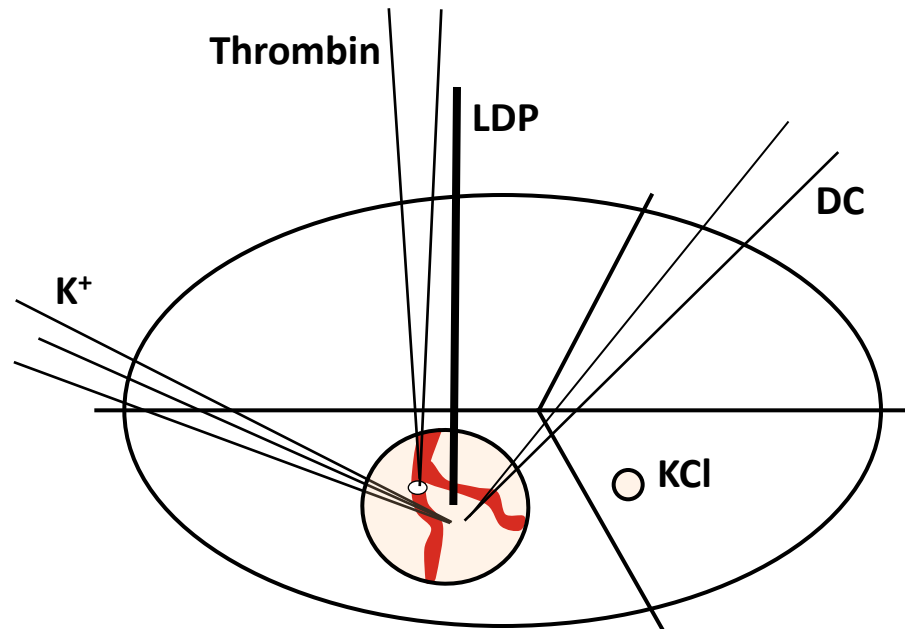


CONFIRM VENOUS RECORDINGS



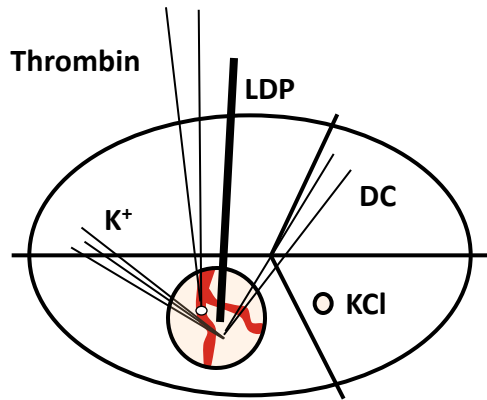
VENOUS THROMBOSIS

VENOUS THROMBOSIS: Thrombin (2500 i.u./ml) was injected into a pial vein (~1-2 mm from midline) using a glass micropipette. Clot was allowed to stabilize for 10 min prior to inducing SD.

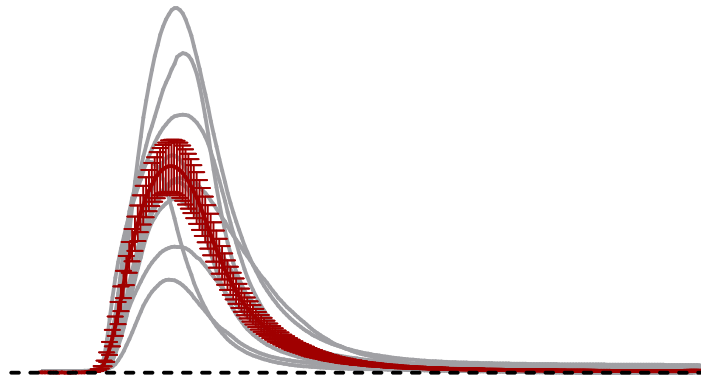


VENOUS THROMBOSIS

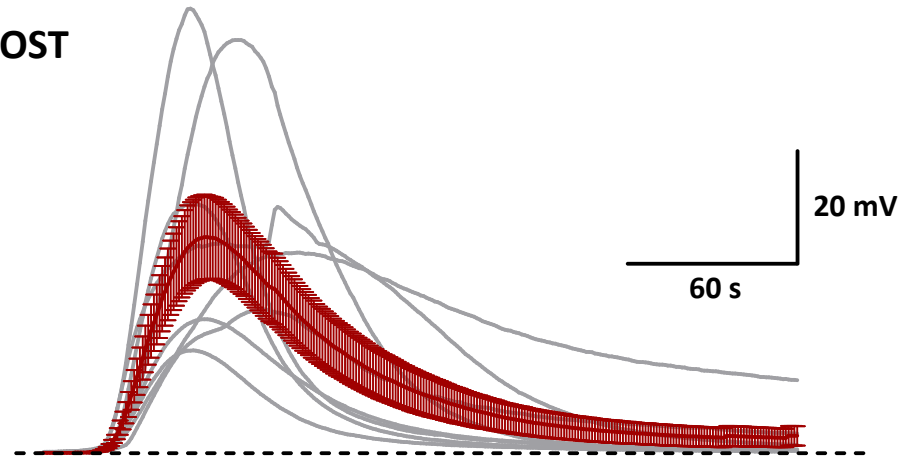
(Cortical K^+ Recordings)



PRE



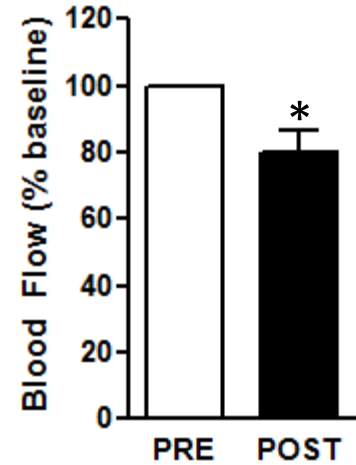
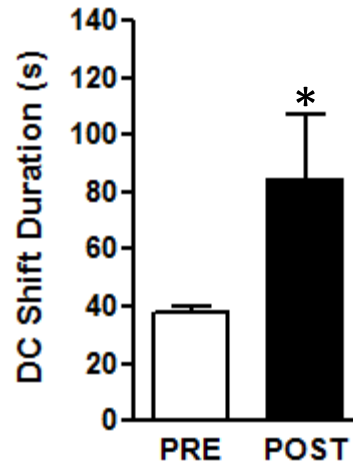
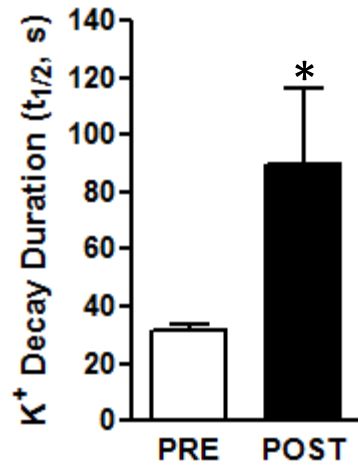
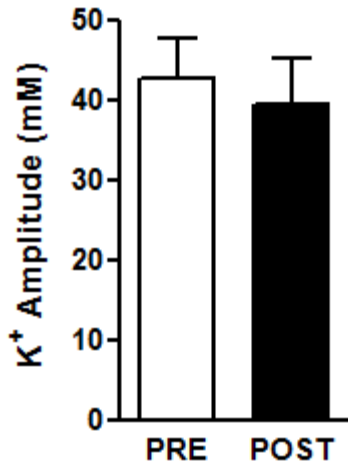
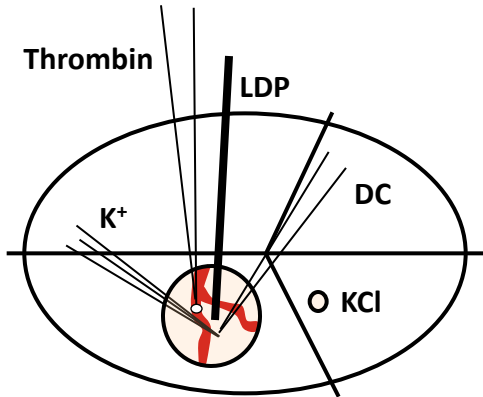
POST



Resting $[K^+]_e$ was increased by 0.72 ± 0.50 mM post-Thrombin injection.

VENOUS THROMBOSIS

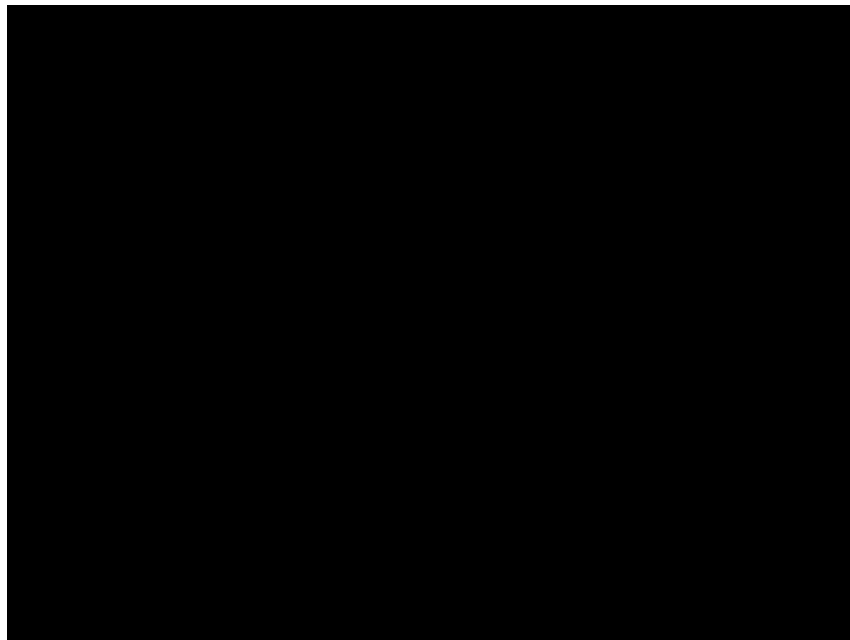
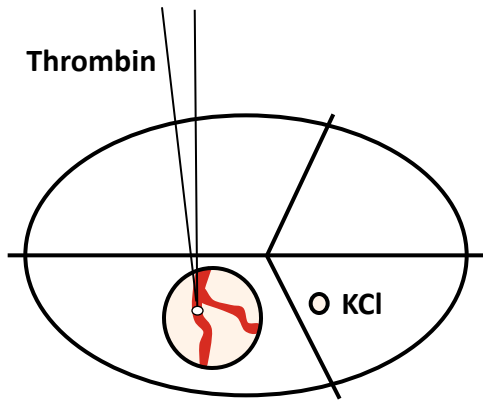
(Averaged Data)



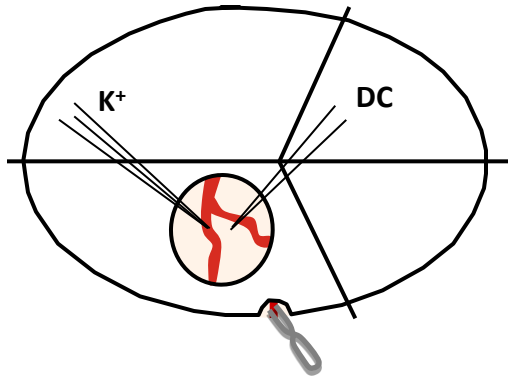
50% of all SD recorded post-Thrombin injection were spontaneous.

