Development and Validation of a Wearable Device for Continuous Assessment and Measurement of Sleep-Associated Cranial Fluid Dynamics and EEG

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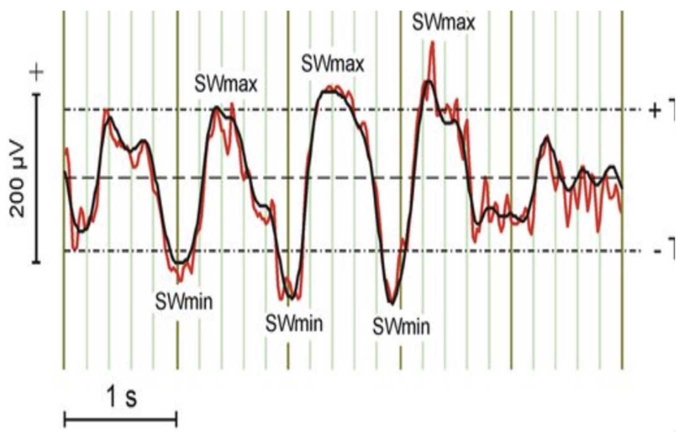
DISCLOSURE

- Paul Dagum, MD PhD; Laurent Giovangrandi, PhD; and Jacob Winebaum are employed by Applied Cognition and own stock in the company
- Jeffrey Iliff, PhD and Miranda Lim, MD PhD receive compensation from Applied Cognition and hold stock options in the company

Glymphatic Flow: A New Biology of the Brain

The newly discovered glymphatic system plays a critical role in the clearance of neurodegenerative proteins and metabolic waste products

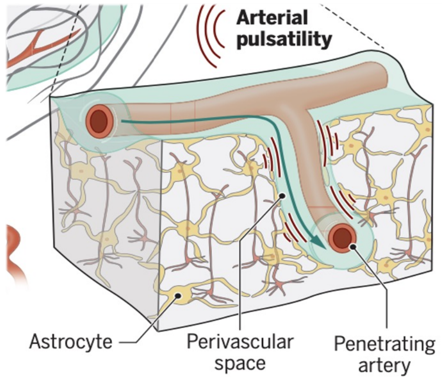


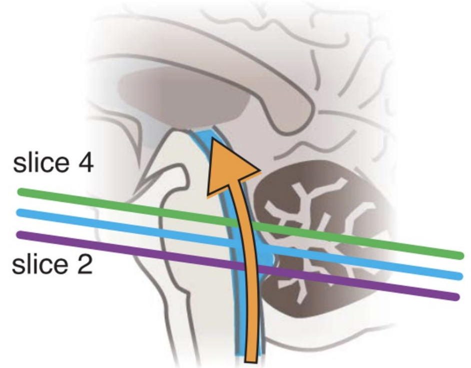
1 Slow Wave Activity

The cleaning power of **slow wave activity (SWA)** during deep sleep is augmented by a 60% increase in the interstitial fluid (ISF) volume created via AQP-channels.

2 Arterial Pulsatility

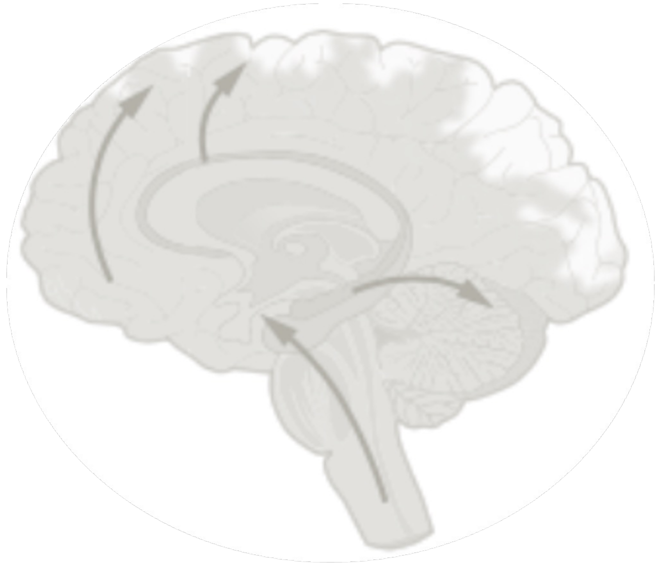
Arterial pulsatility in the brain provides the motive force that moves cerebrospinal fluid (CSF) into the perivascular spaces surrounding major arteries.



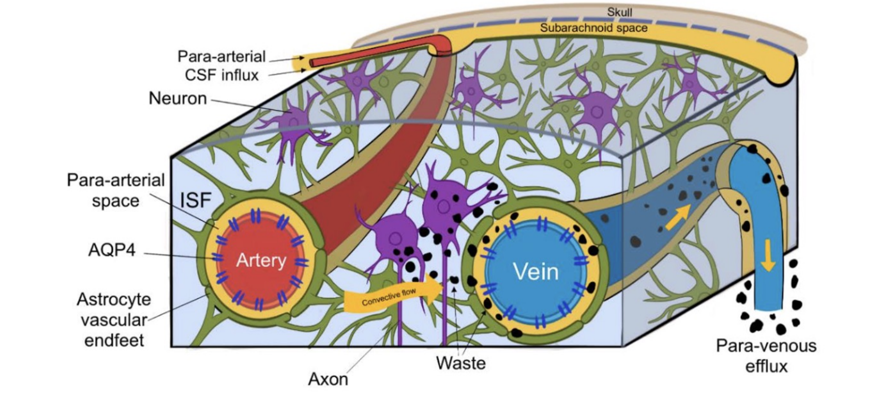


3 Pulsatile Waves of CSF

Pulsatile waves of CSF flow increase waste clearance and are entrained to restorative slow wave oscillations by hemodynamic oscillations and **neurovascular coupling.**



Glymphatic System: a waste clearance pathway in the brain that relies on interchange of cerebrospinal fluid (CSF) and interstitial fluid (ISF).



What we know about glymphatic flow: from pre-clinical to clinical

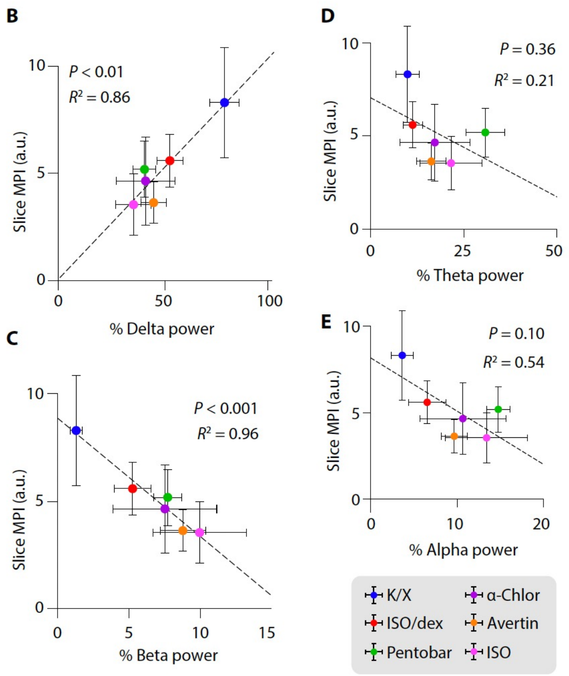
Pre-clinical studies have demonstrated that this new biology has profound effects on brain health and disease

SCIENCE ADVANCES | RESEARCH ARTICLE

NEUROPHYSIOLOGY

Increased glymphatic influx is correlated with high EEG delta power and low heart rate in mice under anesthesia

Lauren M. Hablitz[1], Hanna S. Vinitsky[1], Qian Sun[1], Frederik Filip Stæger[2], Björn Sigurdsson[2], Kristian N. Mortensen[2], Tuomas O. Lilius[2,3], Maiken Nedergaard[1,2]*



- Single measure per rodent before sacrifice
- Different anesthetic protocols
- EEG measured in a different population of rodents

SCIENTIFIC REPORTS
natureresearch

Repetitive Mild Traumatic Brain Injury Alters Glymphatic Clearance Rates in Limbic Structures of Adolescent Female Rats

Jennaya Christensen[1,2], David K. Wright[2], Glenn R. Yamakawa[2], Sandy R. Shultz[2,3] & Richelle Mychasiuk[1,2,4,5*]

