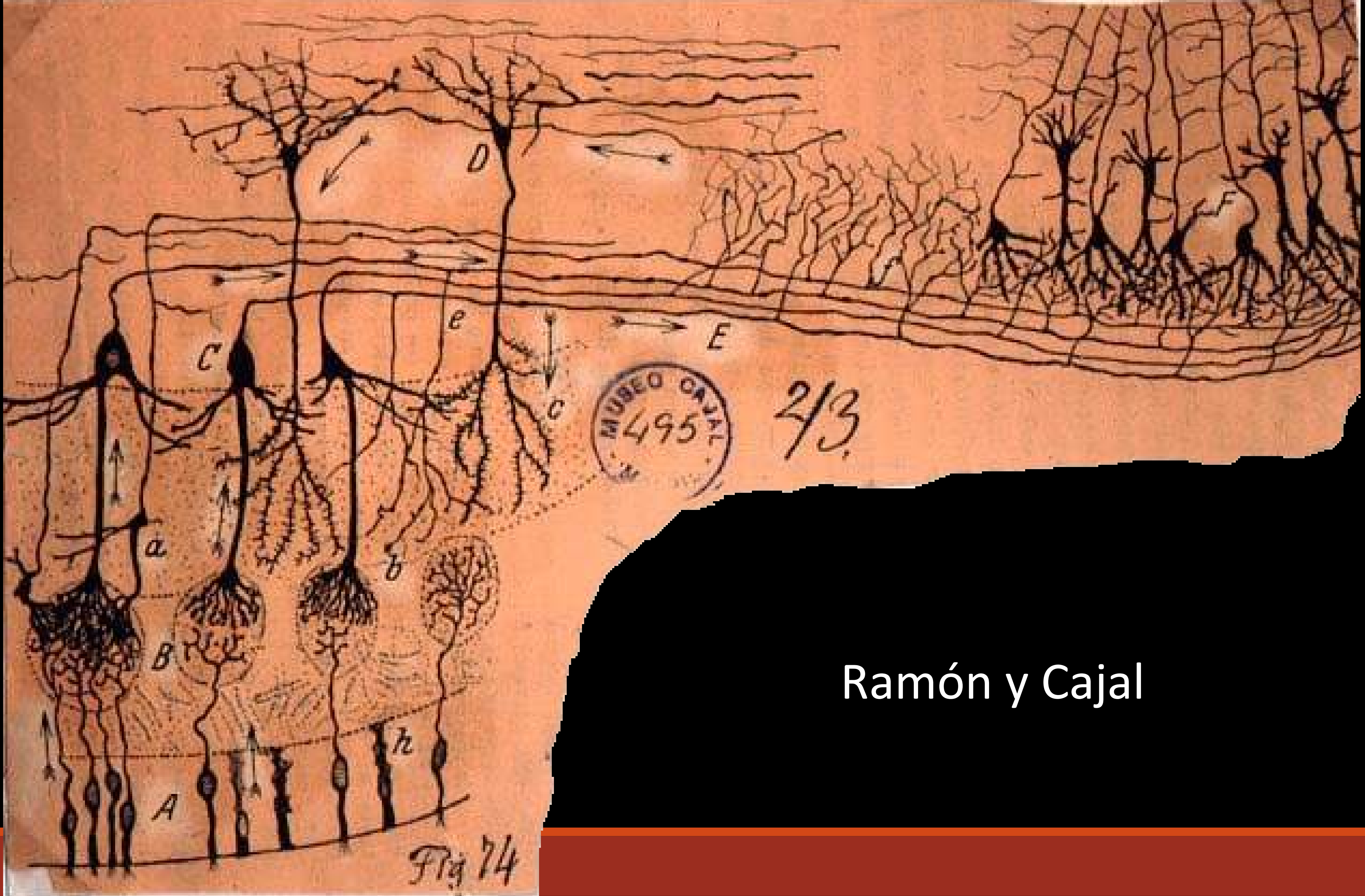
Computational steps towards unwrapping the role of myelin in olfactory signal processing

Justin Losacco, Daniel Ramirez-Gordillo, Jessie Gilmer

Elizabeth A. Gould, Nicolas Busquet, Douglas Shepherd, Robert Dietz, Paco Herson, Fabio M. Simoes de Souza, Anan Li, Nicholas M. George, Wendy B. Macklin



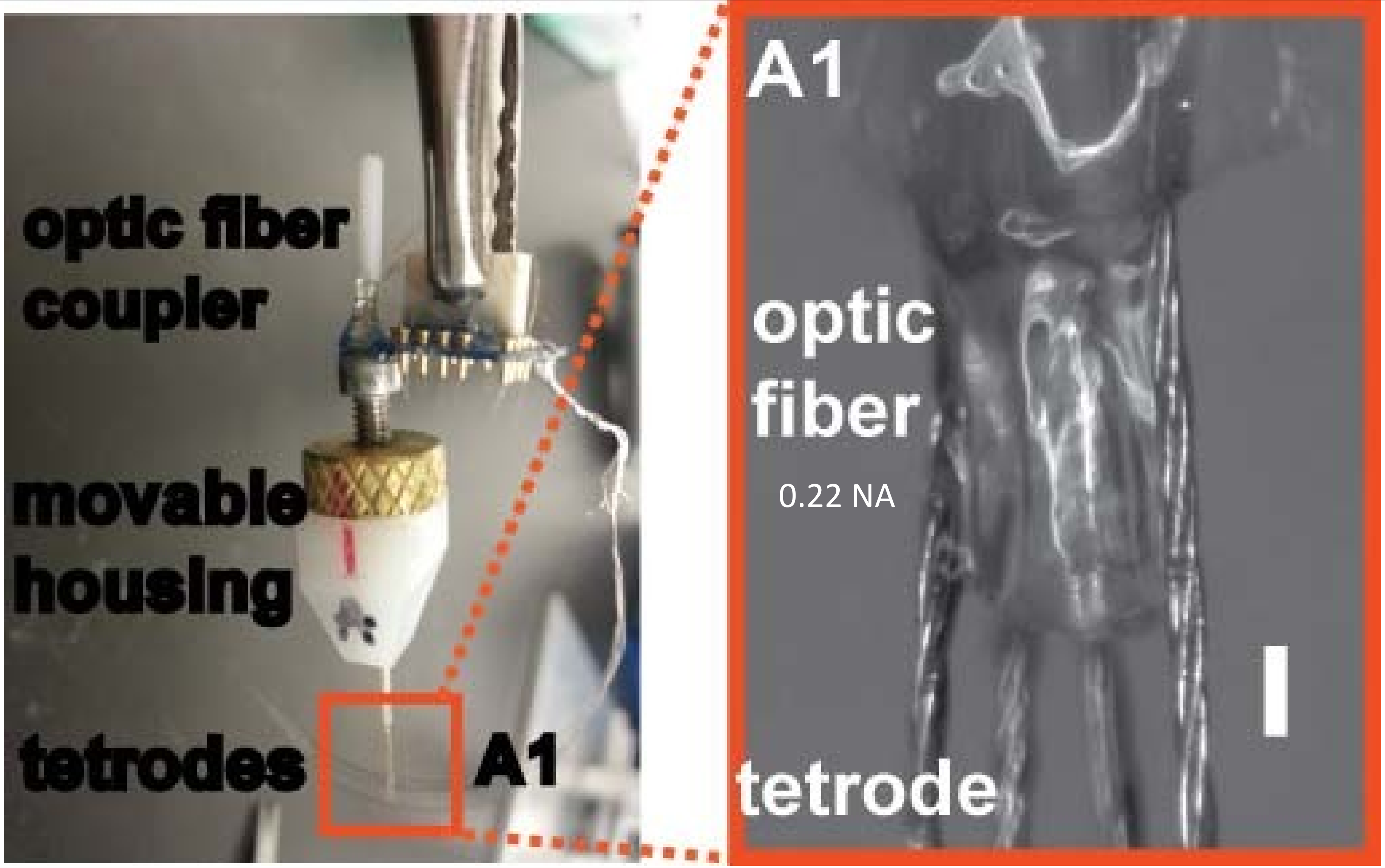
Ramón y Cajal

Computational steps towards unwrapping the role of myelin in olfactory signal processing

- Oscillations are augmented and dimensionality changes as the animal learns to differentiate odorants in the go-no go task

- Mild Myelin Disruption Elicits Early Alteration in Olfactory Behavior and Proliferation in the Subventricular Zone