VENOUS THROMBOSIS

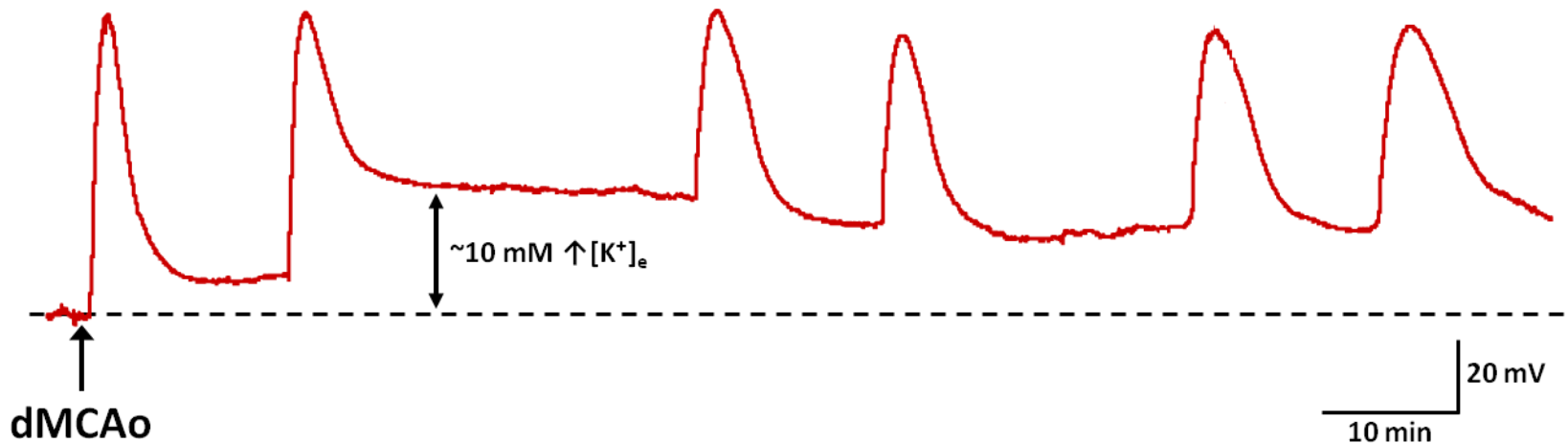
(Laser Speckle Flowmetry)



VENOUS CLEARANCE OF K^+ DURING FOCAL ISCHEMIA

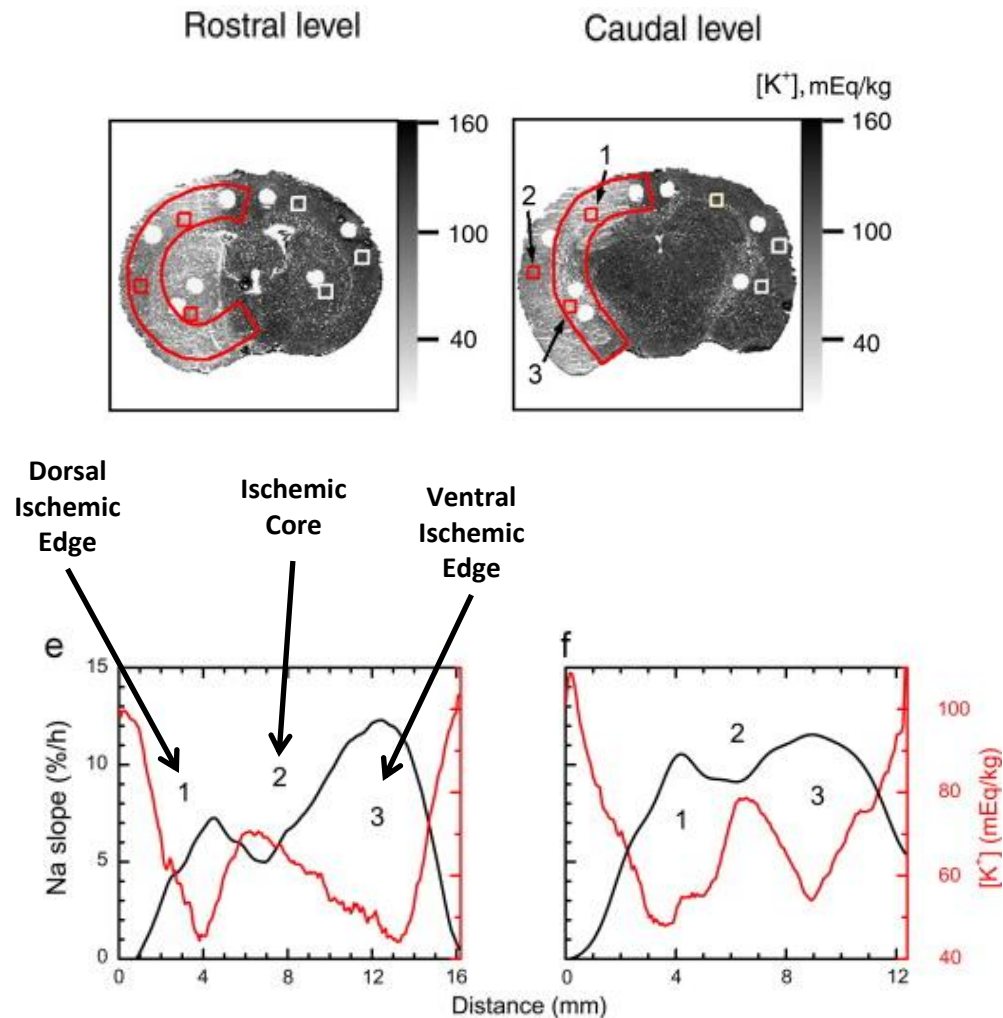


FOCAL ISCHEMIA: K^+ and DC electrodes were placed in the cortex prior to placing the clip on the dMCA. Spontaneous peri-infarct depolarizations (PIDs) were monitored for 2H.

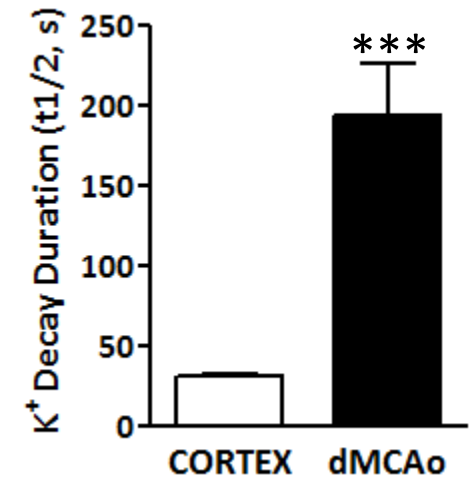
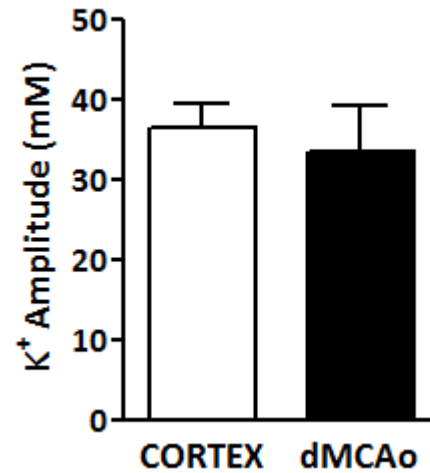
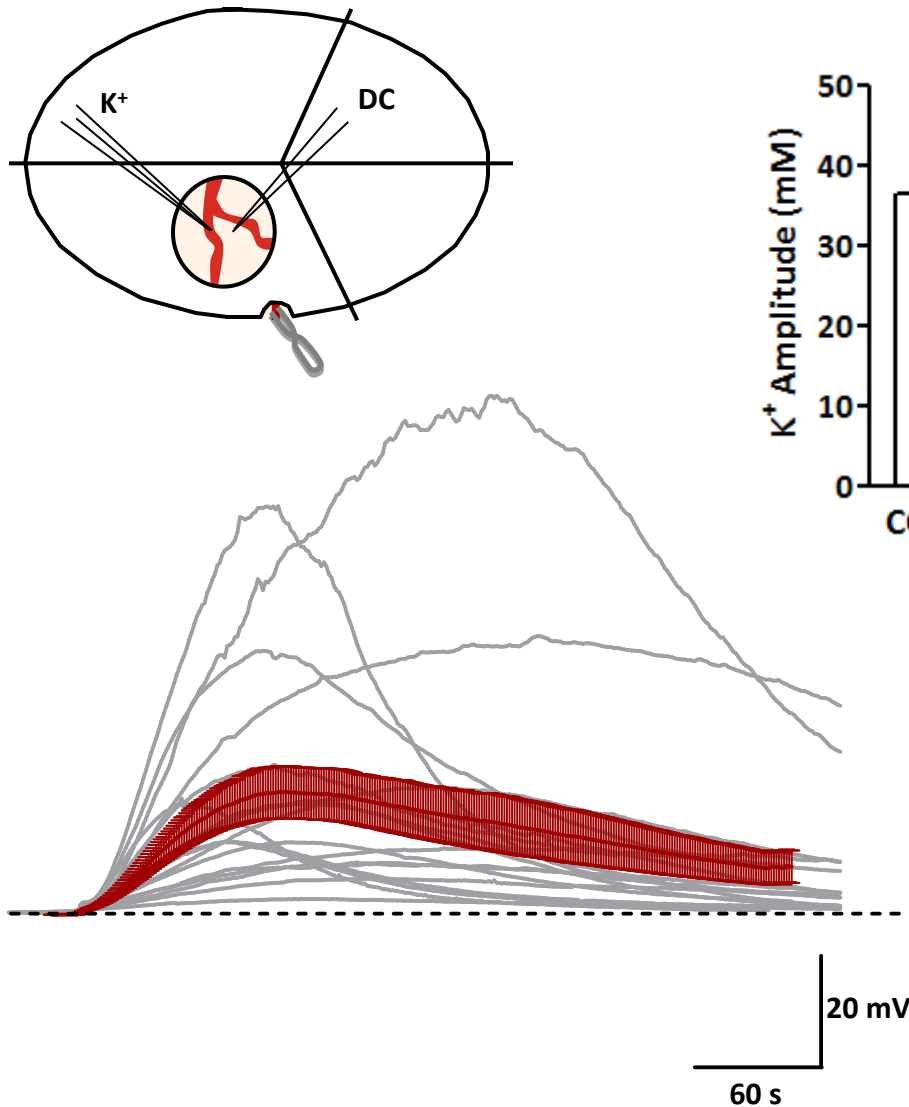