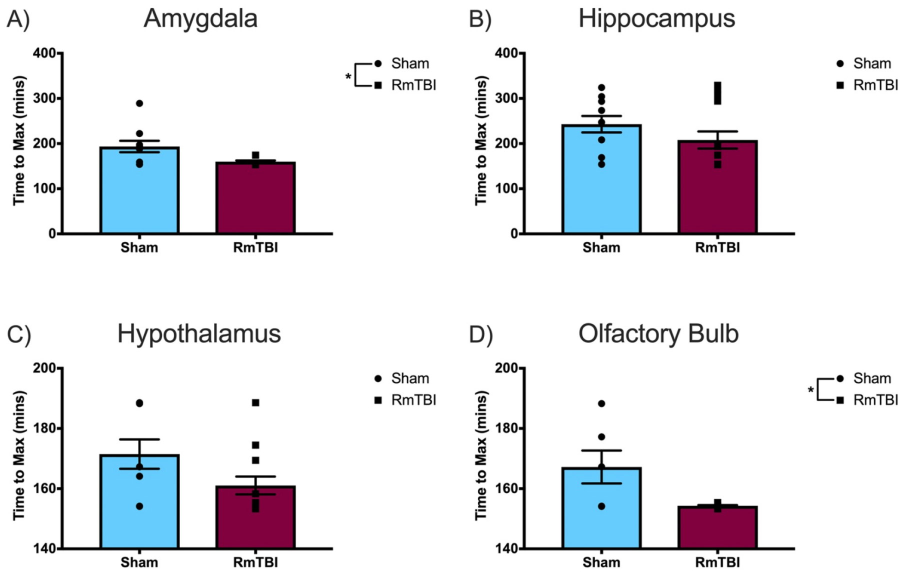
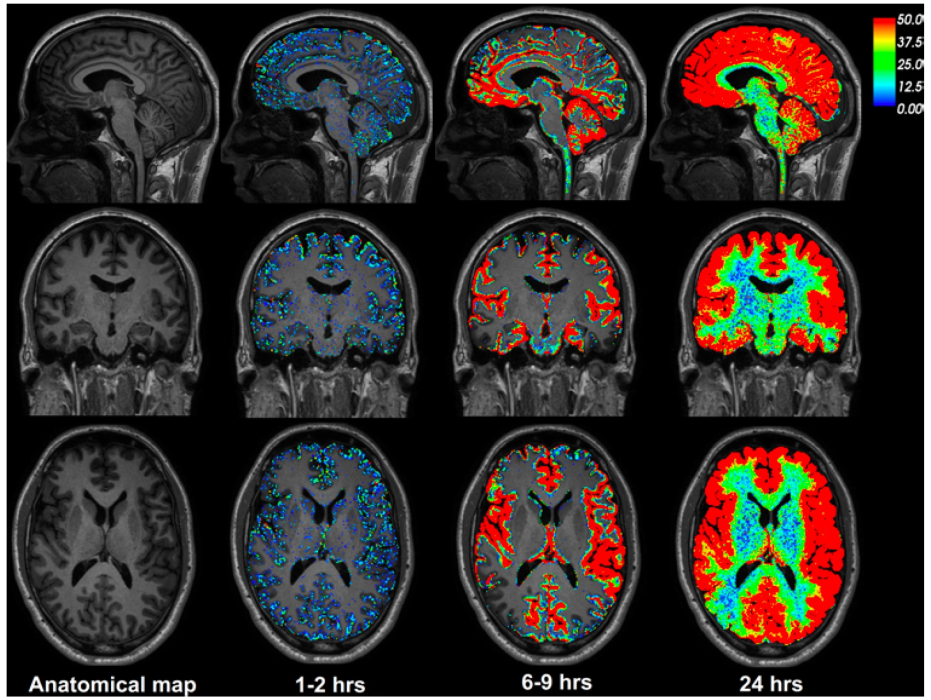


Figure 3. Scatter plot with bar graphs displaying the one-way ANOVA results for the time required to reach maximum signal intensity, a measure of glymphatic *influx*, for the (**A**) amygdala, (**B**) hippocampus, (**C**) hypothalamus, and (**D**) olfactory bulb. Time to max is calculated from the start of contrast agent injection. Means ± standard error; * main effect of injury, $p < 0.05$.

- Animal model of rmTBI
- Showed impact on glymphatic influx/efflux

Brain-wide glymphatic enhancement and clearance in humans assessed with MRI

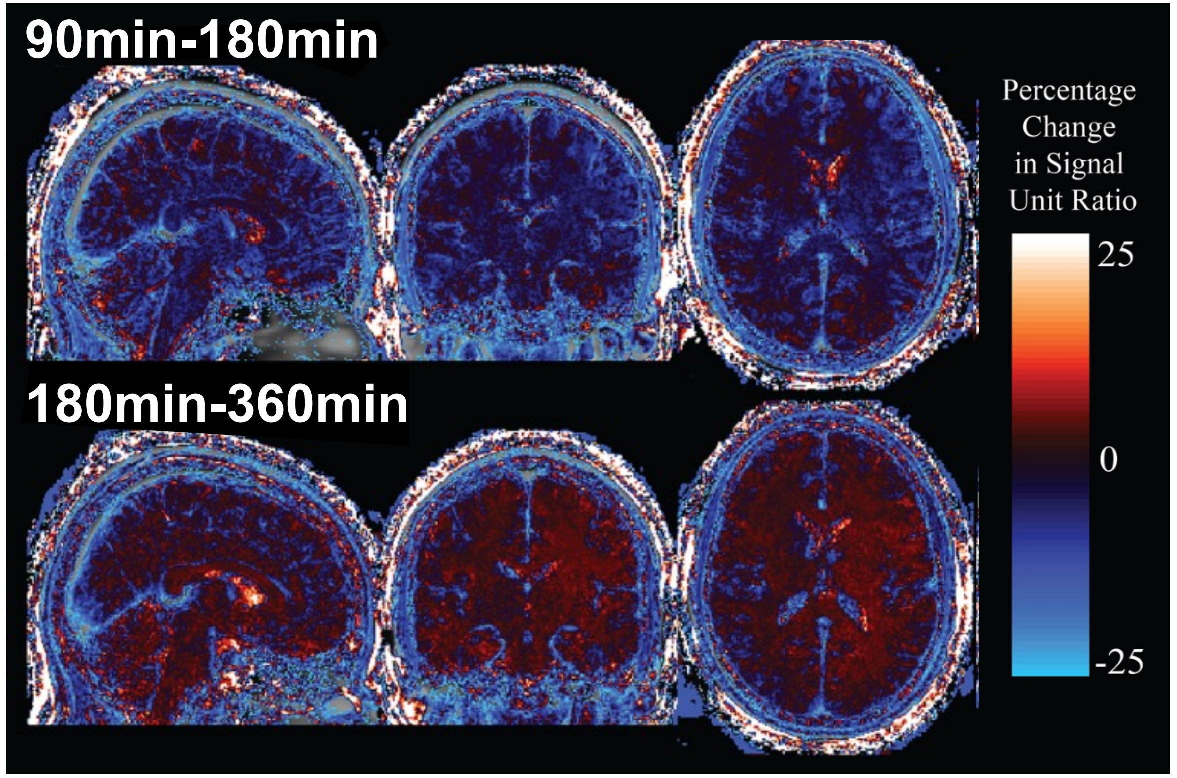
Geir Ringstad,[1,2] Lars M. Valnes,[3] Anders M. Dale,[4,5,6] Are H. Pripp,[7] Svein-Are S. Vatnehol,[8] Kyrre E. Emblem,[9] Kent-Andre Mardal,[3,10] and Per K. Eide[2,11]

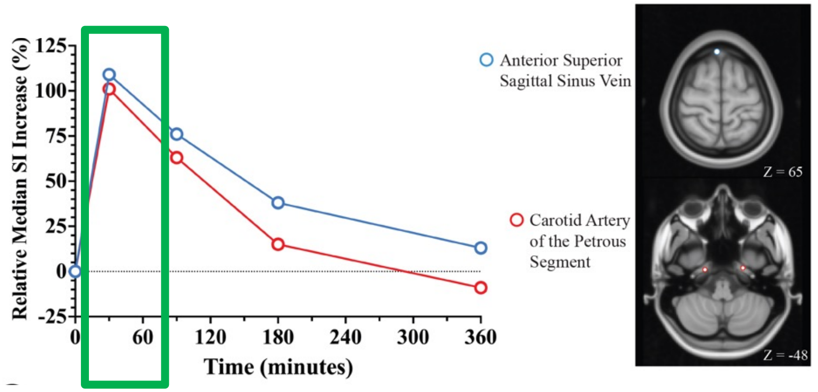


- Intrathecal injection of Gadobutrol
- Serial MRIs over 48 hours with participant supine for the duration of the study

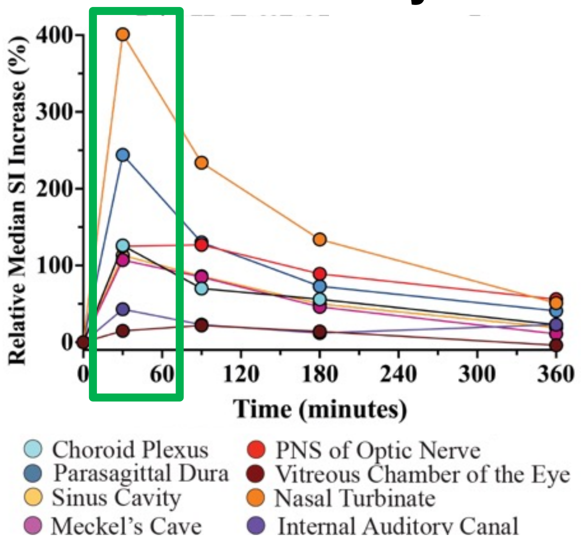
Measurement of glymphatic function using iv contrast-enhanced MRI