Optetrode


optic fiber
coupler
movable
housing
tetrodes
A1
A1
optic
fiber
0.22 NA
tetrode

Go-no Go Associative Learning Task

Optetrode implantation

↓

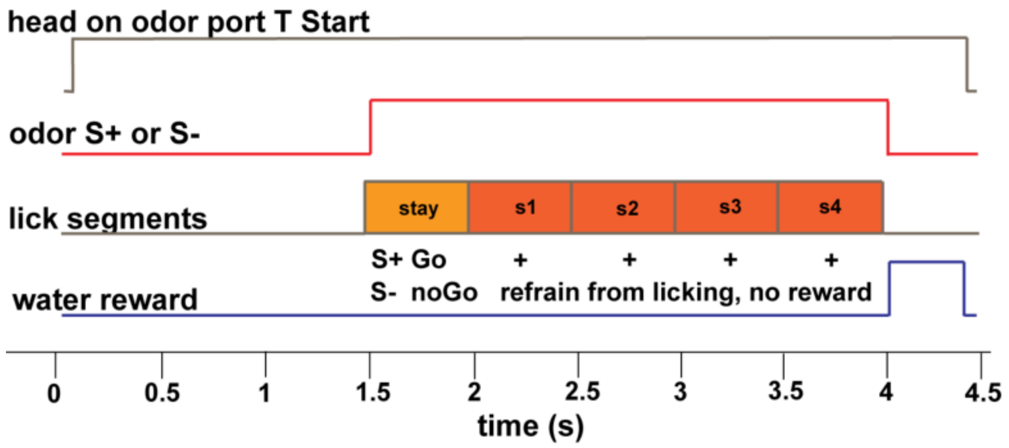
Electrophysiological recordings of neurons during an associative learning task (i.e. go-no go)

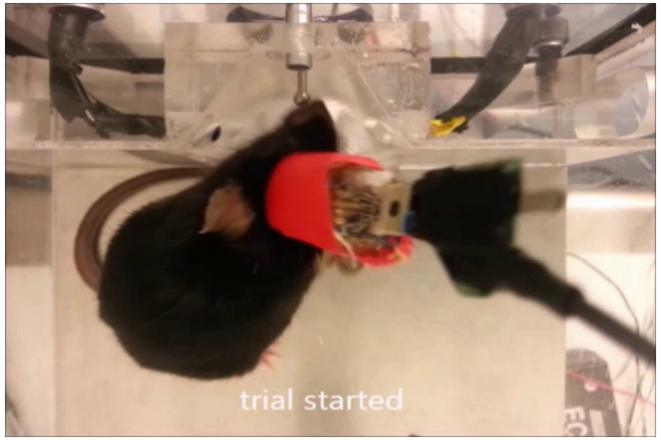
↓

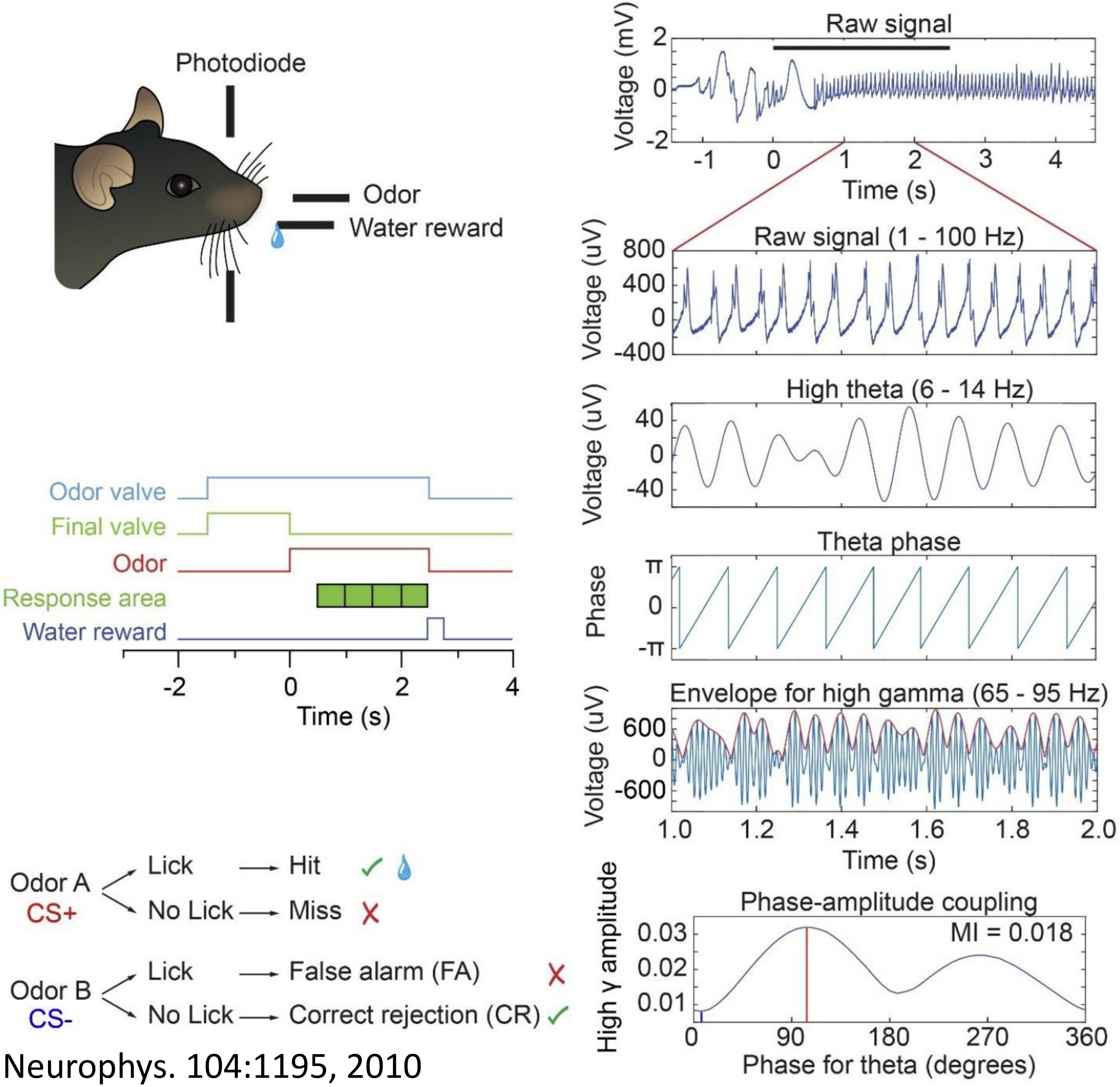
Light delivery protocol

↓

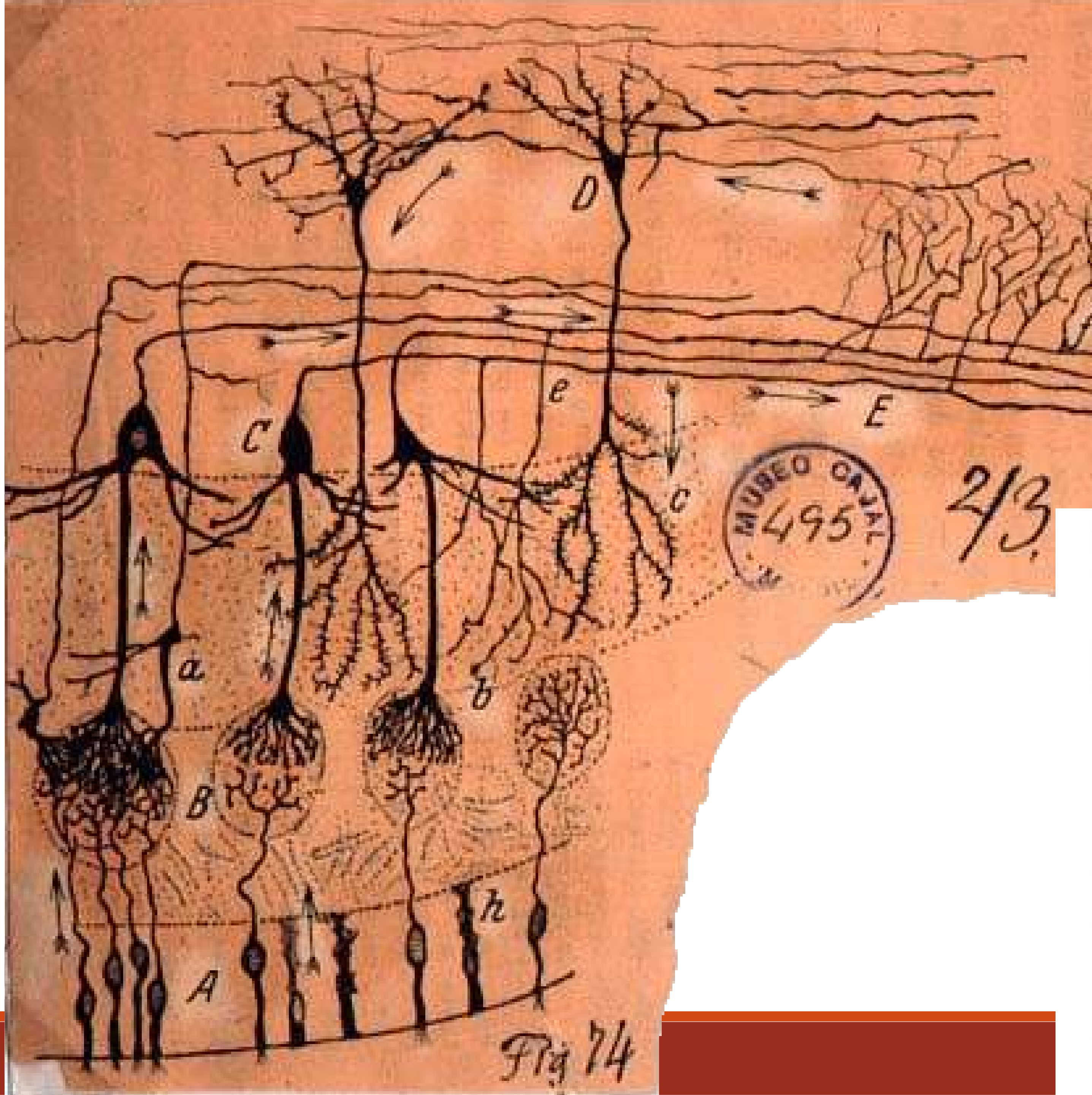
Off-line spike sorting analysis single and multi-unit identification