Average increase in baseline $[K^+]_e$ after the 1st PID was $8.4 \pm 7.1 \text{ mM}$.

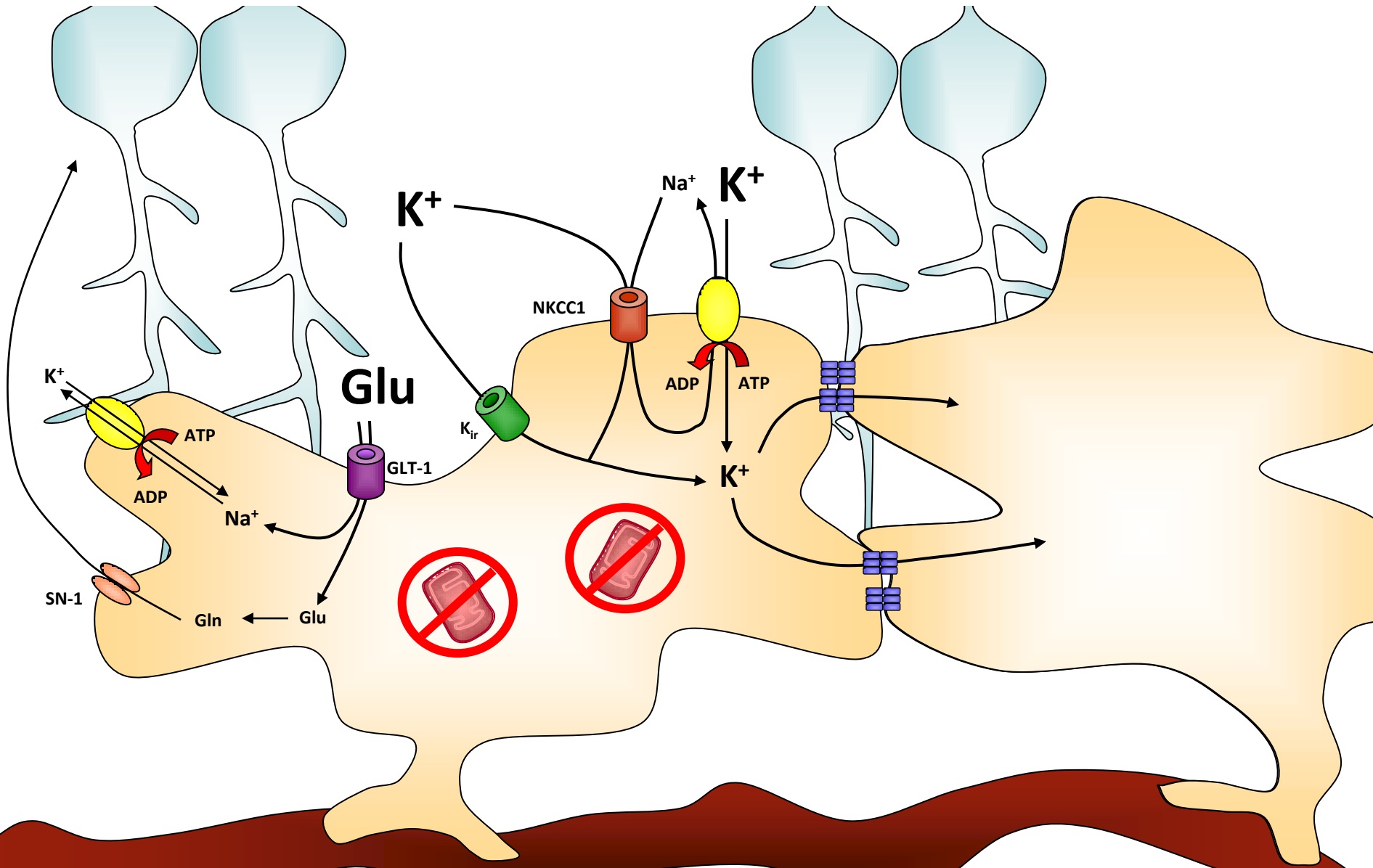
CHANGES IN TISSUE K^+ FOLLOWING ISCHEMIA



VENOUS CLEARANCE OF K^+ DURING FOCAL ISCHEMIA

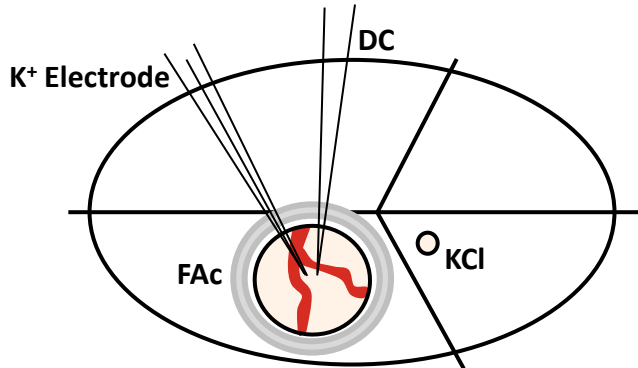


ASTROCYTE CLEARANCE OF $[K^+]_e$

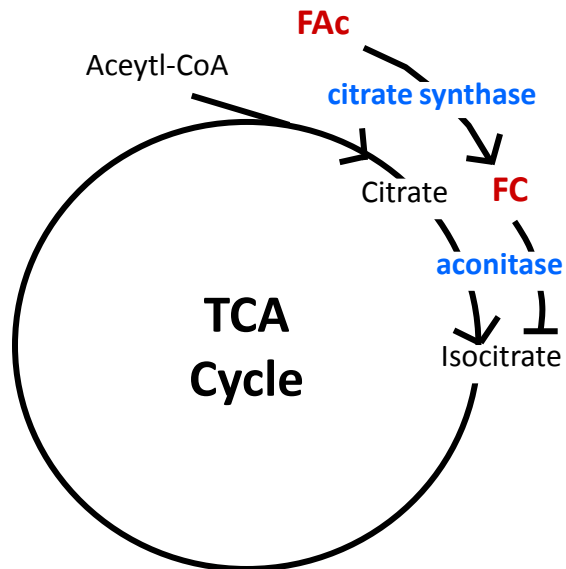
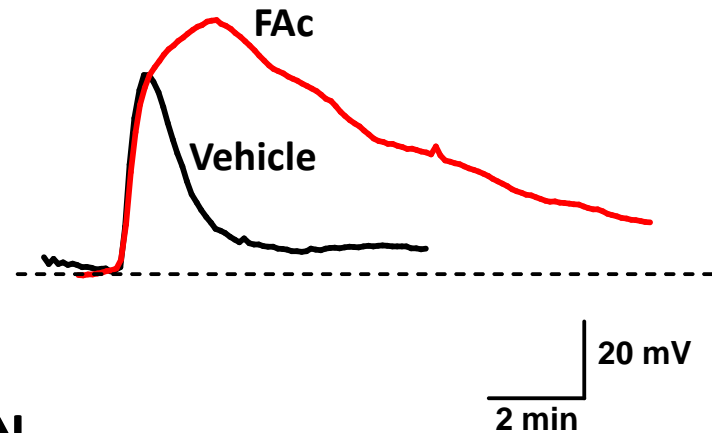


SELECTIVE INHIBITION OF ASTROCYTE OXIDATIVE METABOLISM

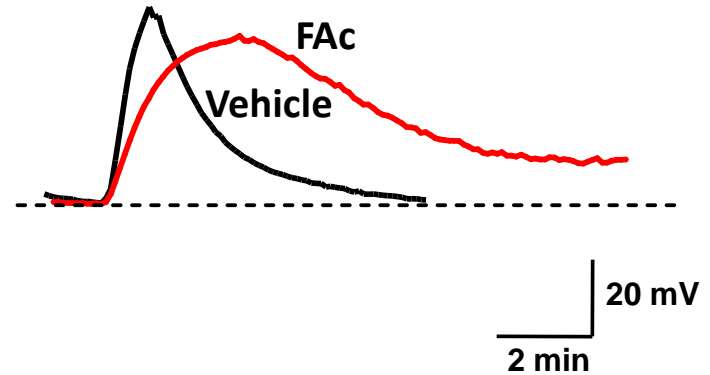
(FAc: 10 mM, 90 min)