The current gold standard measurement of glymphatic flow in humans requires an iv bolus of contrast followed by serial MRIs

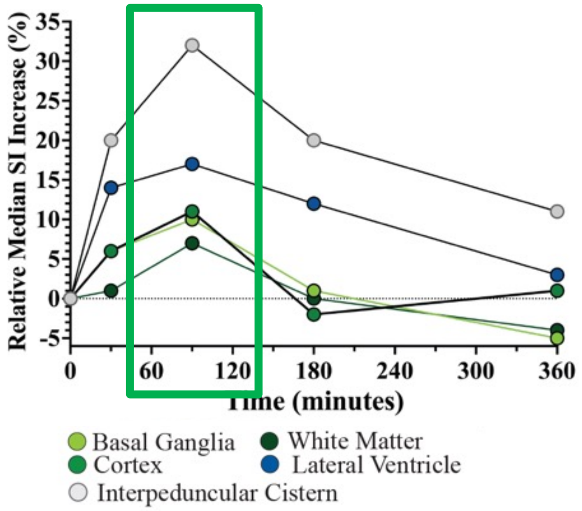




Non-Parenchymal



Parenchyma/CSF



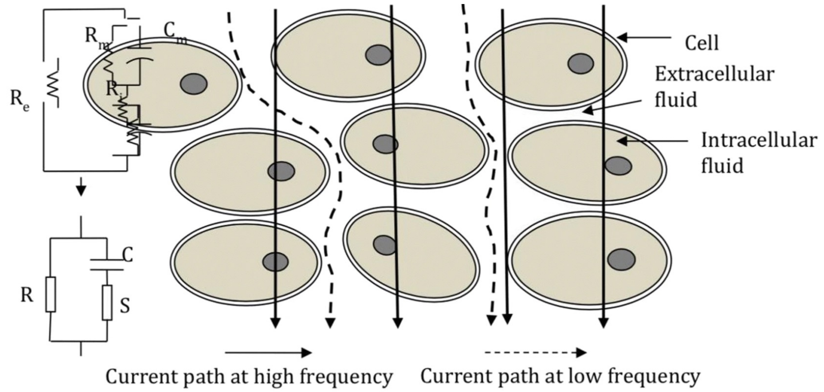
Richmond et al. *Eur J Neurosci* 2023

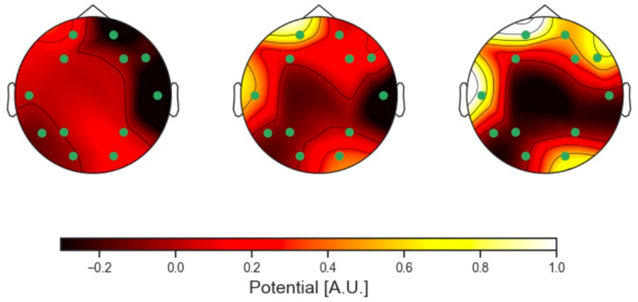
The lack of continuous in-human measurement of glymphatic function is limiting our understanding of this transformative biology and its potential in therapeutic discovery

The Science of How We Measure Glymphatic Flow*

A

Our direct measurement of glymphatic flow measures the change in brain parenchymal resistance (or flow at a given pressure gradient) using continuous spatial measurements of conduction through the brain at different frequencies





B

Our device sensors also **measure key physiologic drivers of glymphatic flow** using novel approaches to radically miniaturize and simplify instrumentation, allowing for continuous overnight measurements

- Slow wave activity
- Arterial pulsatility
- Pulsatile waves of CSF

Glymphatic System: a waste clearance pathway in the brain that relies on the interchange of cerebrospinal fluid (CSF) and interstitial fluid (ISF)

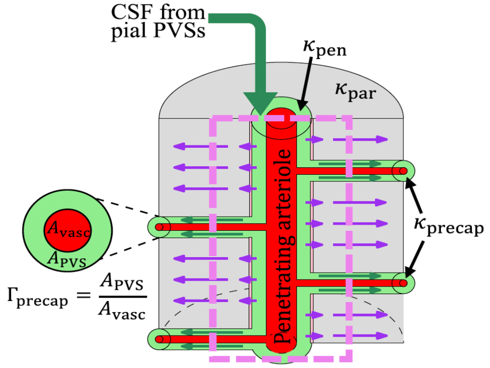
C

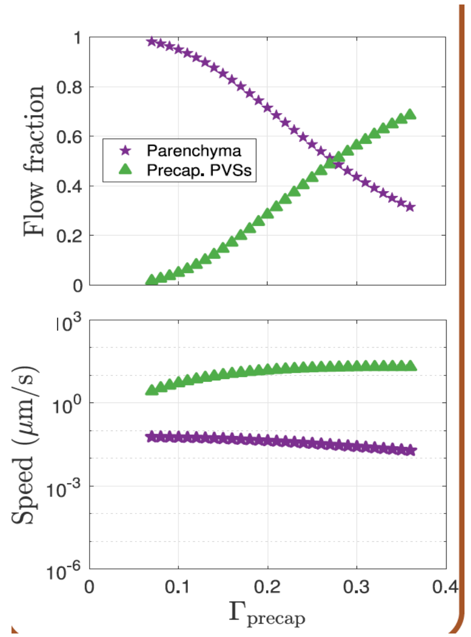
Network models from Nedergaard's group reveal that **change in brain resistance in sleep is the key driver to glymphatic flow**

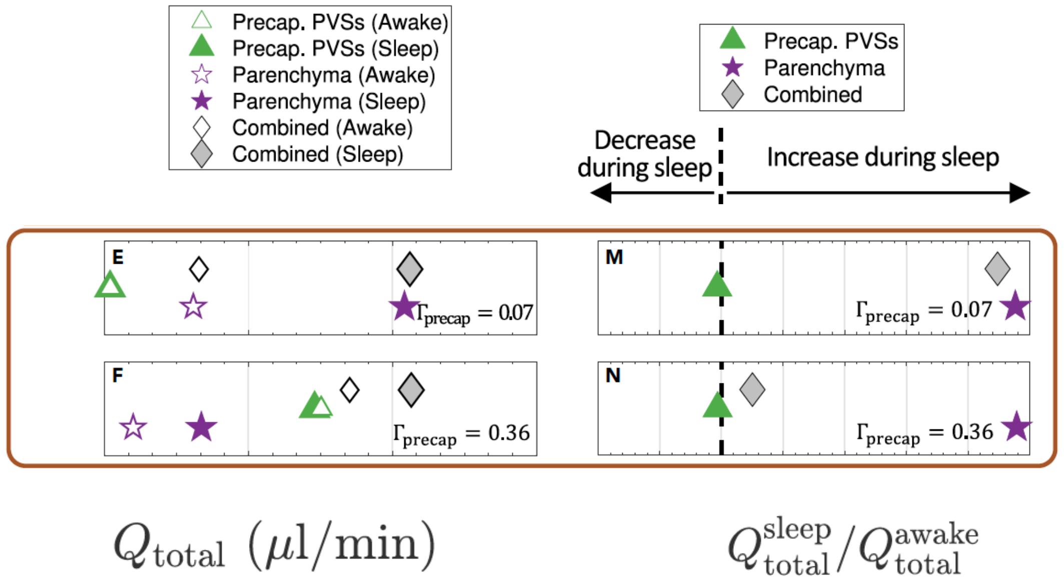
Article

A network model of glymphatic flow under different experimentally-motivated parametric scenarios

Jeffrey Tithof,[1,2,5,*] Kimberly A.S. Boster,[1] Peter A.R. Bork,[3] Maiken Nedergaard,[3,4] John H. Thomas,[1] and Douglas H. Kelley[1]