PAC caclulation: Tort et al. J. Neurophys. 104:1195, 2010



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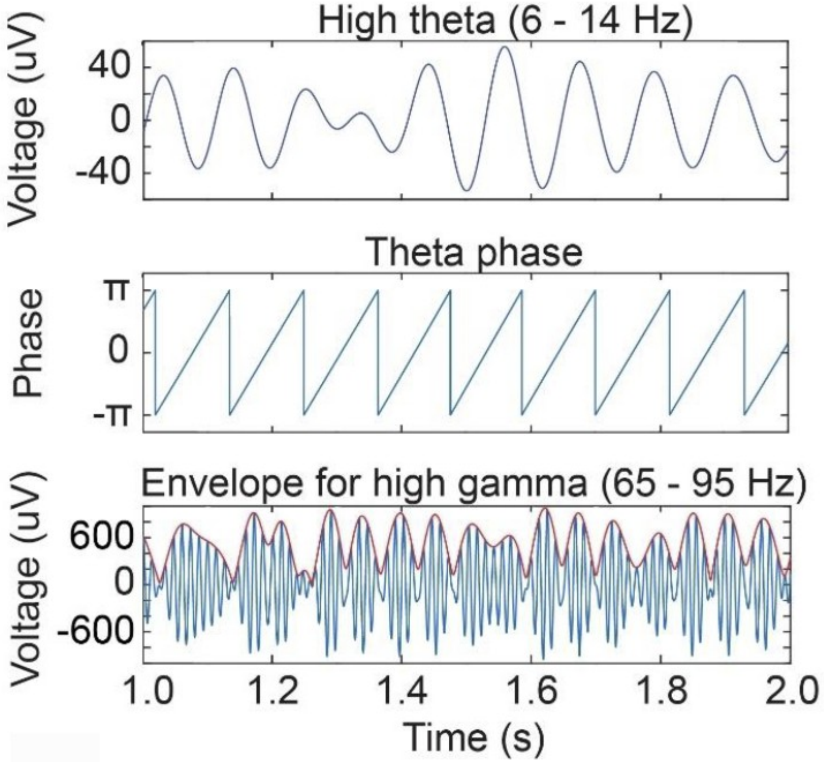
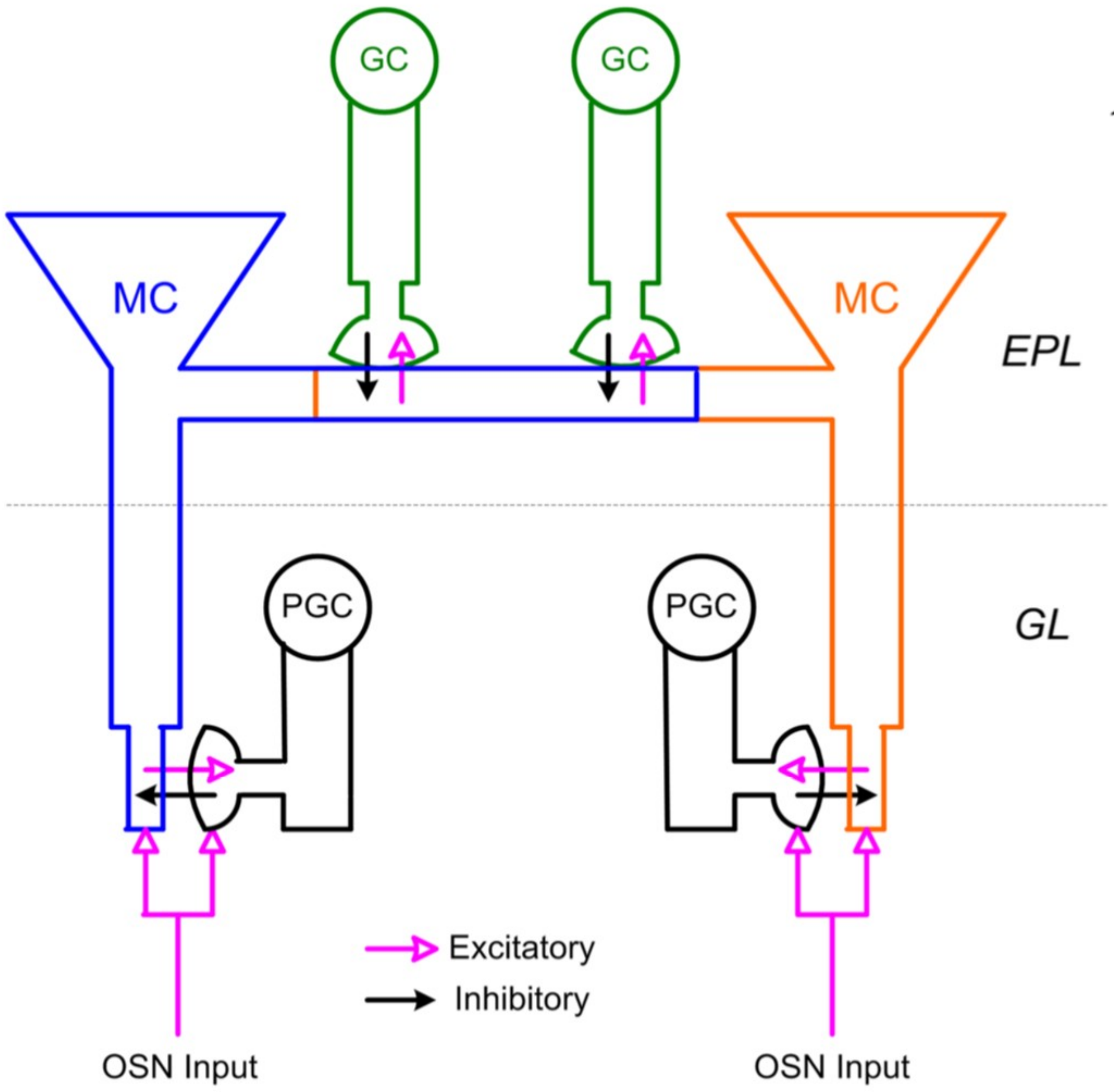
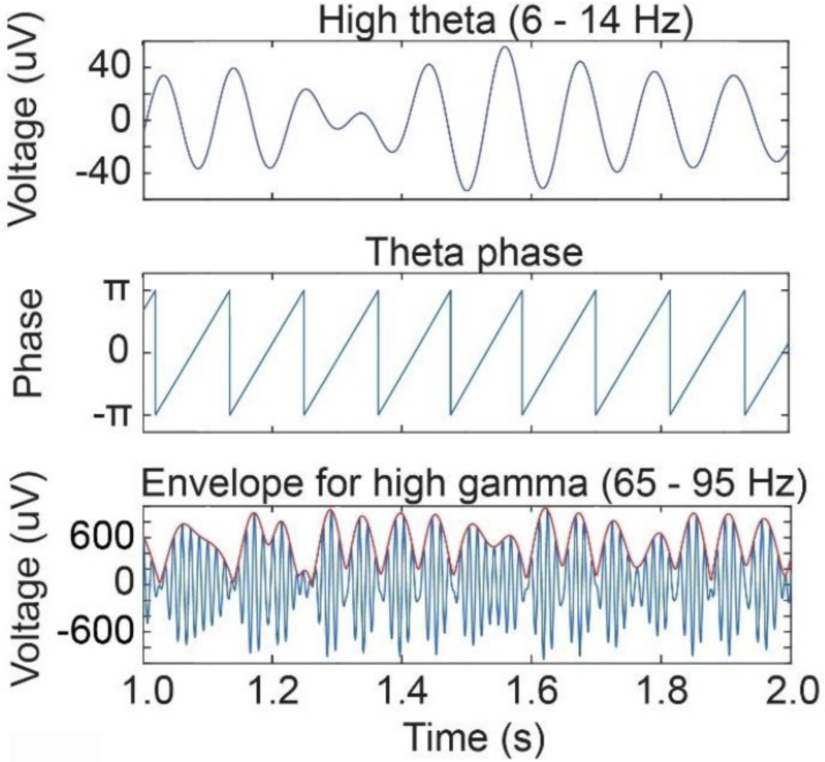