CORTEX

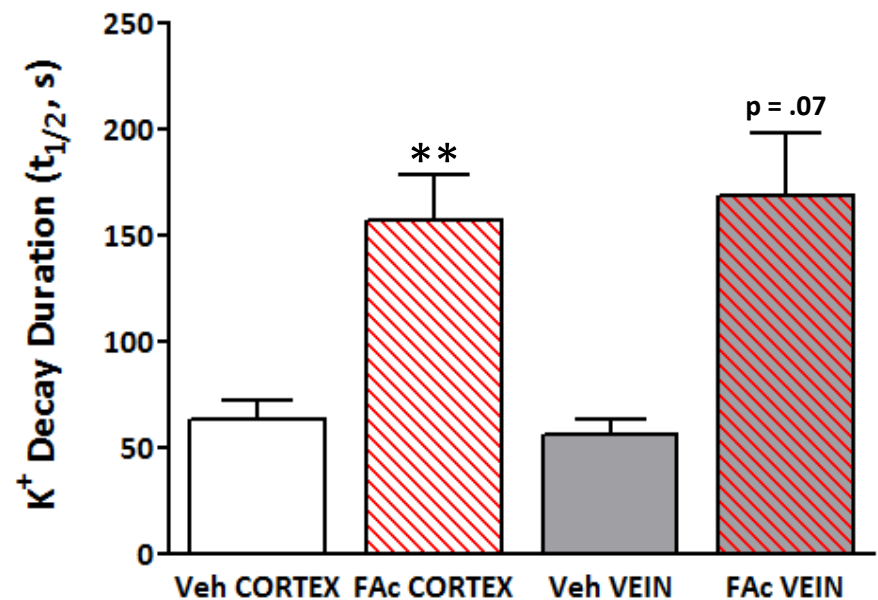
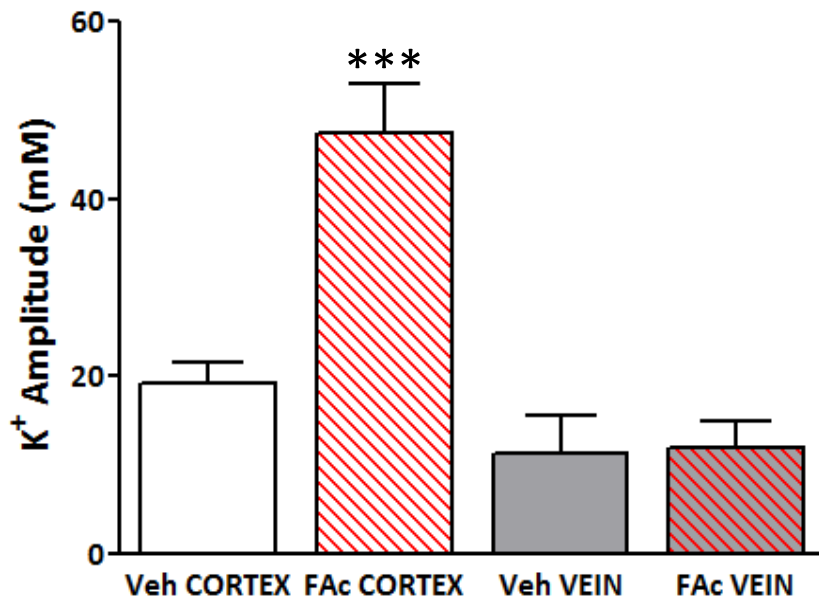
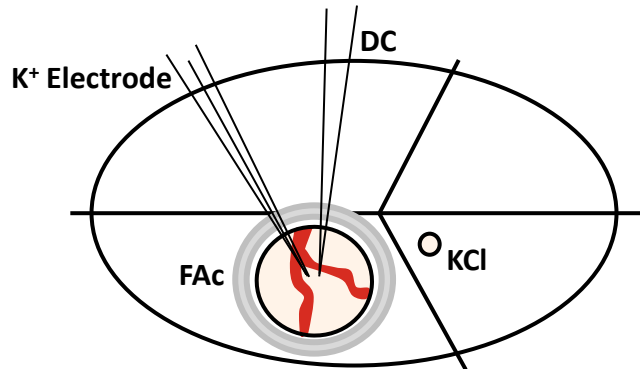


VEIN

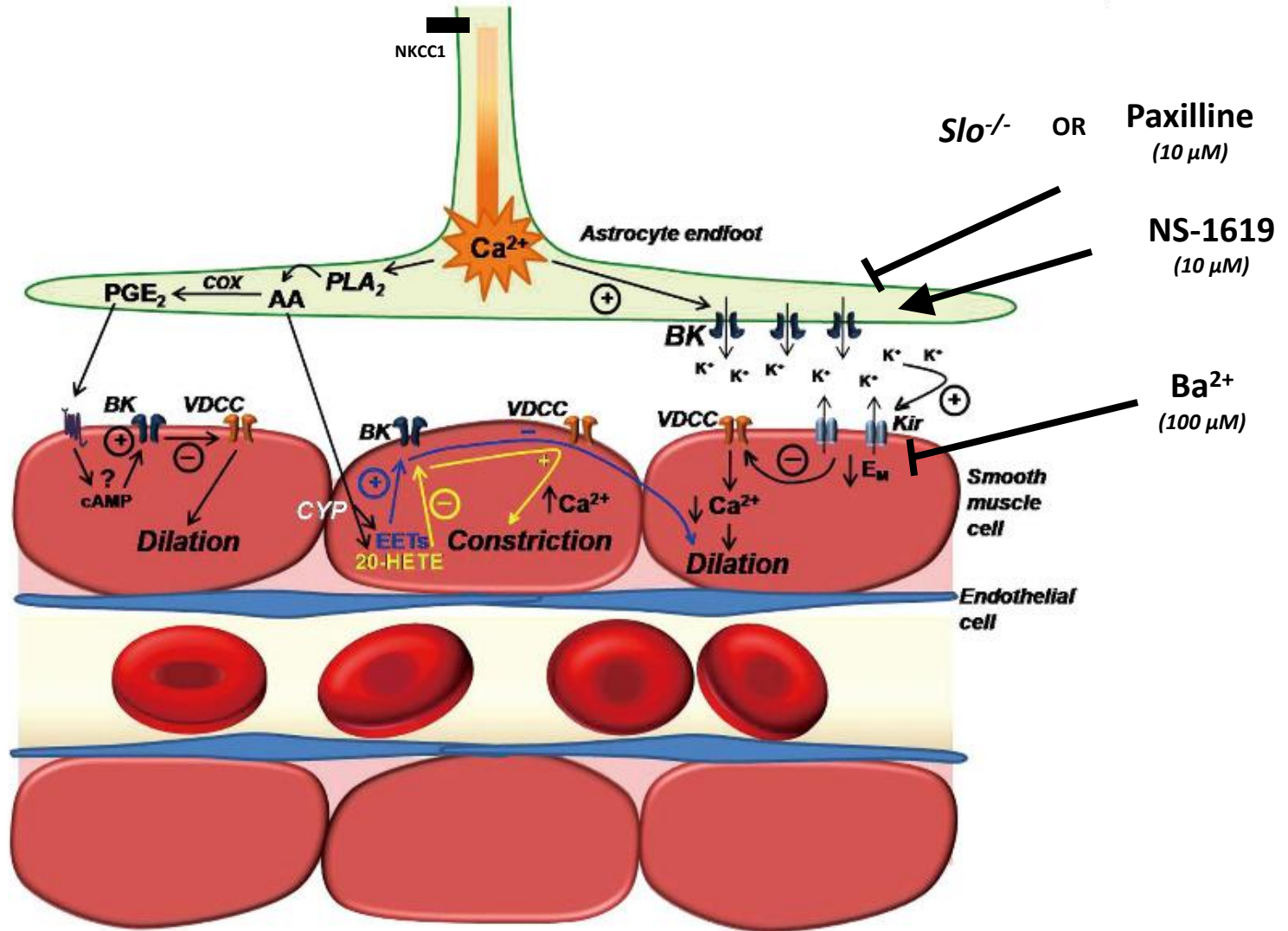


SELECTIVE INHIBITION OF ASTROCYTE OXIDATIVE METABOLISM

(FAc: 10 mM, 90 min)

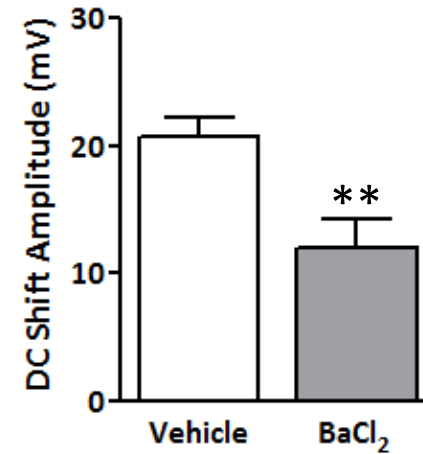
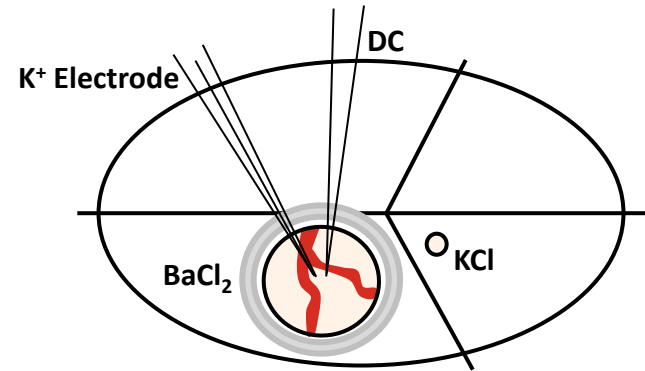
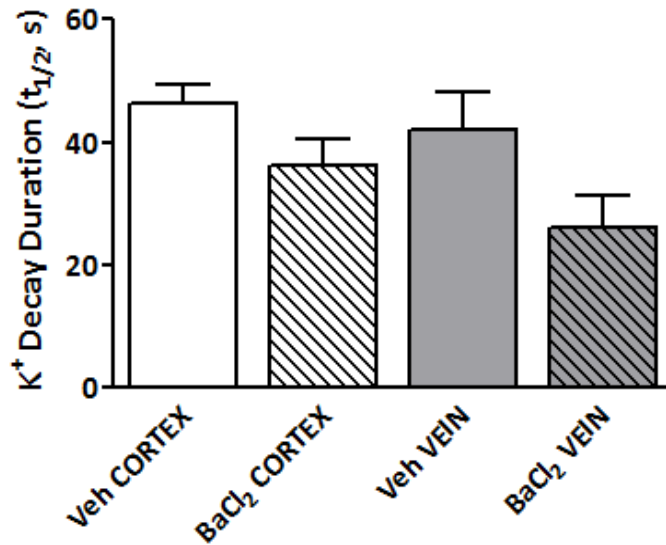
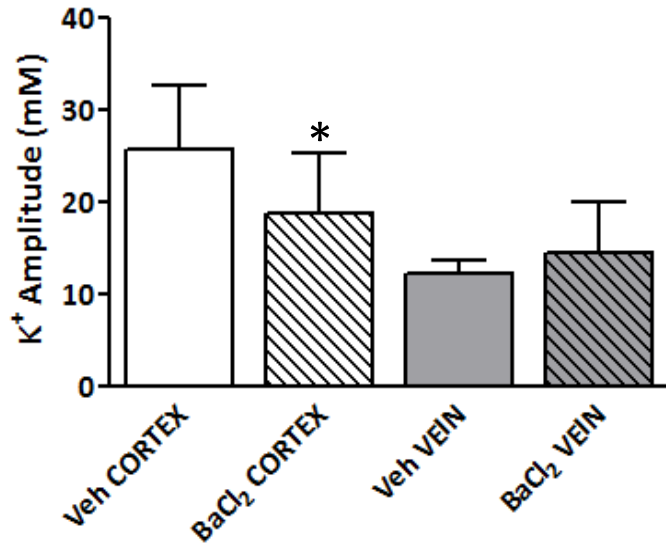