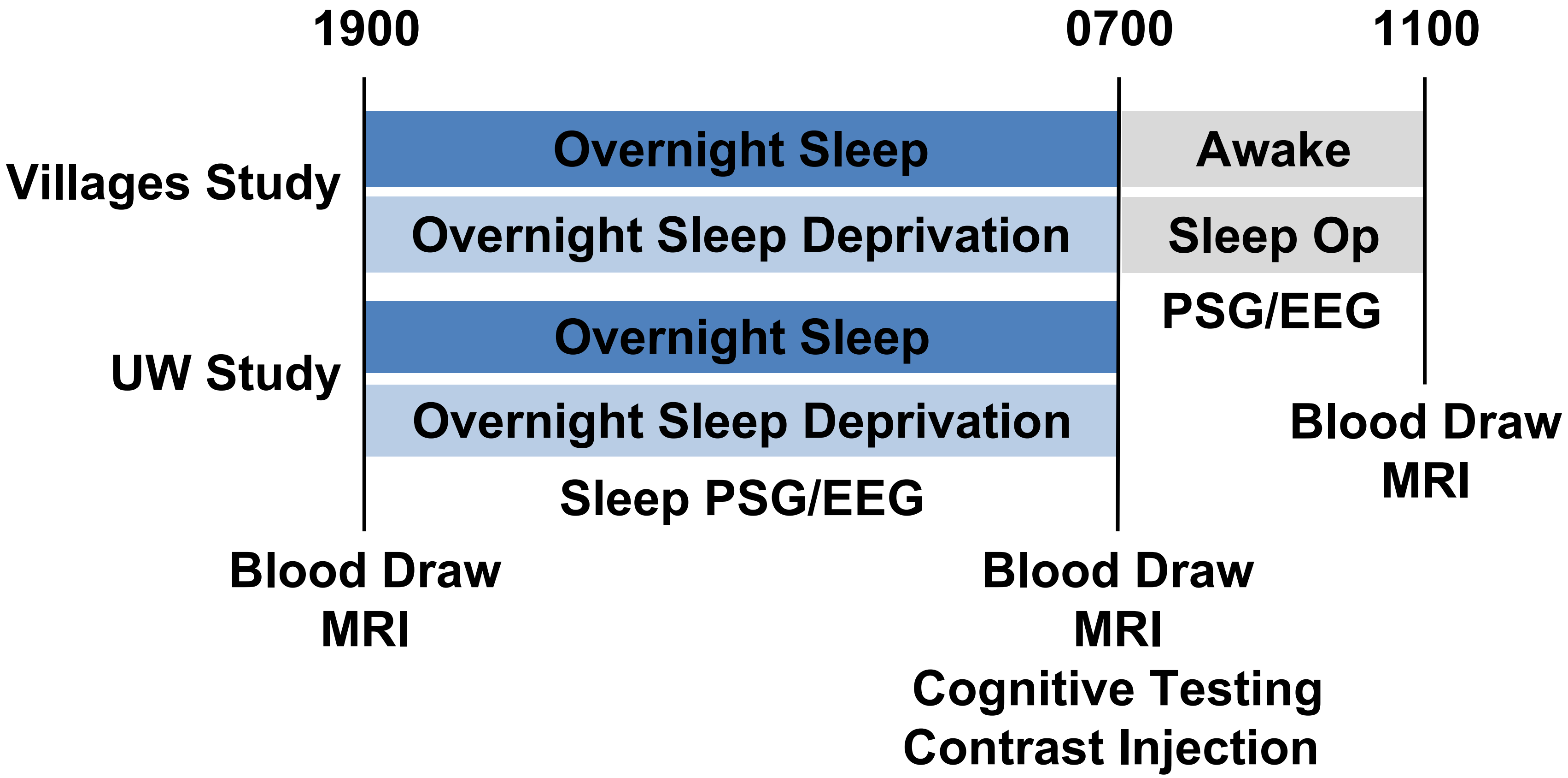
*Dagum et al Patent US20230080140A1

Benchmarking Study: Primary Objective

To demonstrate that our investigational device is as good or better at **measuring glymphatic function** than gold-standard neuroimaging and can do so **continuously during sleep which is currently not possible**

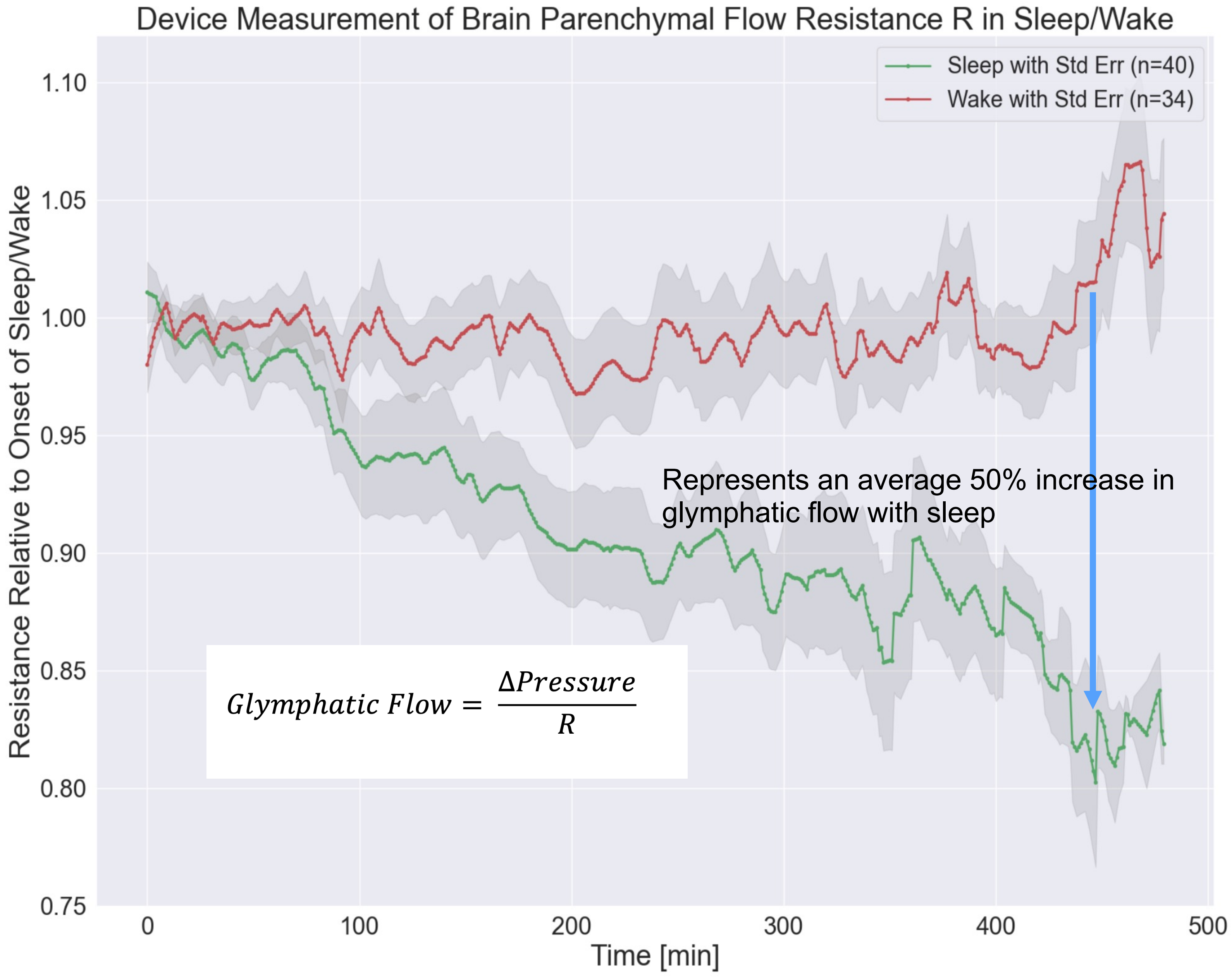
Clinical studies **completed in June**:

- Benchmarking study is a 30 participant randomized cross-over design at the University of Florida
  - Compares our device to CE MRI neuroimaging of glymphatic function
  - Identifies neurophysiology and blood biomarker correlates with device measurements
- Replication study is a comparable 16 participant randomized cross-over design at the University of Washington



Study Results: First-ever Continuous Measure of Glymphatic Flow in Humans

Participants who slept had lower brain resistance to glymphatic flow (p < 0.0005)