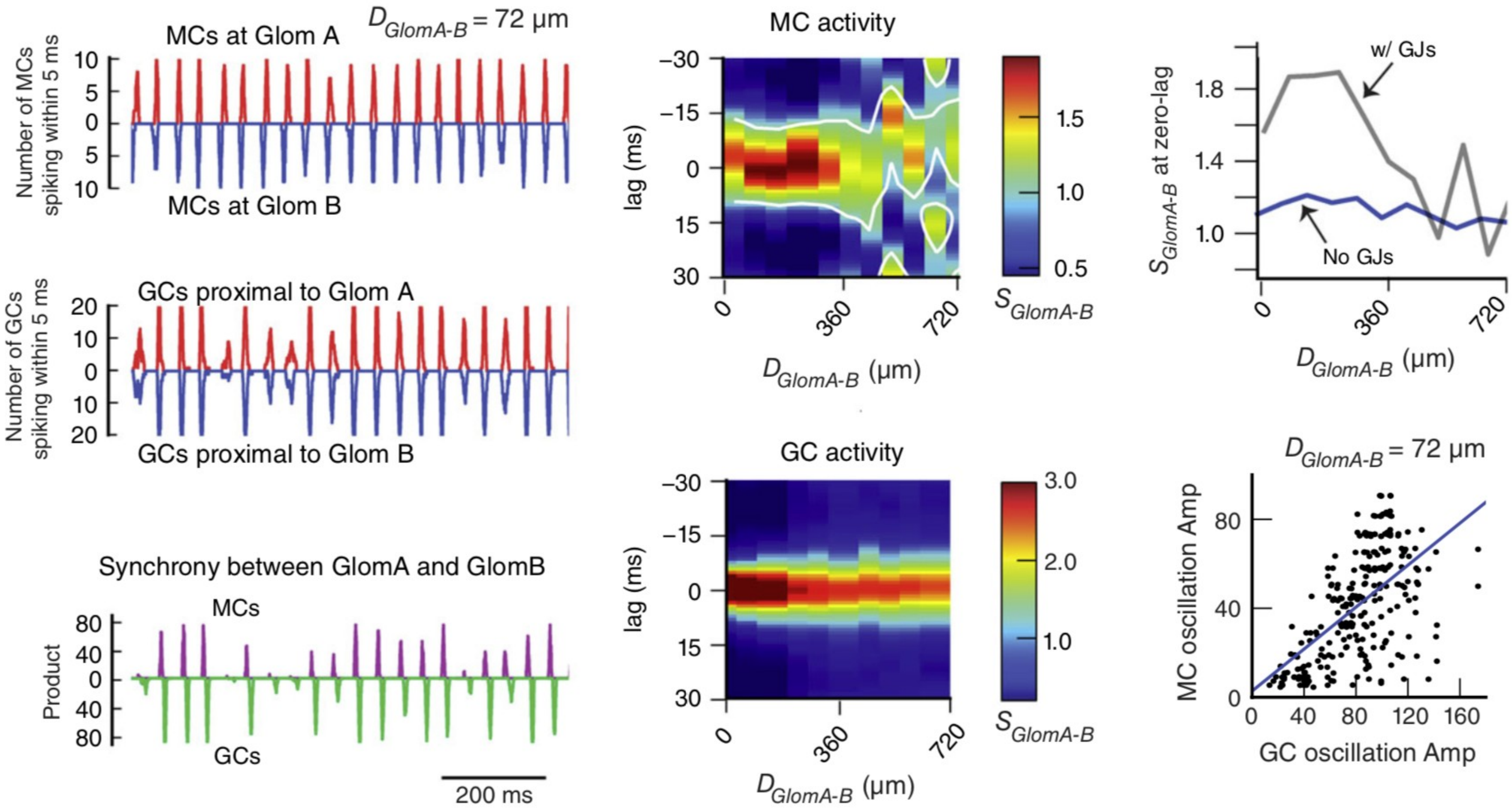
High theta (6 - 14 Hz)
Voltage (uV)
40
0
-40
Theta phase
Phase
π
0
-π
Envelope for high gamma (65 - 95 Hz)
Voltage (uV)
600
0
-600
1.0
1.2
1.4
1.6
1.8
2.0
Time (s)





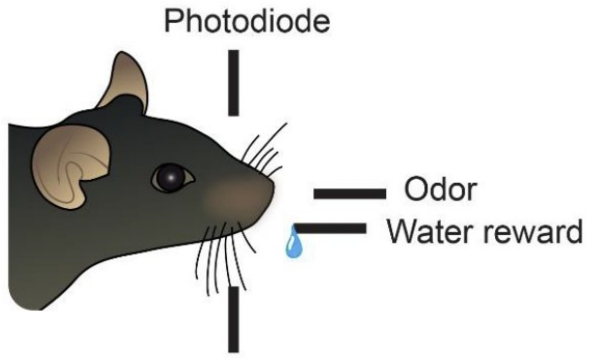
Li and Cleland. PLOS Comp. Biol. 13:e1005760, 2017

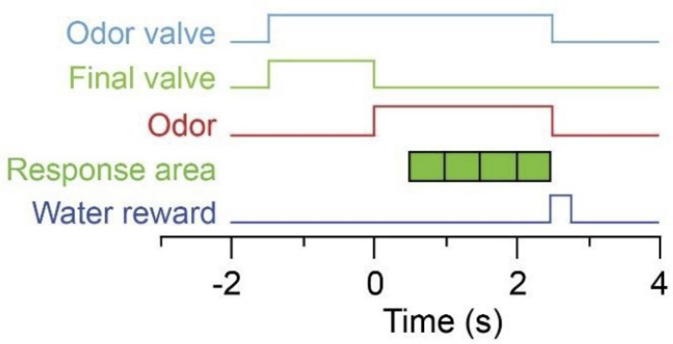
Granule cell inhibition gives rise to gamma frequency oscillations, and these oscillations are augmented by gap junctions between mitral cells

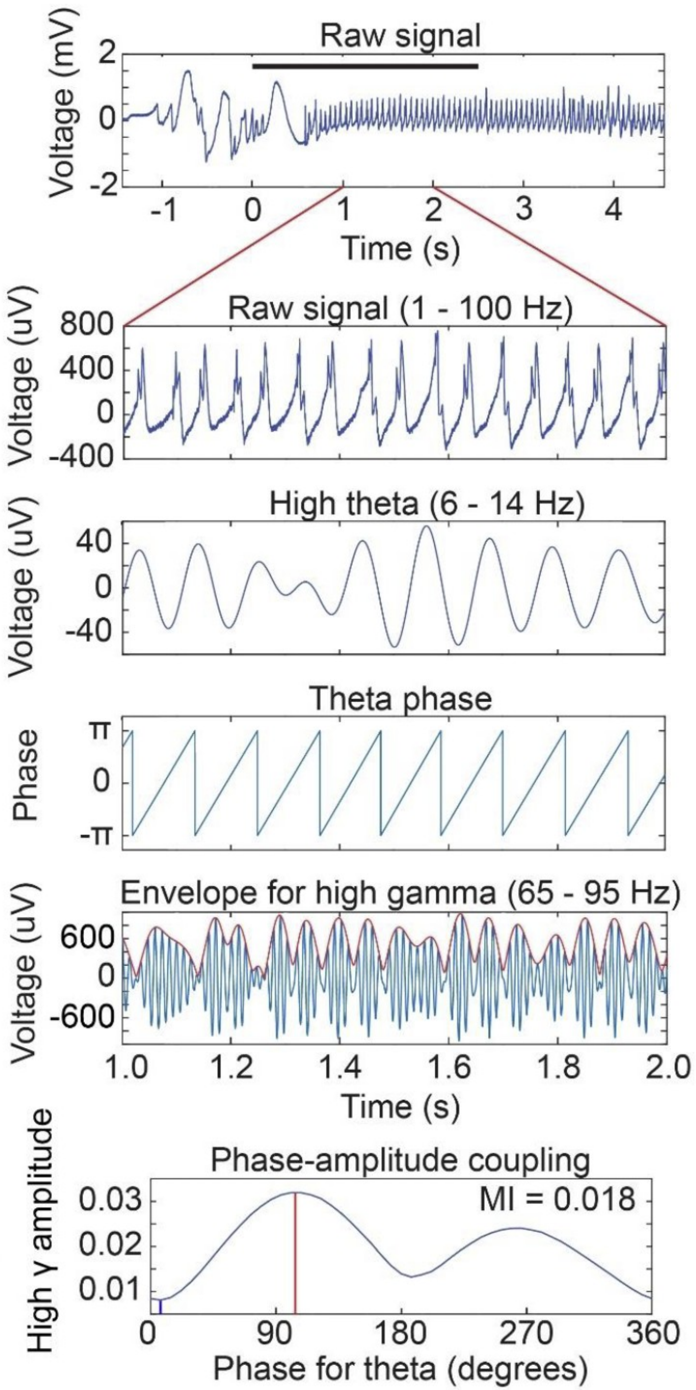


Pouille et al J. Physiol. 595:5965, 2017

Estimating Phase Amplitude Coupling (PAC)