K⁺ SIGNALLING BETWEEN ASTROCYTES AND VASCULATURE



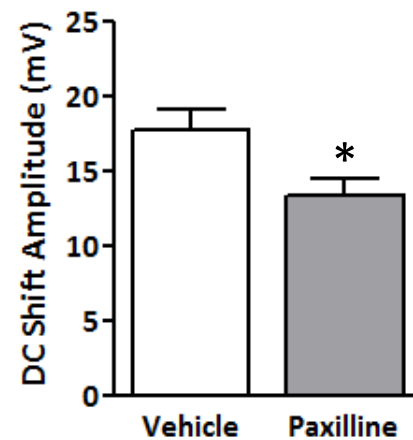
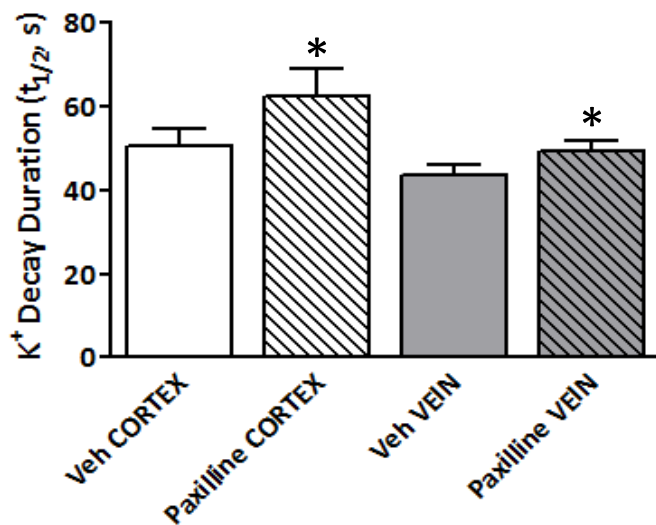
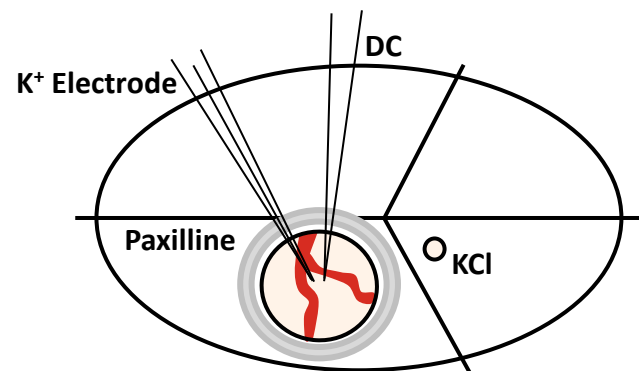
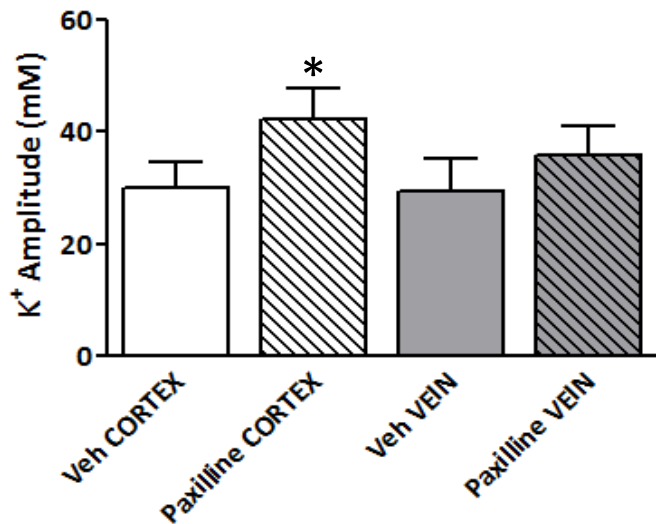
BaCl₂: K_{ir} ANTAGONIST

(100 μM, 30 min)

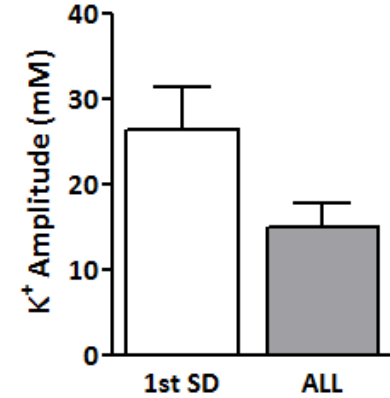
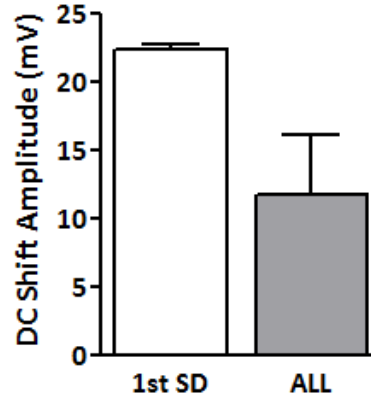
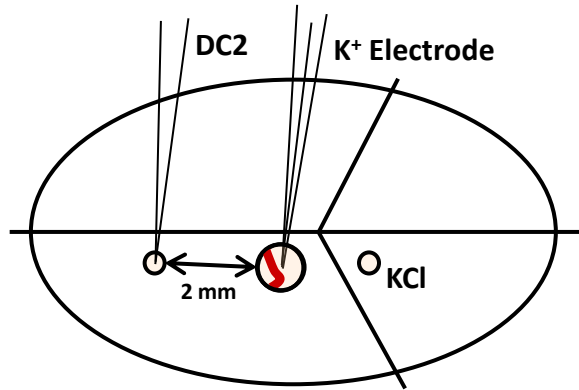


PAXILLINE: BK CHANNEL ANTAGONIST

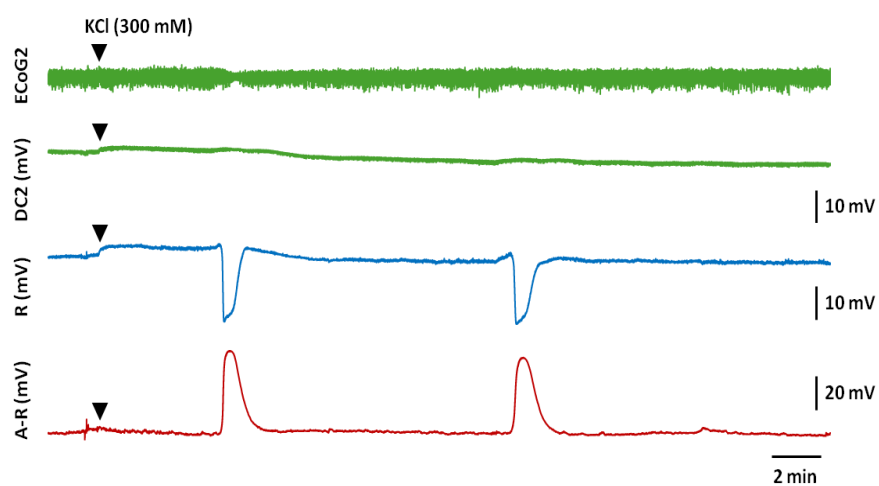
(0.5-20 μ M, 30 min)



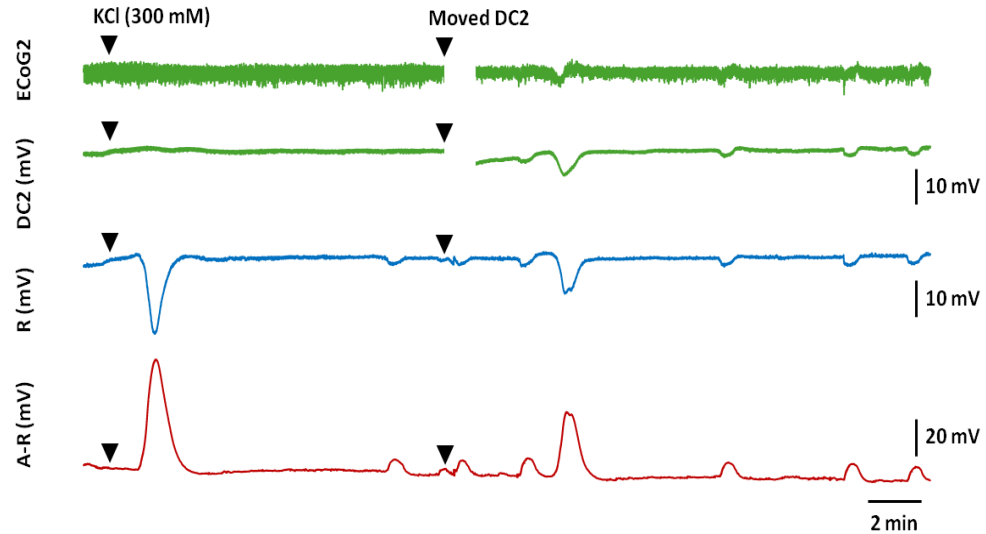
BK KNOCKOUT MOUSE (*Slo^{-/-}*)