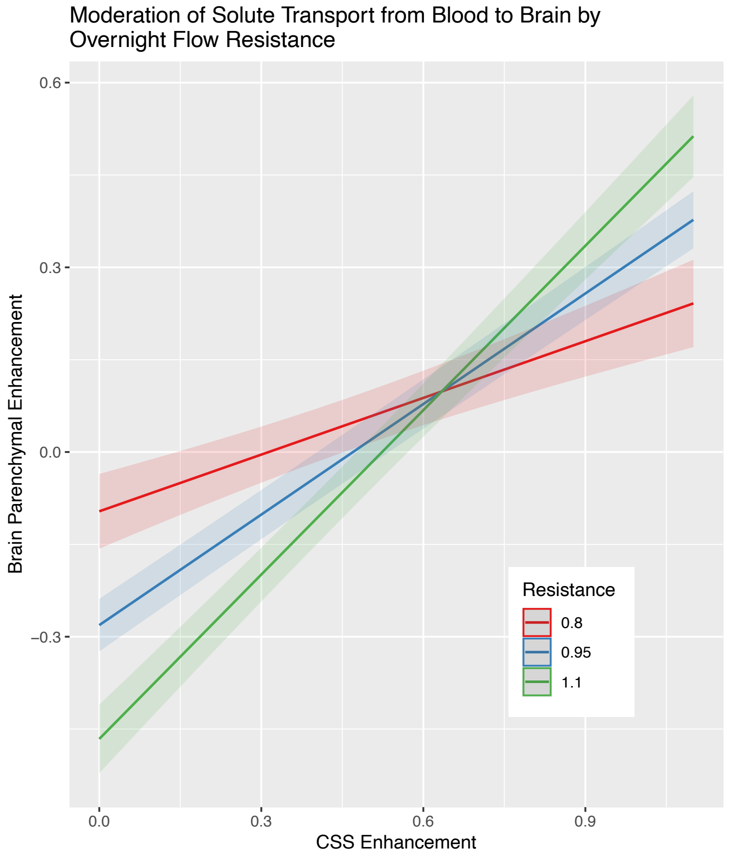
Significant Study Findings

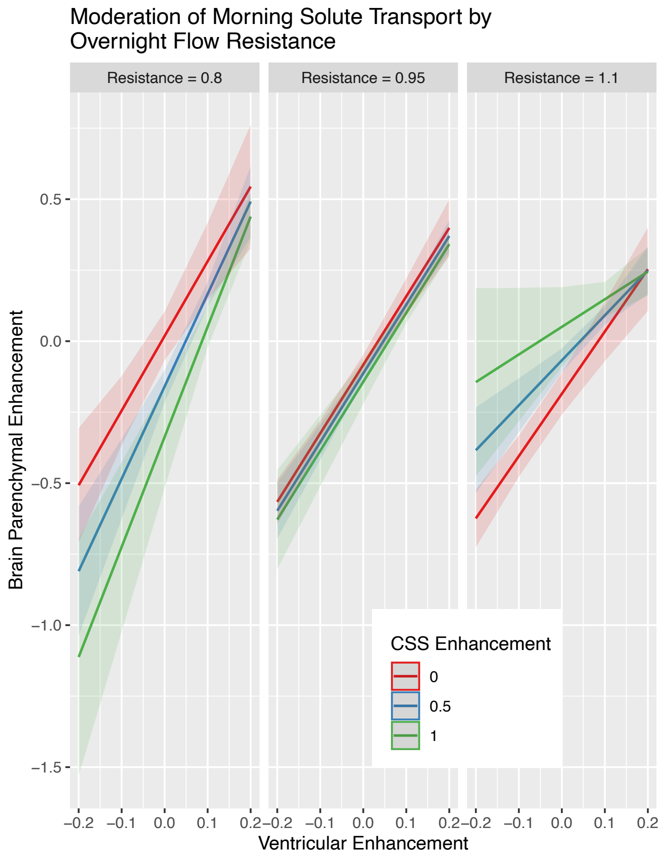
- Resistance (p= 0.01, 0.011, 0.039, 0.044), *not* sleep/wake assignment (three p=NS, one p = 0.025), was the **significant moderator of MRI contrast movement** into the brain with sleep
- Changes in resistance correlated with changes in sleep EEG band power **replicating pre-clinical animal studies** (all p < 0.005 except beta p = 0.018)
- Changes in overnight resistance (p = 0.005), *not* sleep/wake assignment (p=NS), **predicted performance in morning cognitive test battery**

Change in resistance was a robust predictor of contrast movement from the blood and CSF compartments through the brain parenchyma*

Lower resistance steepened the relationship between CSF-parenchymal contrast (glymphatic influx) and flattened the relationship between blood-parenchymal contrast (clearance to blood)

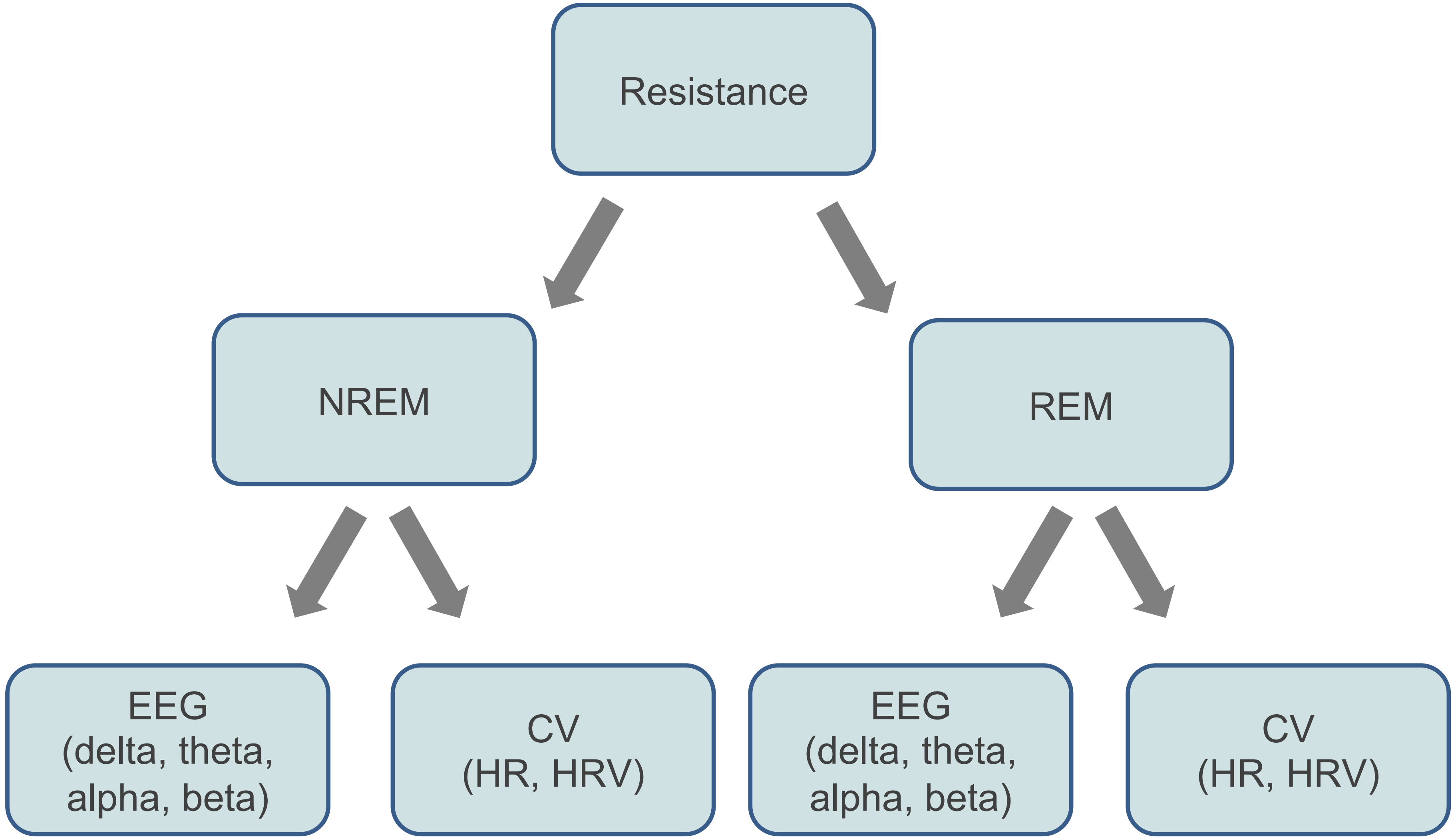
	Moderation of Morning Brain Solute Transport by Overnight Resistance		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.4074	-0.0167 – 0.8315	0.063
Overnight Resistance	-1.2833	-2.5195 – -0.0471	**0.044**
Morning CSS Contrast	0.3372	-3.5942 – 4.2685	0.868
Morning Ventricular Contrast	-0.5336	-0.9721 – -0.0952	**0.018**
Resistance:CSS	8.3833	1.9704 – 14.7962	**0.011**
Resistance:Ventricles	1.3555	0.0839 – 2.6272	**0.039**
CSS:Ventricles	1.9429	-1.9562 – 5.8419	0.333
Resistance:CSS:Ventricles	-8.8356	-15.4429 – -2.2283	**0.010**
Random Effects			
σ^2	0.001		
$\tau_{00\ pid}$	0.002		
ICC	0.632		
N_{pid}	29		
Observations	357		
Marginal R^2 / Conditional R^2	0.874 / 0.954		





Brain parenchymal resistance is dynamically coupled to sleep neurophysiology in humans

We used *continuous device recordings* of resistance, EEG and cardiovascular metrics during sleep to identify coupling between sleep neurophysiology and resistance



1. Data was time aligned in the device to within 4 ms
2. Device sleep hypnogram was used to identify REM and NREM sleep
3. EEG power bands (delta, theta, alpha and beta), HR and HRV (sdnn) were computed
4. First difference of all measures were taken (R, powerbands, HR, HRV)
5. Linear, multilinear and linear threshold models were fit

Delta Power Decreased Resistance in NREM Sleep

Delta power showed a threshold effect on resistance requiring a step increase > 7%. A step increase in delta power had a larger effect on R than a similar size step decrease