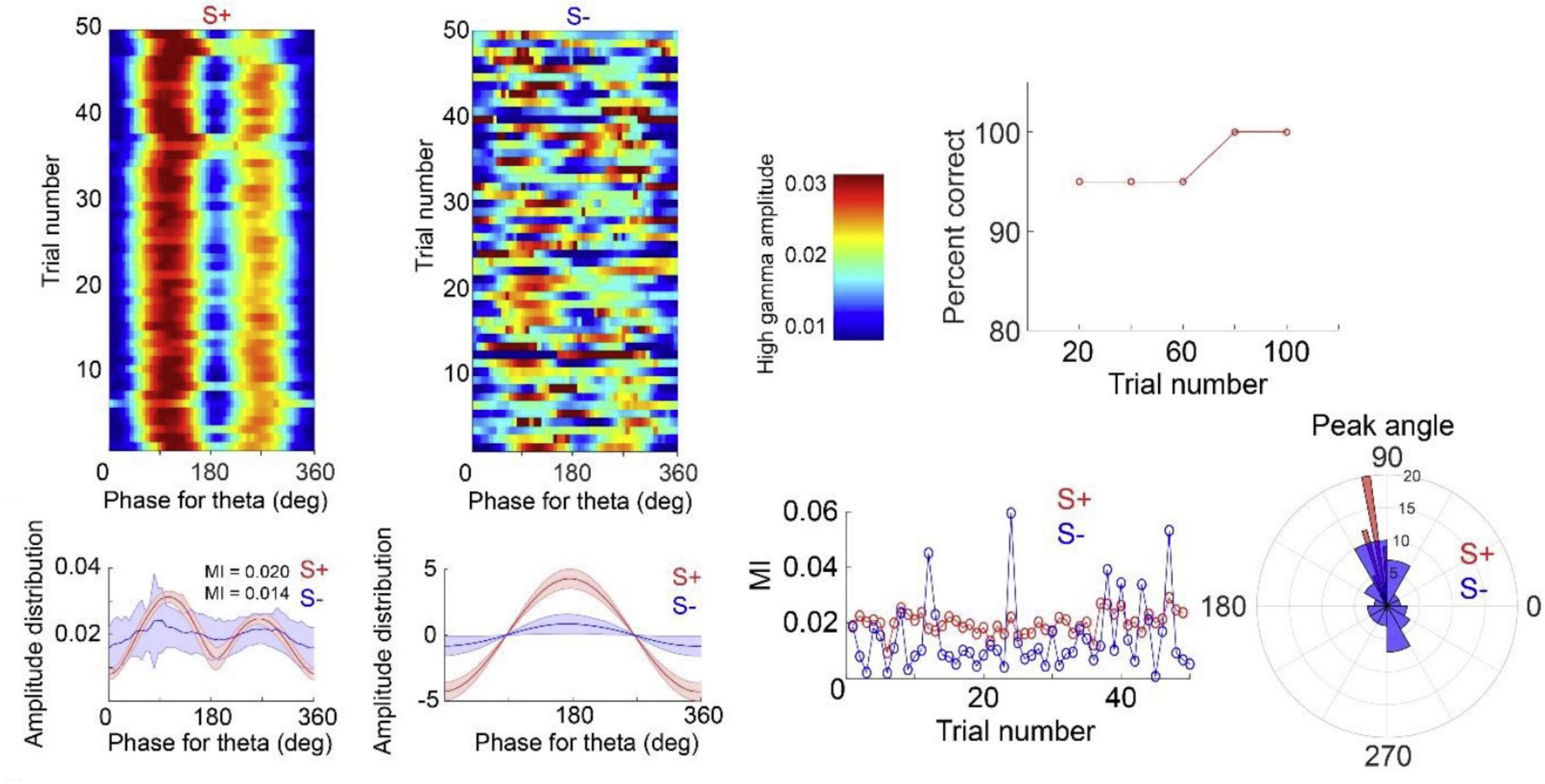




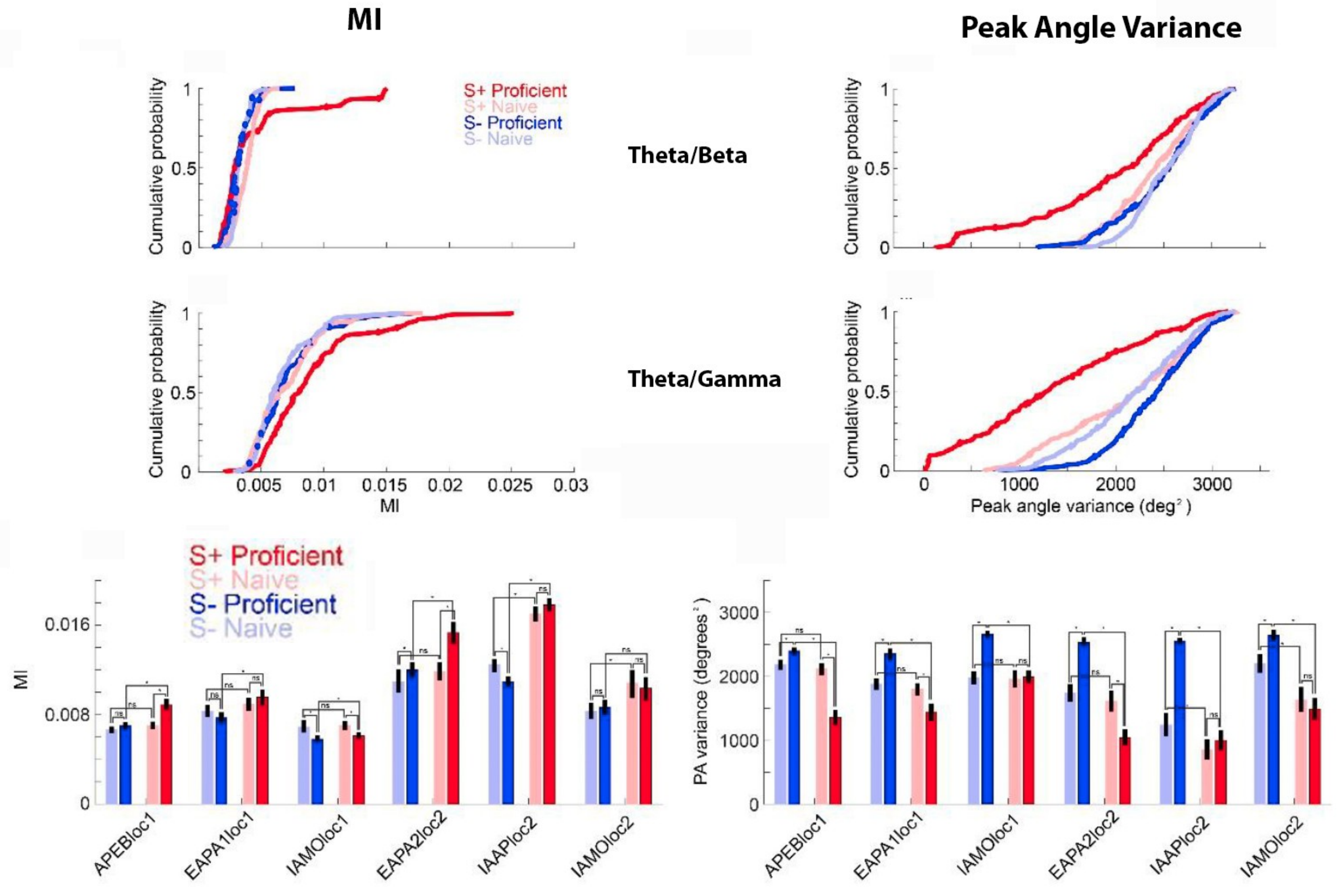


PAC calculation: Tort et al. J. Neurophys. 104:1195, 2010

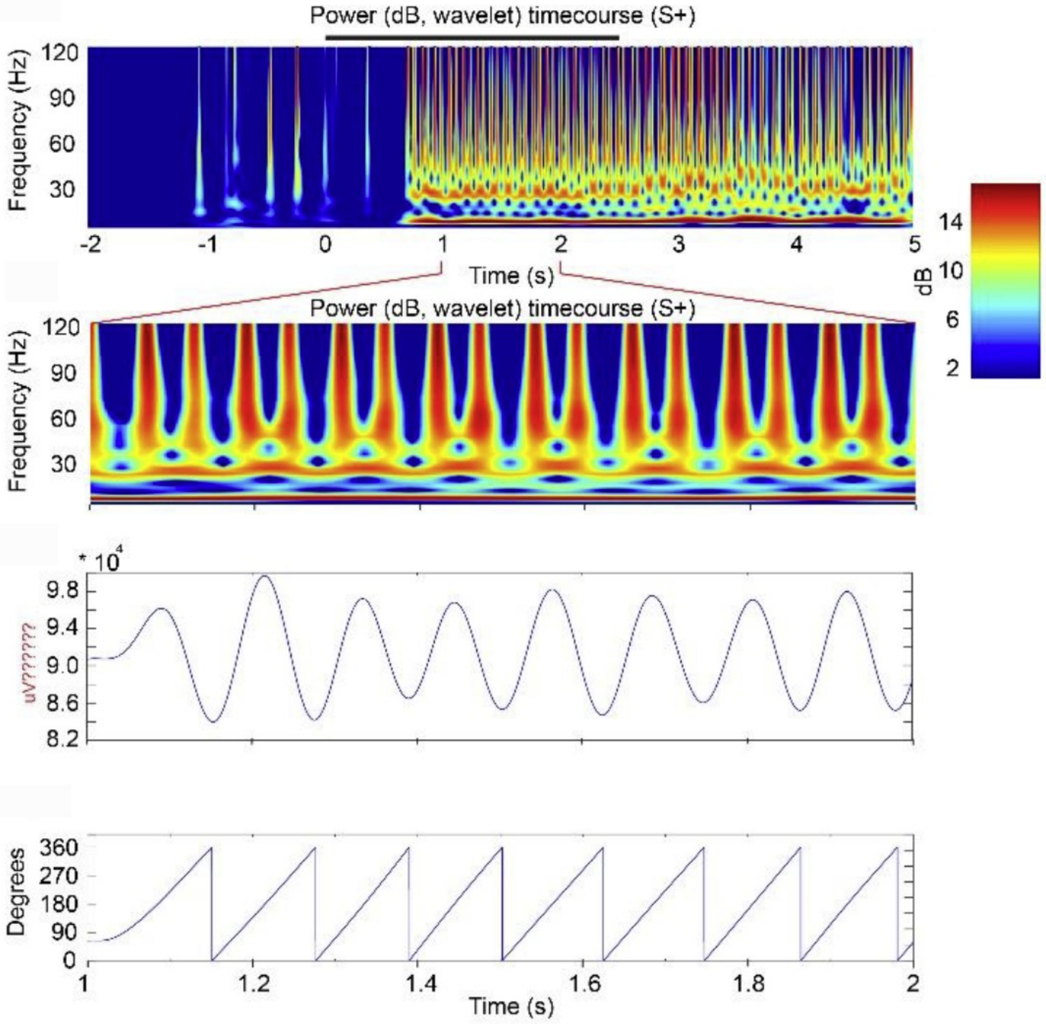
PAC for a proficient mouse

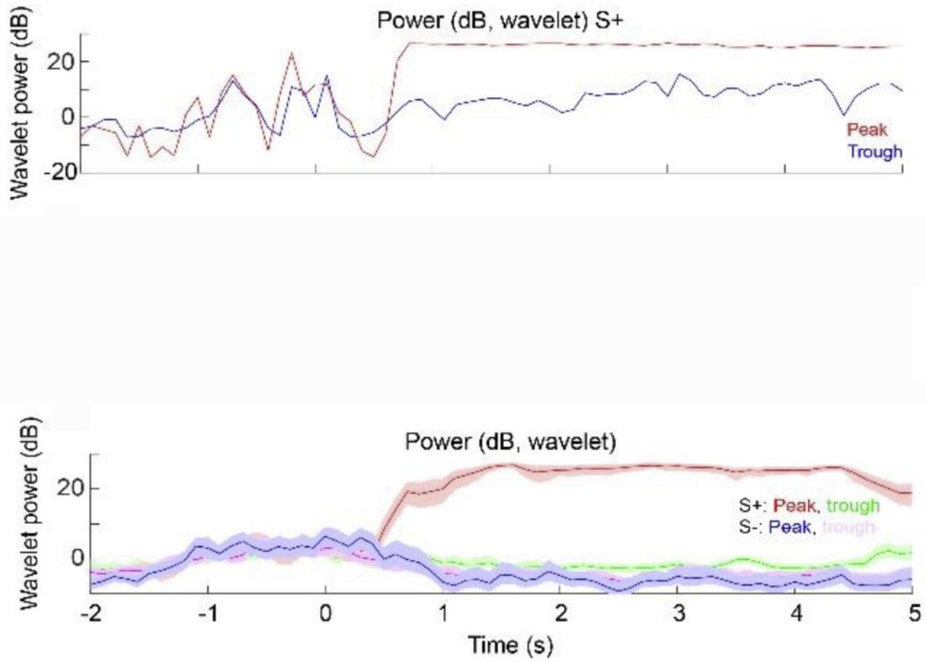


Peak angle variance decreases for S+ and increases for S- as the mouse learns



What happens if the downstream observer of gamma power uses a window at the peak or trough of the theta oscillation?