VEIN

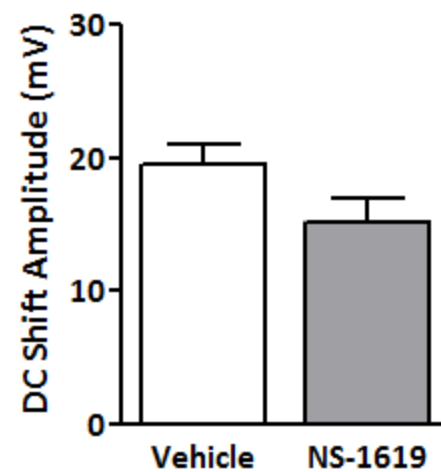
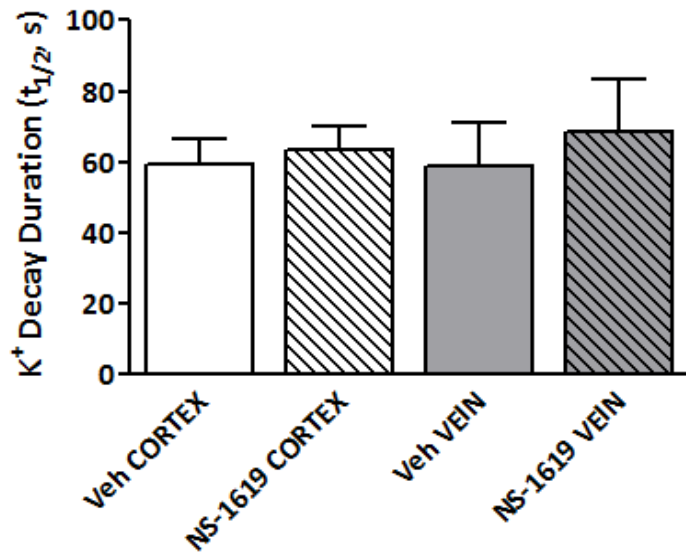
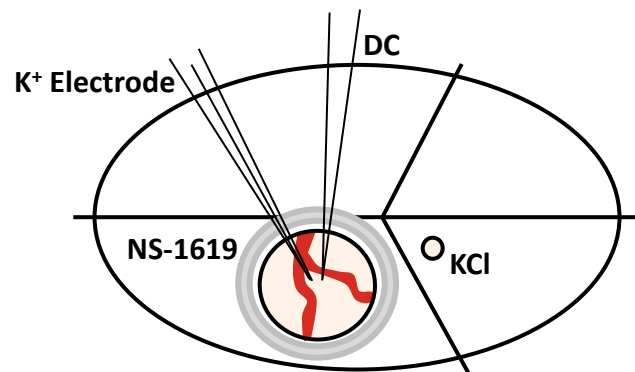
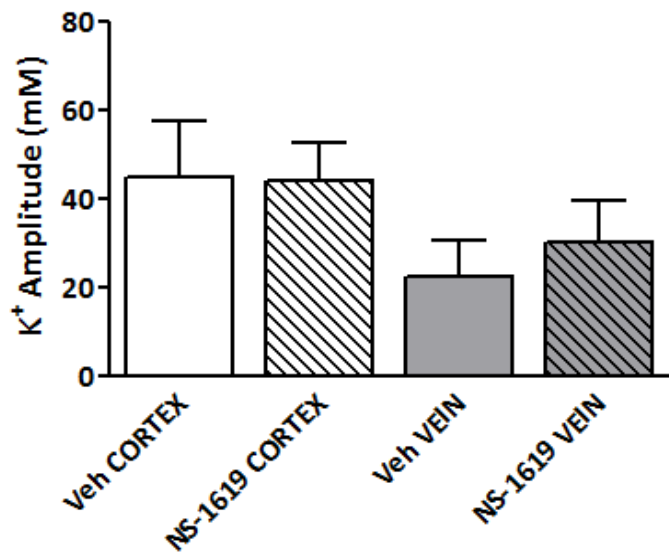


CORTEX



NS-1619: BK CHANNEL AGONIST

(10 μ M, 30 min)



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- Role of Astrocytes in Vascular K⁺ Clearance
- Role of K⁺ Channels in Venous K⁺ Clearance

3. Future directions

FUTURE DIRECTIONS

- 1. Can we determine at what level K^+ is entering the vasculature?**
 - Use *in vivo* TPM to image vascular increases in K^+ .
 - Asante Potassium Green (APG2)
 - Good photostability
 - Better K^+ selectivity (over Na^+) than previous indicators
 - Rapid loss of dye *in vivo* (i.a. injection)
- 2. Are there differences in K^+ clearance in different regions of the brain during focal ischemia/stroke?**
 - Simultaneously with LSF
 - Determine if there are differences in K^+ clearance in penumbral vs. non-ischemic tissue.

ACKNOWLEDGEMENTS

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The Lab

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Fanny Herisson

Qin Tao

Daniel Von Bornstädt*

Ying Wei

Nilufer Yalcin

Esther Yu

Yi Zheng

Funding

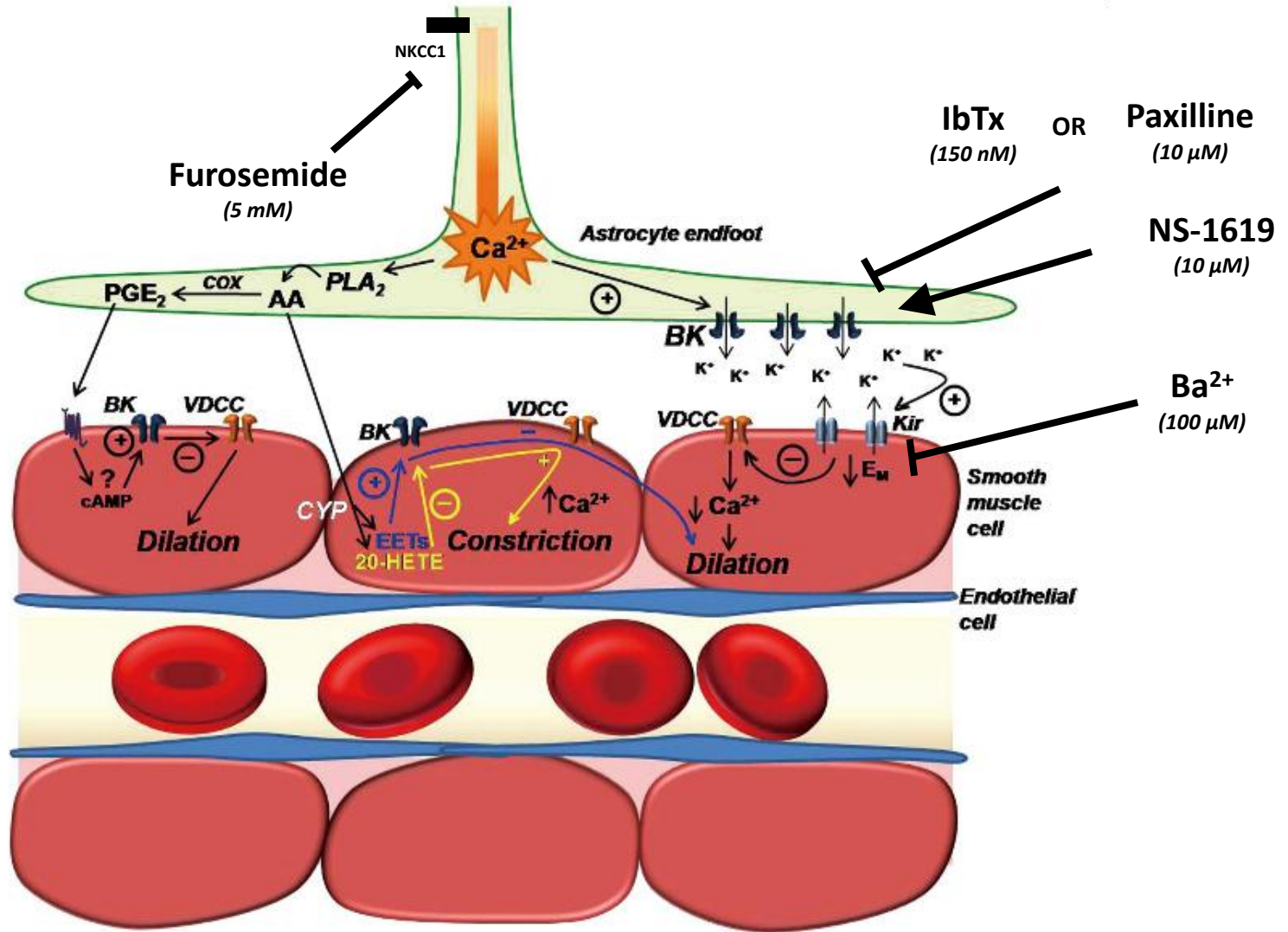
AHA Postdoctoral Fellowship Award, Seidel (PI)

MGH Internal Sundry: Heitman Foundation, Ayata (PI)

NINDS: 2P50NS051343-06, Ayata (PI Project 2)

Leducq Foundation: Transatlantic Centers of Excellence Award 2012D000293, Ayata (PI MGH)