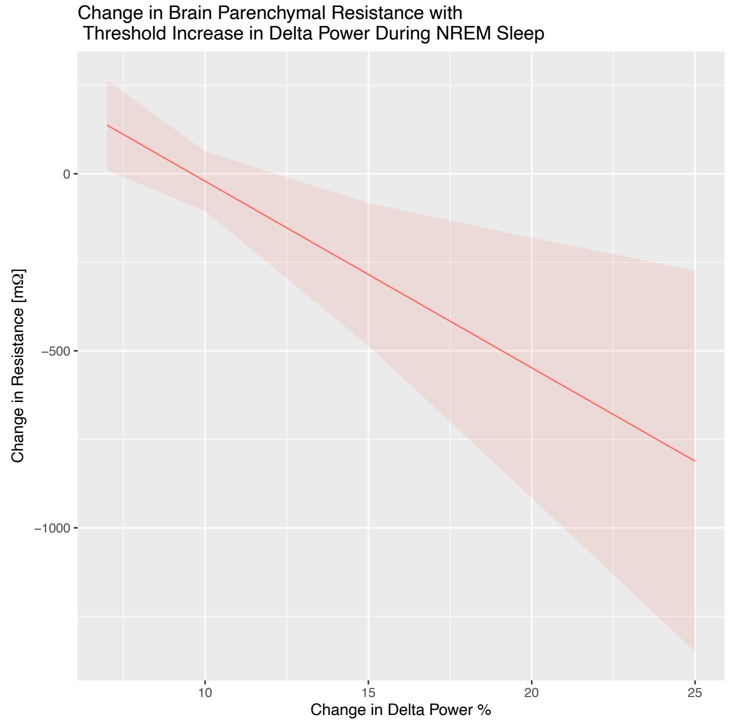
Change in Brain Parenchymal Resistance with Threshold Increase Above 7% in Delta Power During NREM Sleep

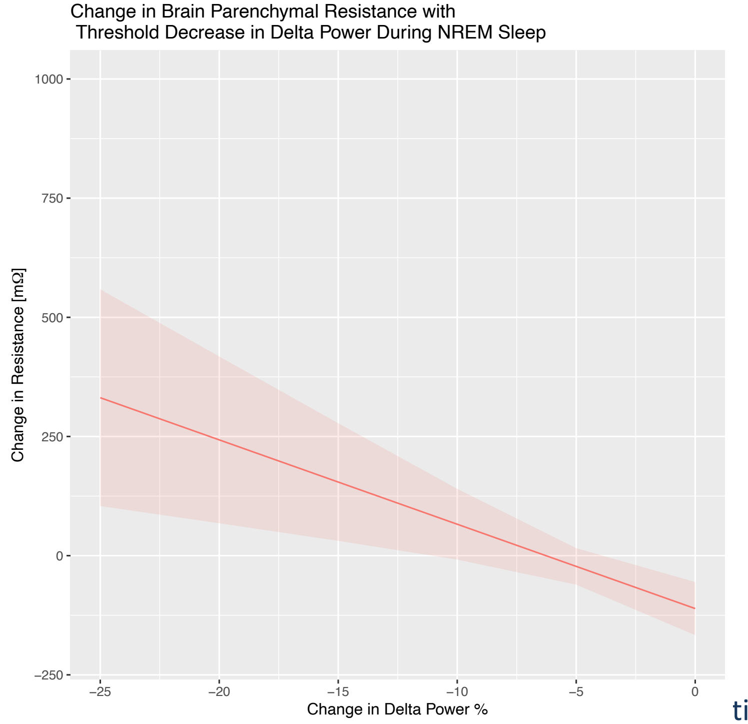
Predictors	*Estimates*	*CI*	*p*
(Intercept)	506.4601	157.0480 – 855.8723	**0.005**
Delta Power %	-52.7240	-87.5650 – -17.8830	**0.003**
Observations	90		
R^2 / R^2 adjusted	0.093 / 0.083		

Change in Brain Parenchymal Resistance with Decrease Below Zero in Delta Power During NREM Sleep

Predictors	*Estimates*	*CI*	*p*
(Intercept)	-110.9821	-166.7174 – -55.2468	**<0.001**
Delta Power %	-17.6942	-28.3488 – -7.0397	**0.001**
Observations	708		
R^2 / R^2 adjusted	0.015 / 0.013		

Does this explain why morning brain parenchymal resistance does not return to evening levels?



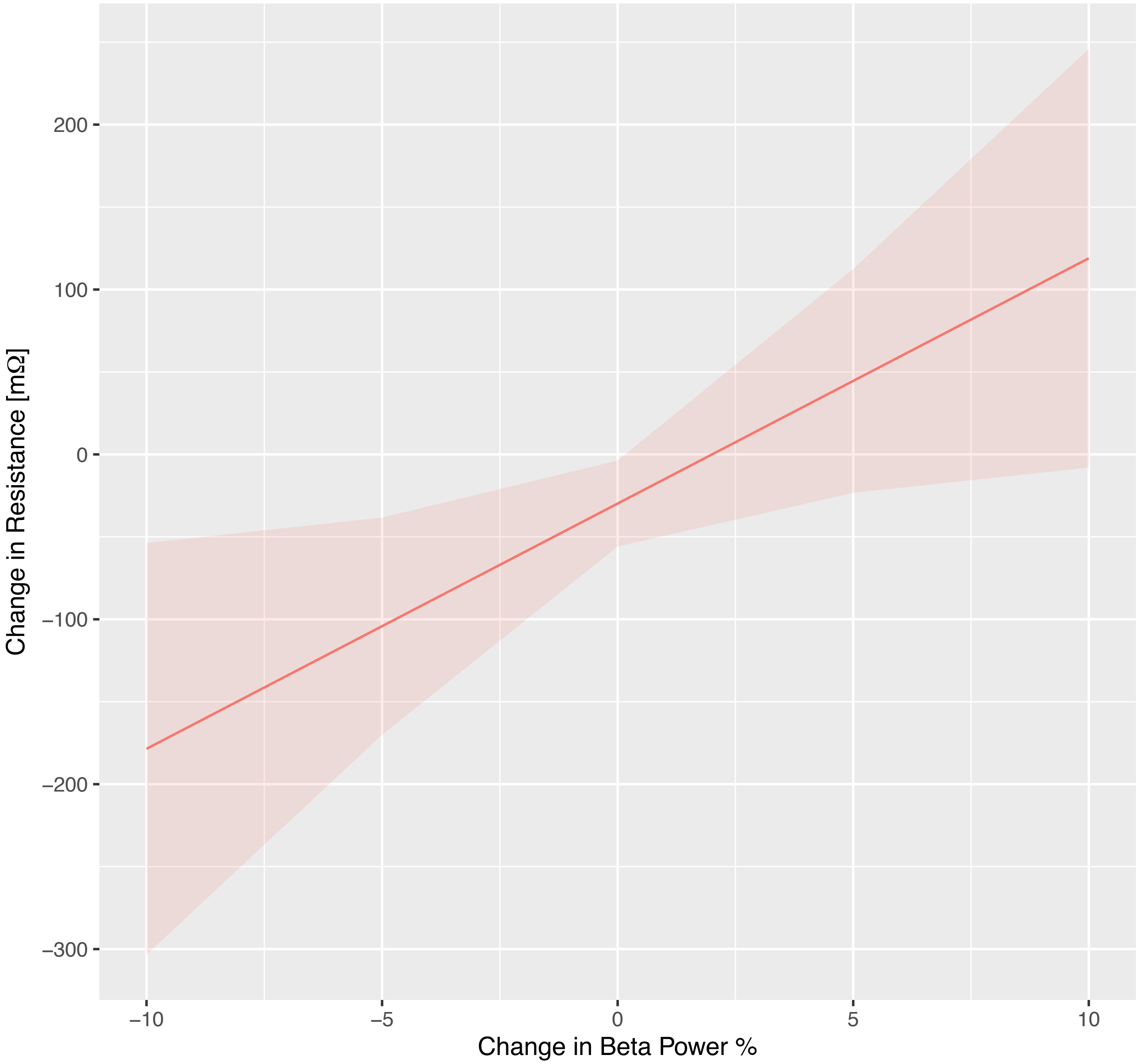


Beta Power Increased Resistance in NREM Sleep

A step increase/decrease in beta power led to a step increase/decrease in resistance during NREM sleep

	Change in Brain Parenchymal Resistancewith with Change in Beta Power During NREM Sleep		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-29.8051	-55.8753 – -3.7348	**0.025**
Beta Power %	14.8749	2.5577 – 27.1922	**0.018**
Observations	1402		
R^2 / R^2 adjusted	0.004 / 0.003		

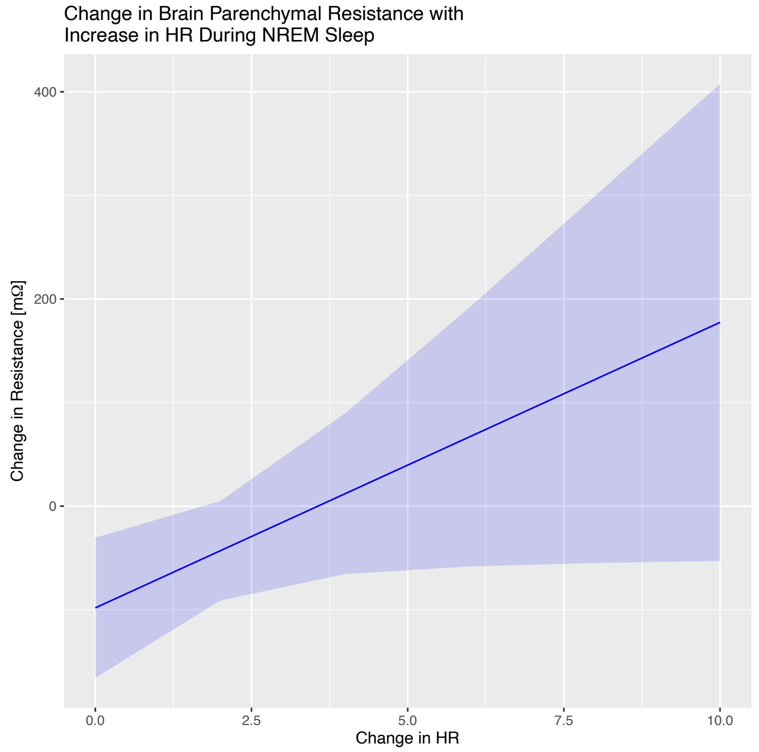
Change in Brain Parenchymal Resistance
with Change in Beta Power During NREM Sleep