Confidential. Losacco, Ramirez-Grodillo et al, manuscript in preparation

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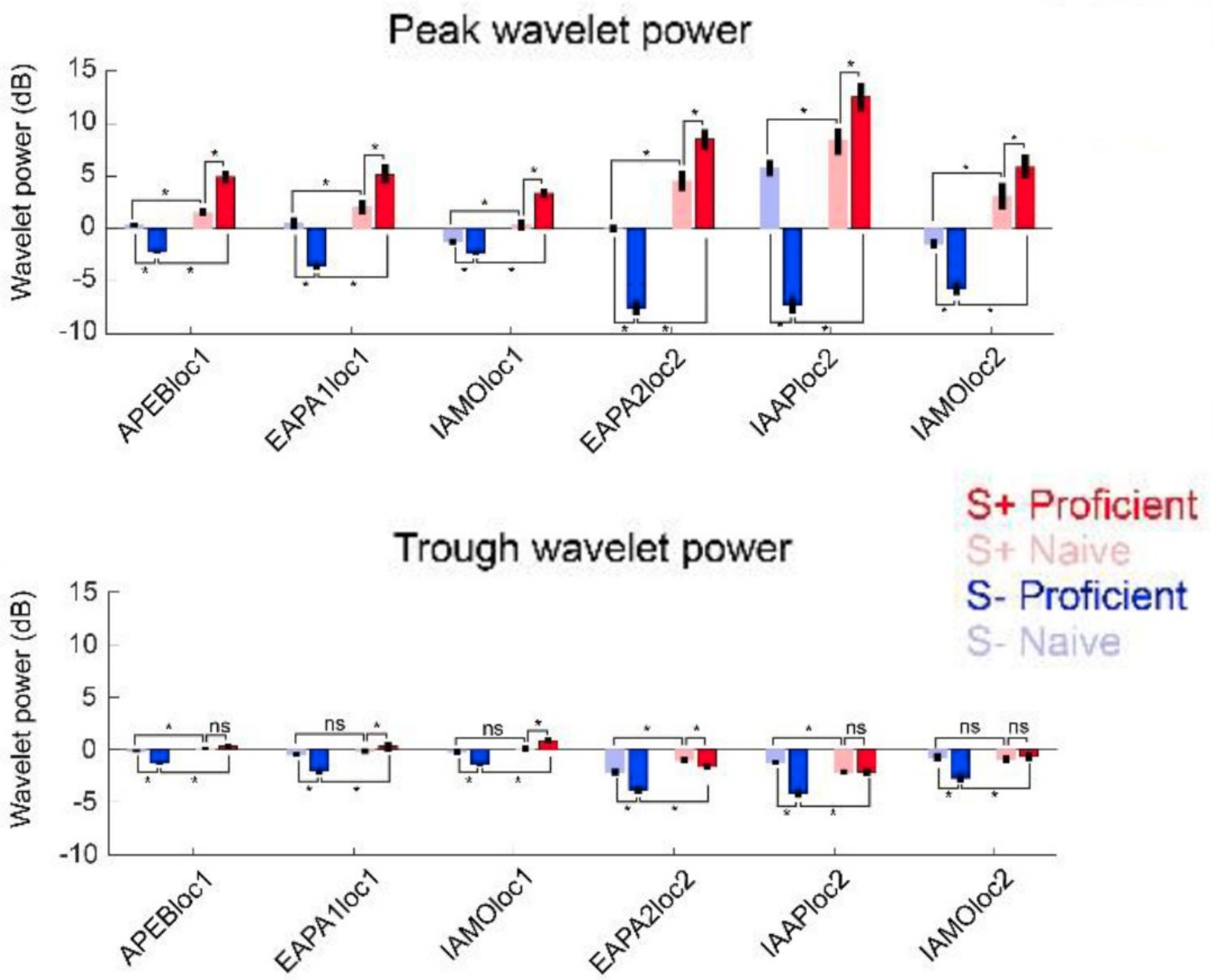
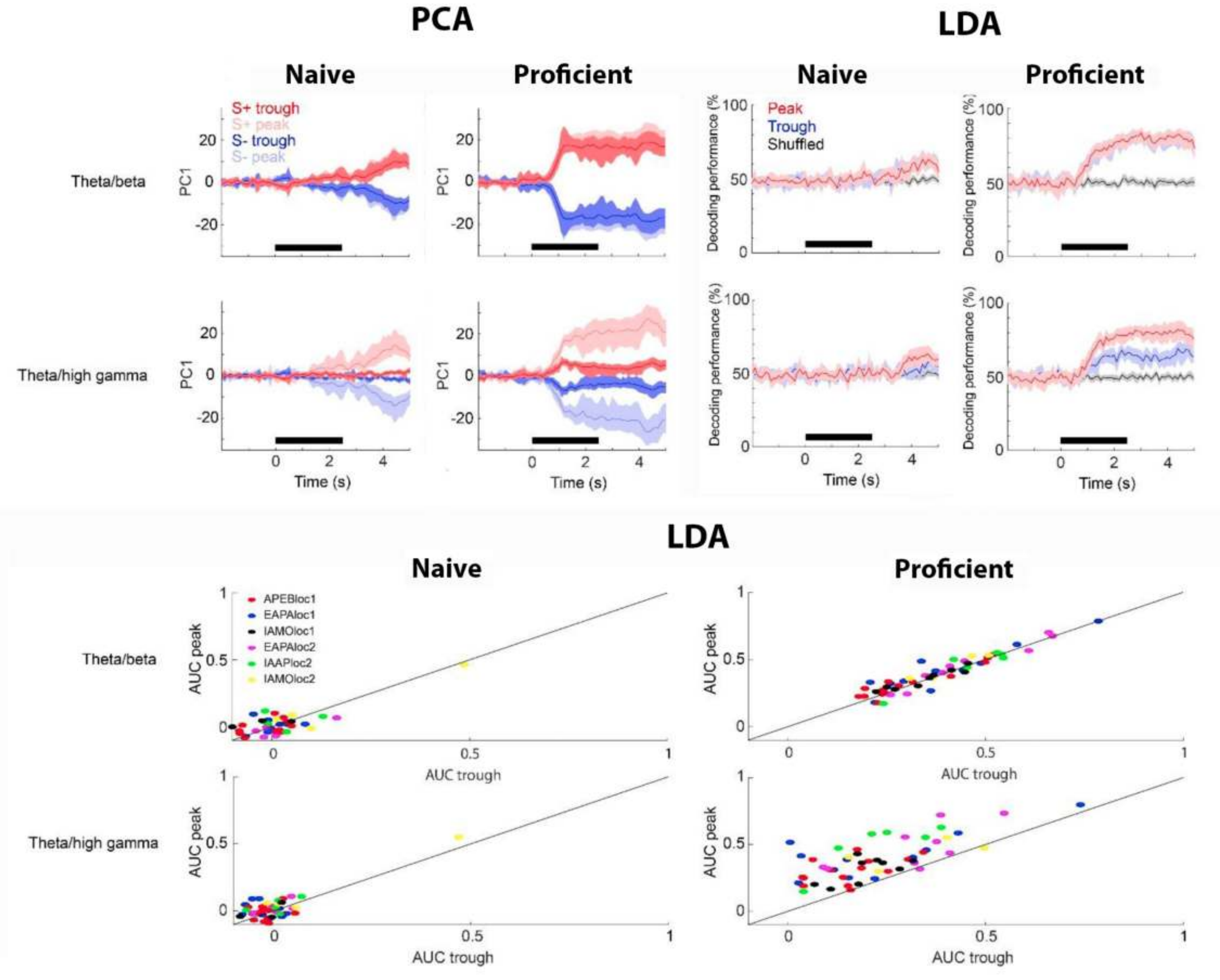