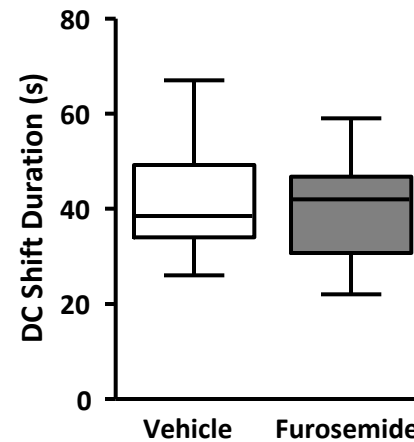
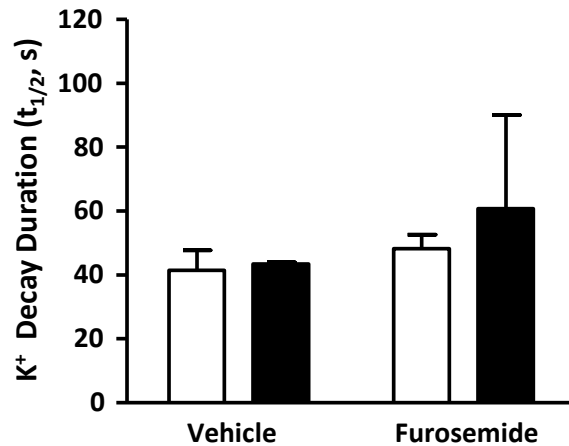
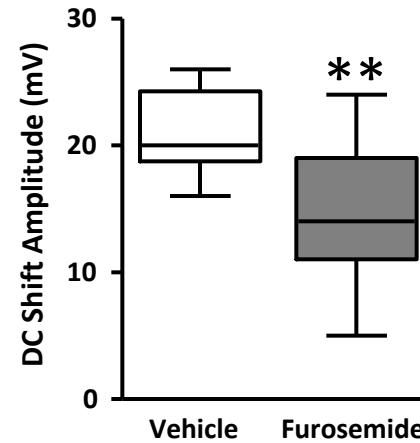
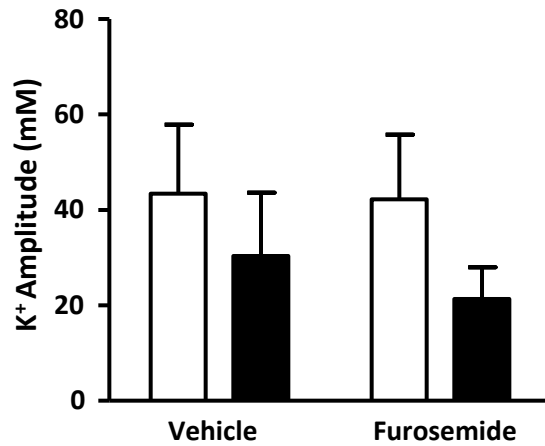
K⁺ SIGNALLING BETWEEN ASTROCYTES AND VASCULATURE



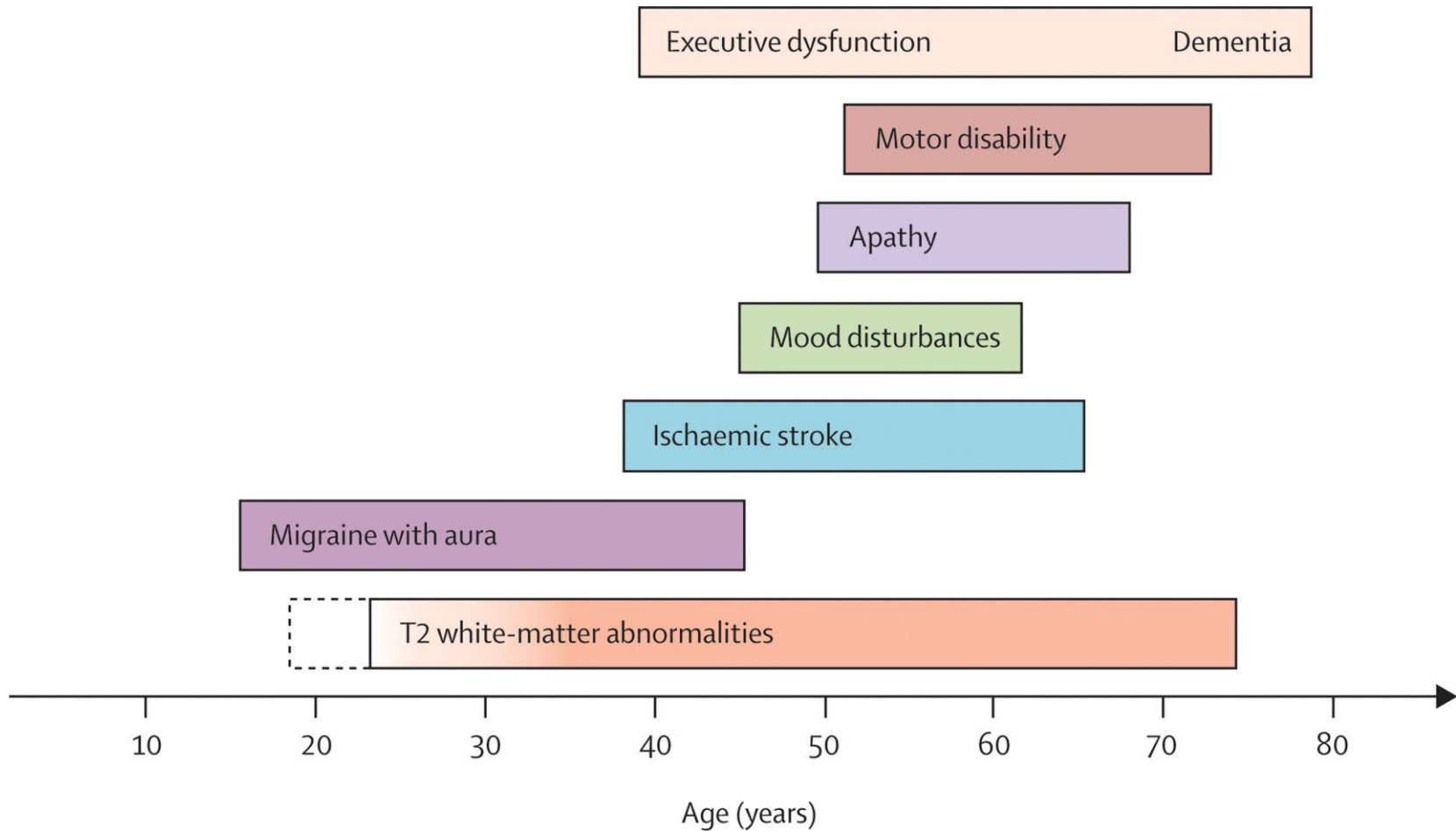
FUROSEMIDE: NKCC1 ANTAGONIST

(5 mM, 30 min)

□ CORTEX (n=5)
■ VEIN (n=5)



CLINICAL MANIFESTATION OF CADASIL

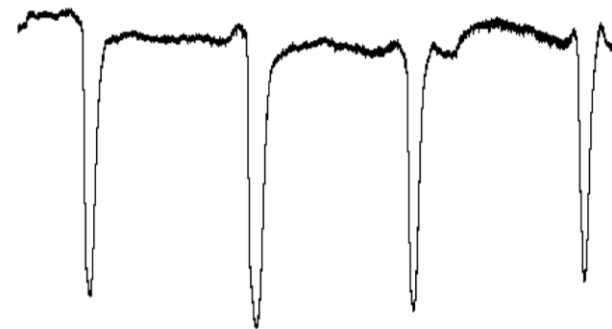
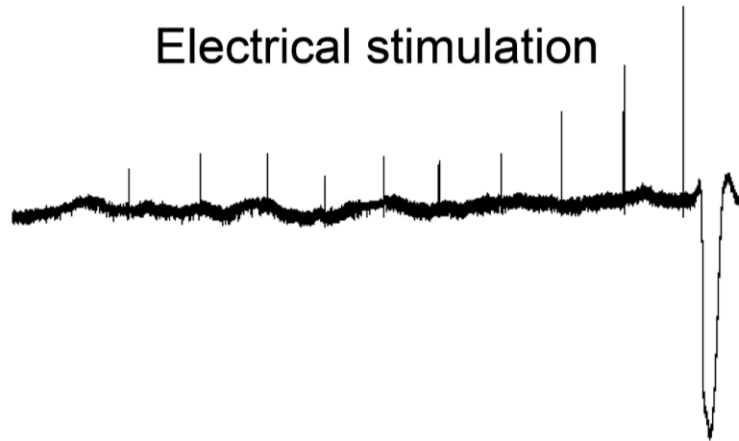


SD SUSCEPTIBILITY INCREASED IN CADASIL MUTANTS

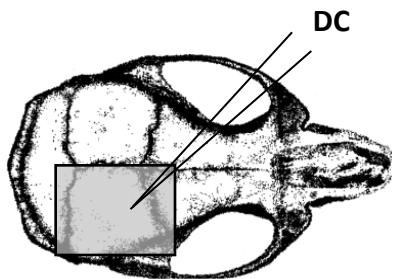
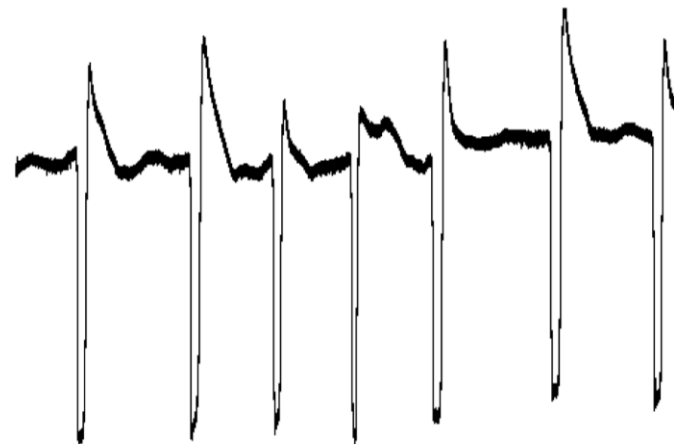
Electrical stimulation