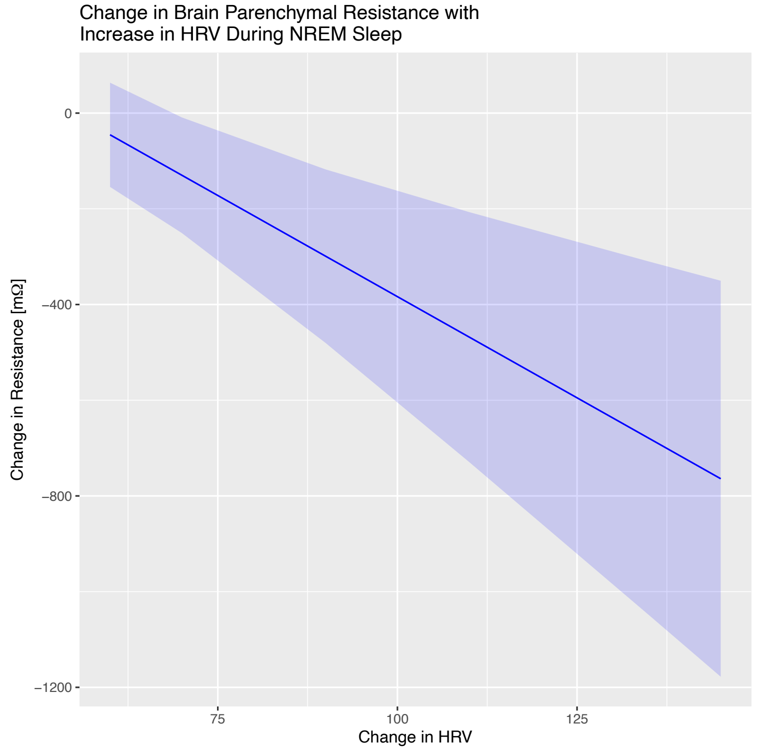
HR and HRV had Opposite Effects on Resistance During NREM Sleep

Both where threshold above zero with a step increase in HR leading to a *step increase* in R and a step increase in HRV leading to a *step decrease* in R

	Change in Brain Parenchymal Resistance with Threshold Increase Above 0.28bpm in HR During NREM Sleep		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-98.1940	-165.8378 – -30.5502	**0.005**
HR	27.5603	0.2205 – 54.9001	**0.048**
Observations	450		
R^2 / R^2 adjusted	0.009 / 0.006		



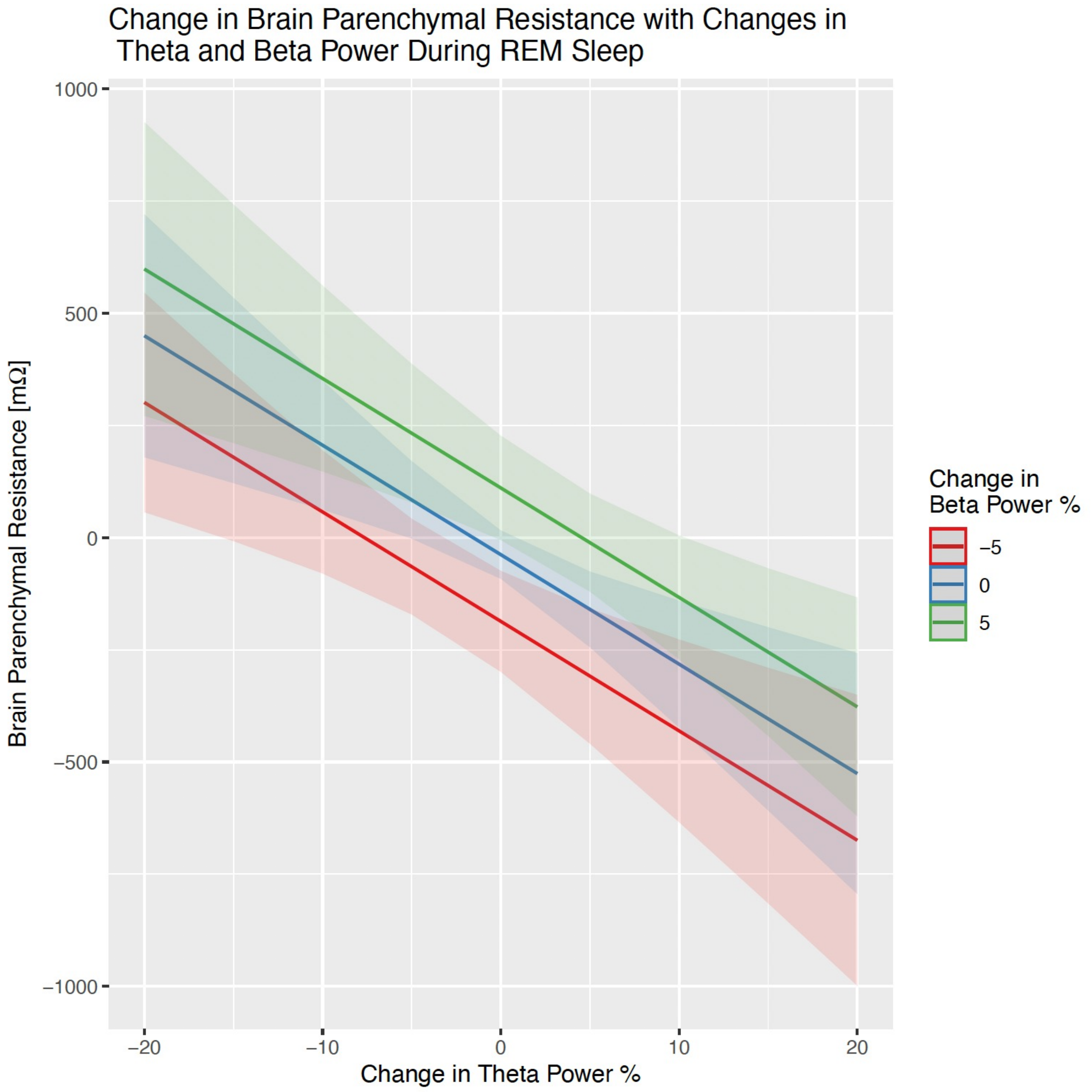
	Change in Brain Parenchymal Resistance with Threshold Increase Above 34 ms in HRV During NREM Sleep		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	462.0403	170.2266 – 753.8540	**0.002**
HRV	-8.4560	-13.0770 – -3.8349	**<0.001**
Observations	86		
R^2 / R^2 adjusted	0.136 / 0.126		



Theta Power Decreased and Beta Power Increased Resistance in REM Sleep

A step increase (decrease) in theta power (beta power) during REM sleep led to a step decrease (increase) in resistance