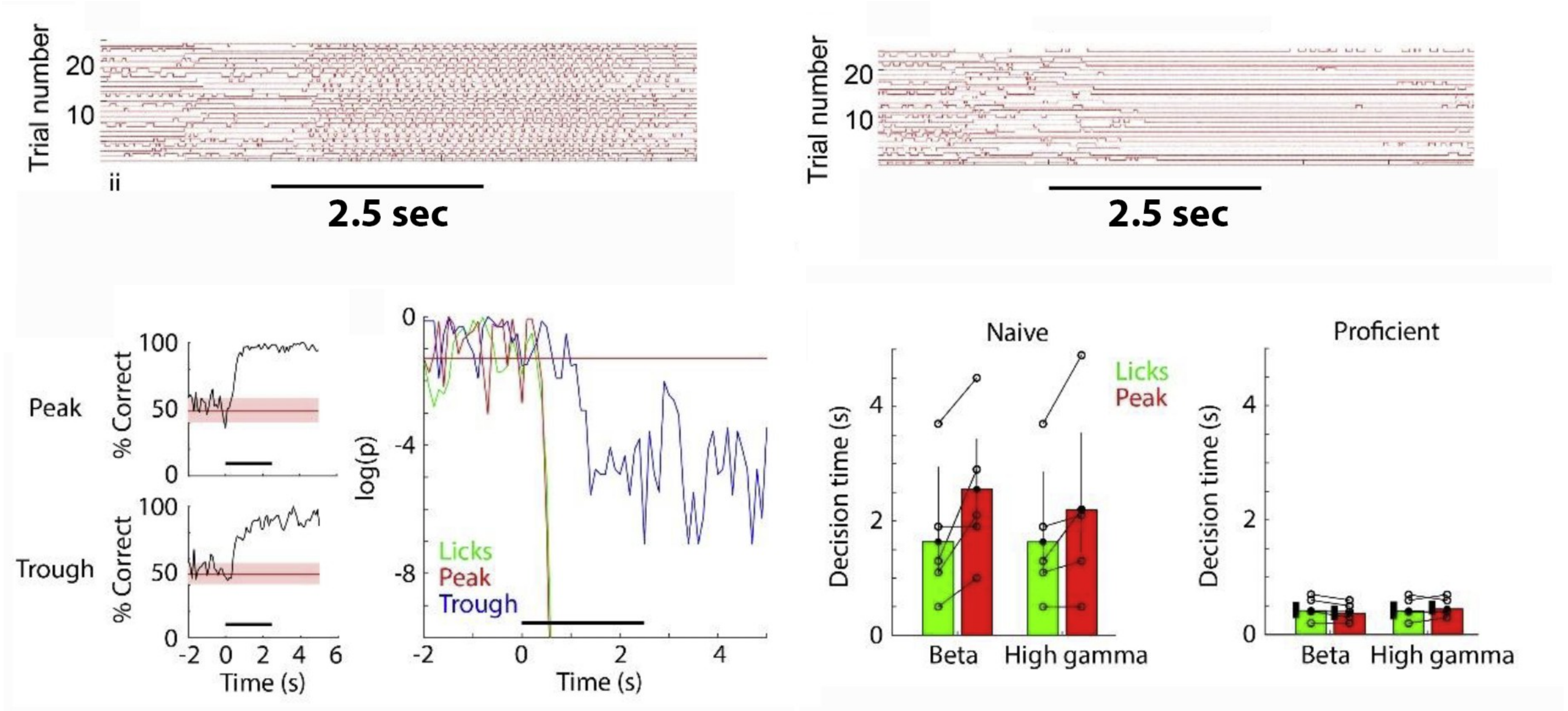


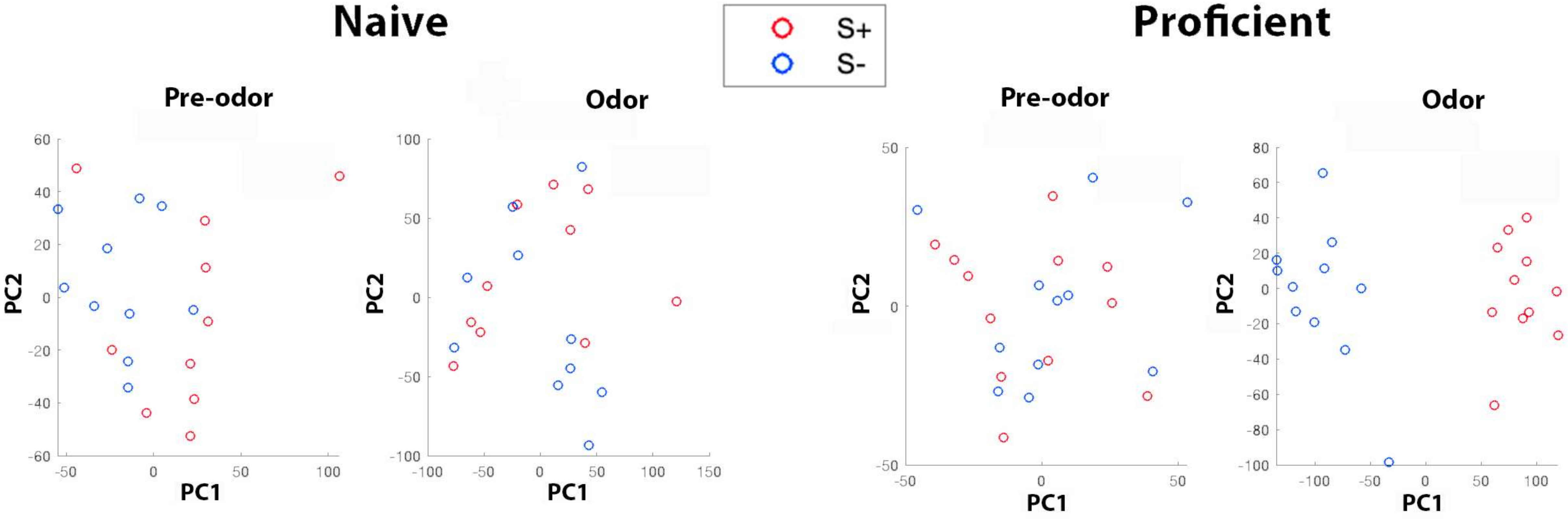
Decoding of odorant identity with linear discriminant analysis using the theta peak-referenced gamma power preforms better than trough-referenced gamma power