KCl stimulation

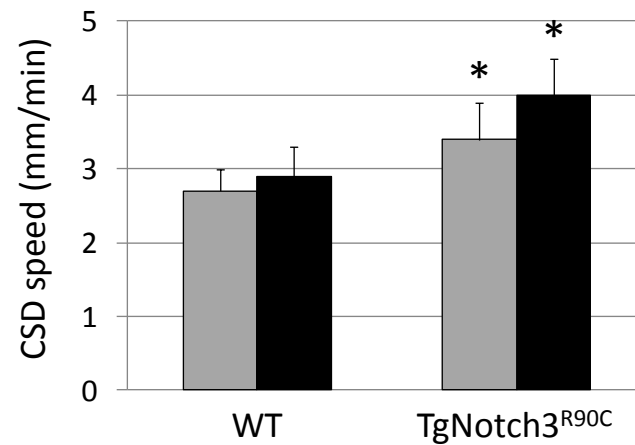
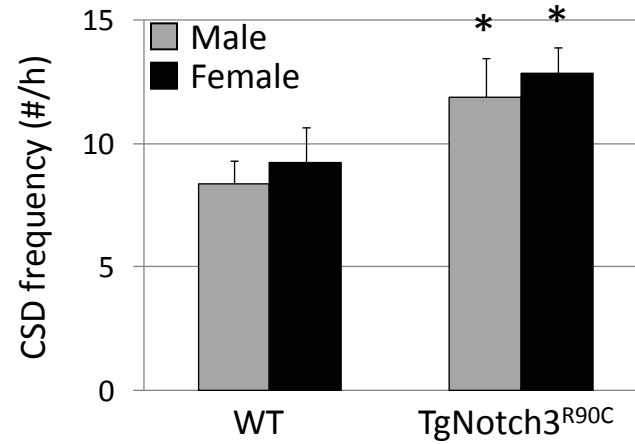
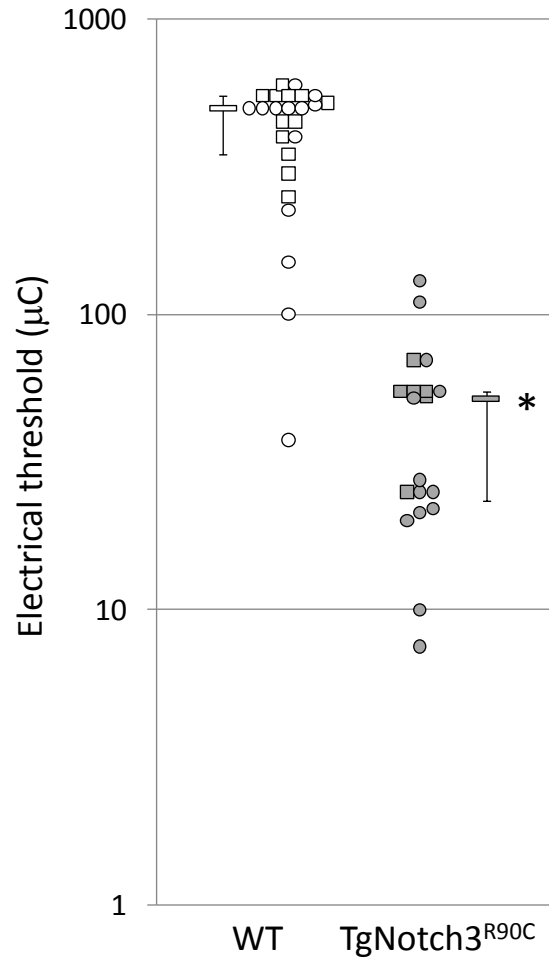
WT



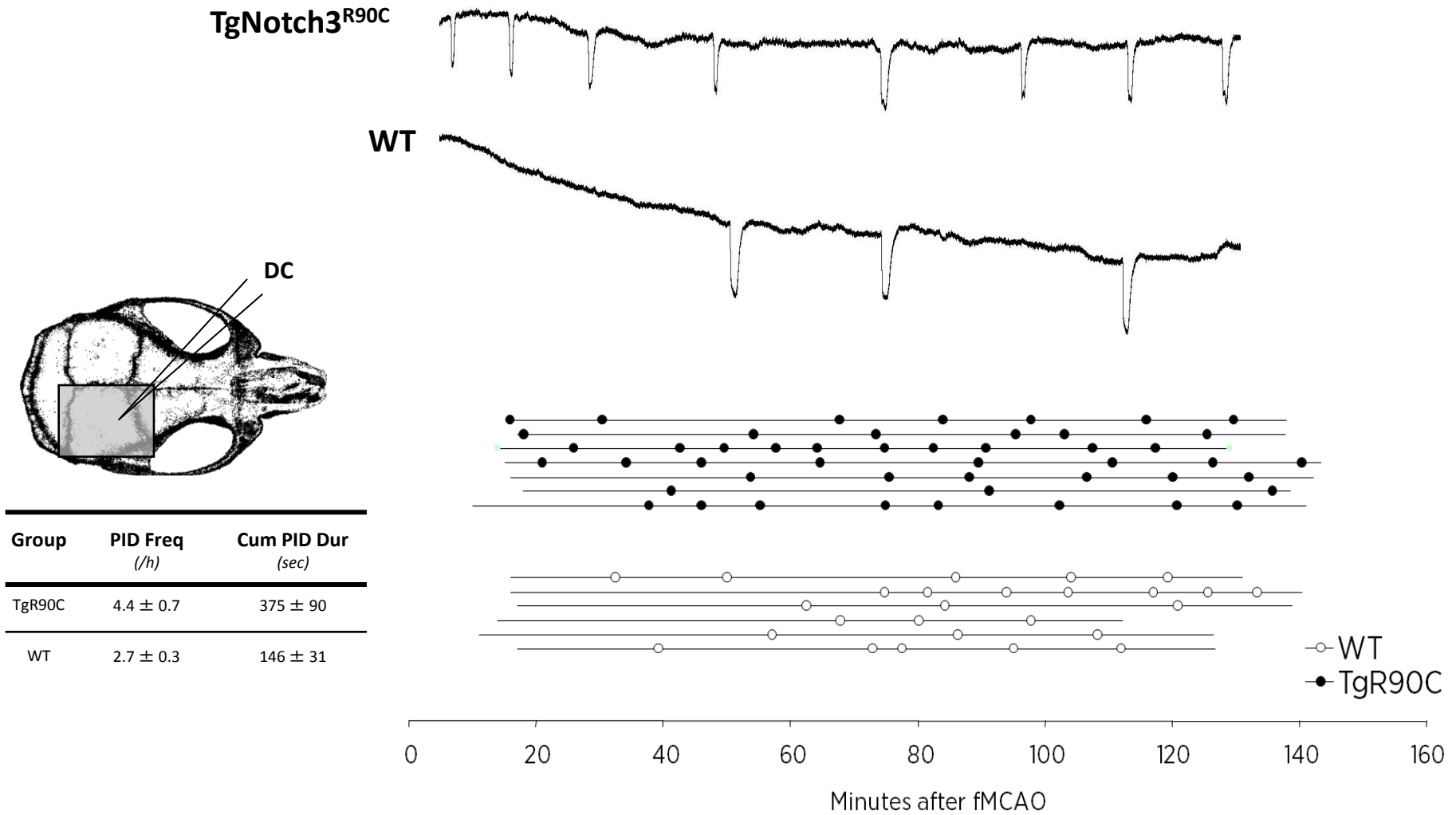
TgNotch3^{R90C}



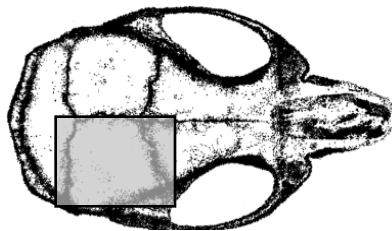
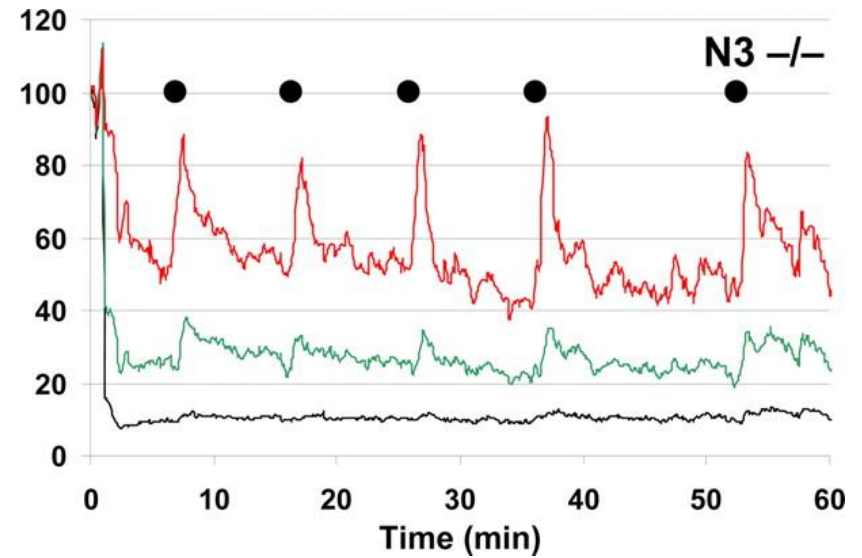
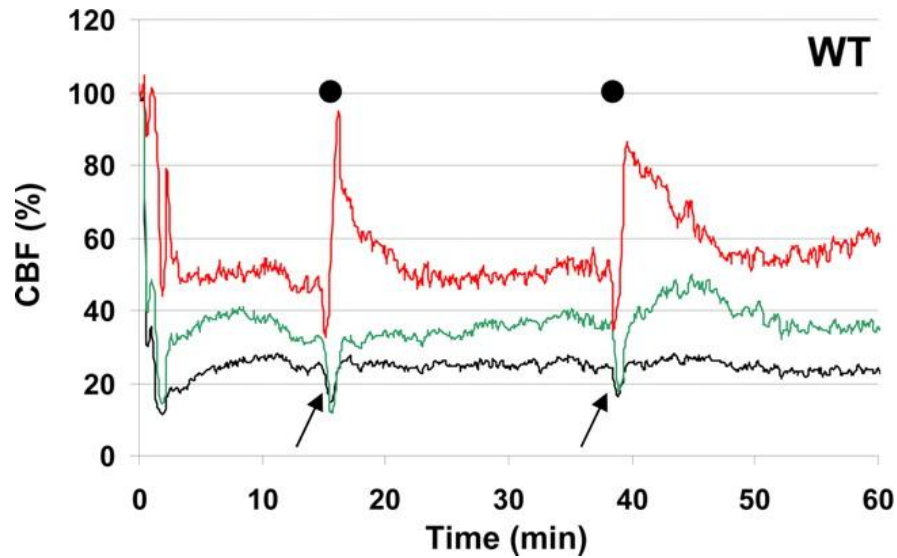
SD SUSCEPTIBILITY INCREASED IN CADASIL MUTANTS



INCREASED PID FREQUENCY IN CADASIL MUTANTS



INCREASED PID FREQUENCY IN NOTCH3^{-/-}



LSF

| Group | PID Freq (/h) |
|------------------------|---------------|
| Notch 3 ^{-/-} | 6.0 ± 2.5 |
| WT | 2.9 ± 2.5 |