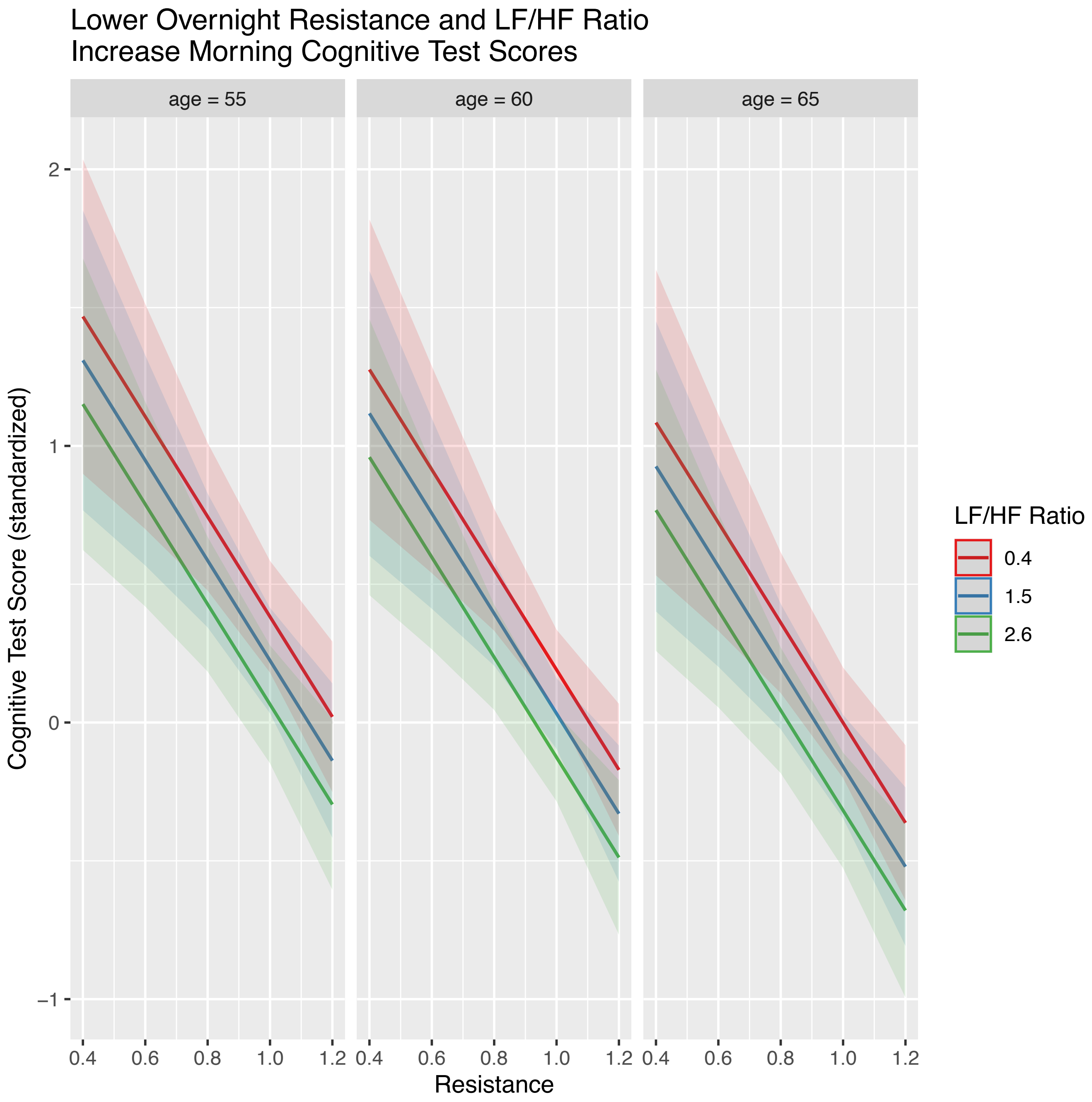
	Change in Brain Parenchymal Resistance with Changes in Theta and Beta Power During REM Sleep		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-37.9764	-91.8900 – 15.9373	0.167
Theta Power	-24.4000	-37.6112 – -11.1888	**<0.001**
Beta Power	29.7429	9.5468 – 49.9390	**0.004**
Observations	353		
R^2 / R^2 adjusted	0.042 / 0.036		



Why Do We Care? Lower overnight resistance maintained sleep-sensitive cognitive performance

Participants who had lower overnight resistance, or greater glymphatic flow, performed better on a multi-domain cognitive battery in the morning

	Predictors of Cognitive Standardized Test Scores		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.36	2.40 – 6.31	**<0.001**
Overnight Mean Resistance	-1.81	-2.71 – -0.91	**<0.001**
HRV LF/HF Ratio	-0.14	-0.22 – -0.07	**<0.001**
Age [yrs]	-0.04	-0.07 – -0.01	**0.010**
Random Effects			
σ^2	0.31		
$\tau_{00\ Cog.Test}$	0.22		
$\tau_{00\ pid}$	0.61		
$N_{Cog.Test}$	5		
N_{pid}	44		
Observations	340		
Marginal R^2 / Conditional R^2	0.173 / NA		



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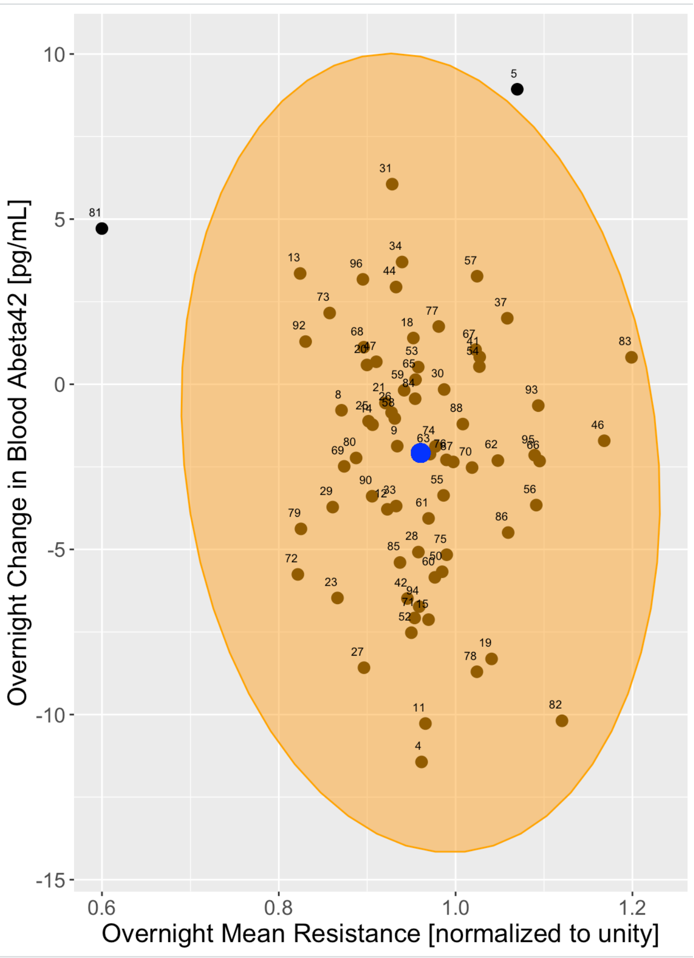
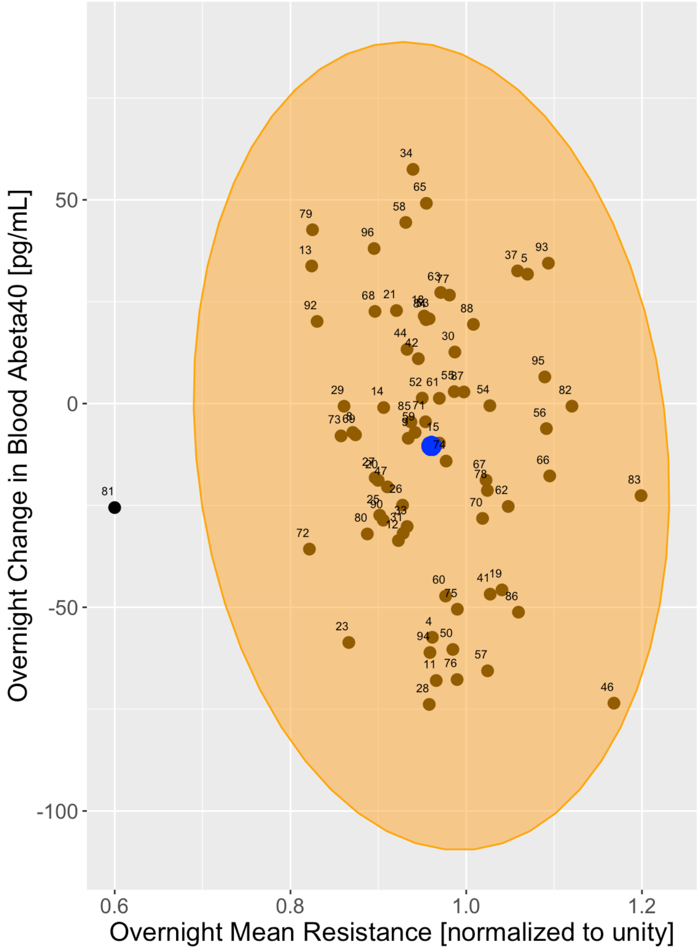
Lower overnight resistance was also weakly associated with greater β amyloid clearance from the brain

Overnight differences in serum Aβ40 and Aβ42 tended to be greater with lower overnight resistance

	Predictors of Overnight Change in Blood Abeta42		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	7.81	-4.54 – 20.15	0.211
Overnight Mean Resistance	-9.48	-21.80 – 2.84	0.129
Sleep Visit	-1.96	-3.87 – -0.04	**0.045**
Observations	70		
R^2 / R^2 adjusted	0.066 / 0.038		

	Predictors of Overnight Change in Blood Abeta40		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	97.47	-8.77 – 203.71	0.072
Overnight Mean Resistance	-103.99	-209.96 – 1.98	0.054
Sleep Visit	-13.97	-30.54 – 2.60	0.097
Observations	71		
R^2 / R^2 adjusted	0.066 / 0.039		





Research Roadmap

With a non-invasive investigational device to measure glymphatic function in the field, we will better understand the pathophysiology and clinical correlates of glymphatic dysfunction to restore cognitive performance and improve injury recovery

Pathophysiology

- Identify the impact of sustained or repeated exposure to extreme environments and trauma
- Identify the impact of diseases such at TBI and mental health on glymphatic function

Clinical Performance Correlates

- Identify the sleep, cognitive and neurological performance correlates of glymphatic pathophysiology

Therapeutic Discovery

- Investigate pharmacological and non-pharmacological interventions to restore glymphatic function