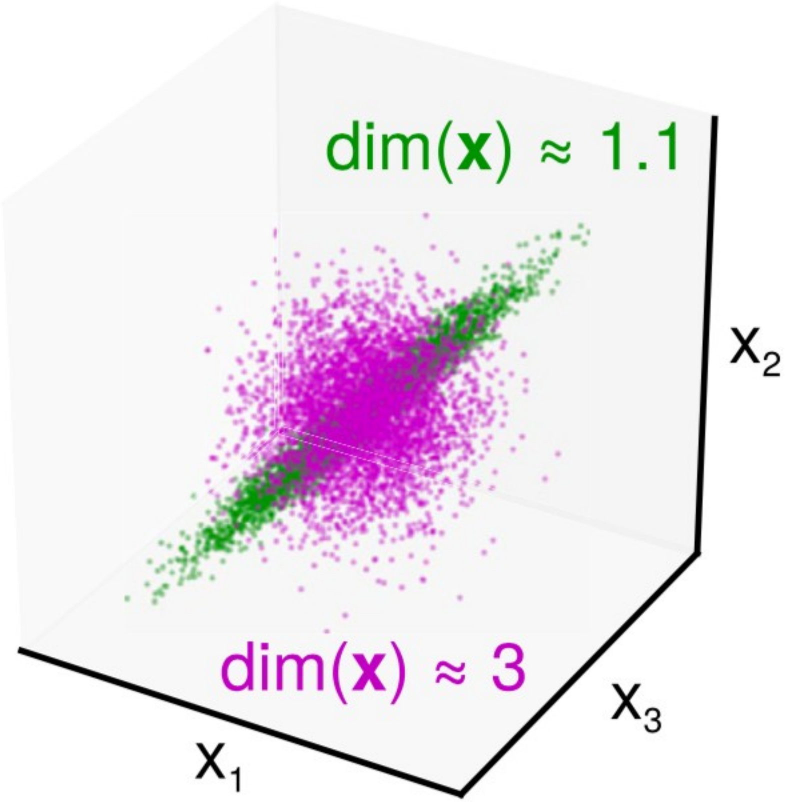
Decision time is the same for theta peak-referenced wavelet power linear discriminant analysis and for lick divergence



Example of principal component separation as the animal learns to differentiate the odorants



What is the dimensionality of the peak-referenced gamma power?

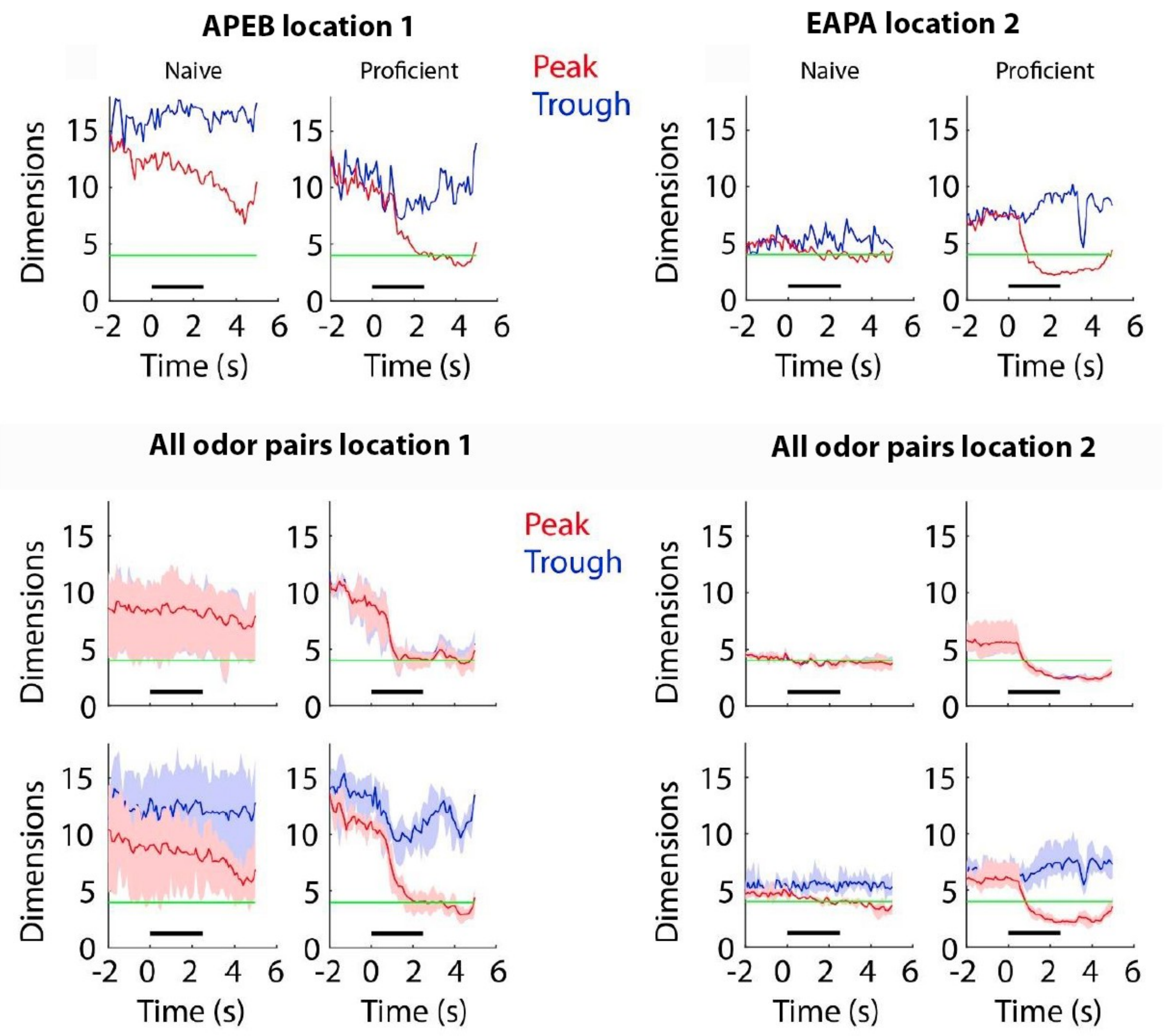


We define the dimension of a system with M degrees of freedom, $\mathbf{x} = (x_1, x_2, \ldots x_M)$, as

$$\dim(\mathbf{x}) = \frac{\left(\sum_{i=1}^{M} \lambda_i\right)^2}{\sum_{i=1}^{M} \lambda_i^2}, \qquad \text{(Equation 1)}$$

where λ_i are the eigenvalues of the covariance matrix of $\mathbf{x}$ computed by averaging over the distribution of inputs to the system (Abbott et al., 2011).

Dimensionality of the peak-phase referenced gamma power differs between locations and changes after learning



Conclusions

- Theta peak-referenced gamma power carries information on odor identity
- Theta trough-referenced gamma power carries significantly less information on odor identity
- As the mouse learns to differentiate the odorant the dimensionality of theta-peak referenced gamma power decreases

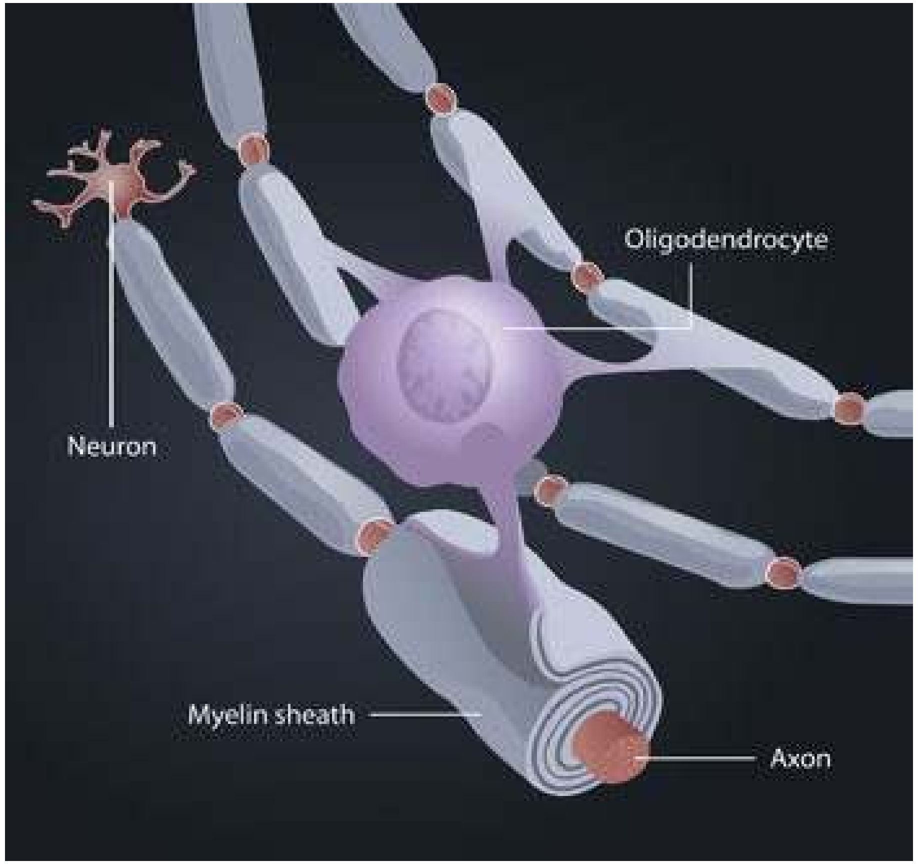
Computational steps towards unwrapping the role of myelin in olfactory signal processing

- Oscillations are augmented and dimensionality changes as the animal learns to differentiate odorants in the go-no go task

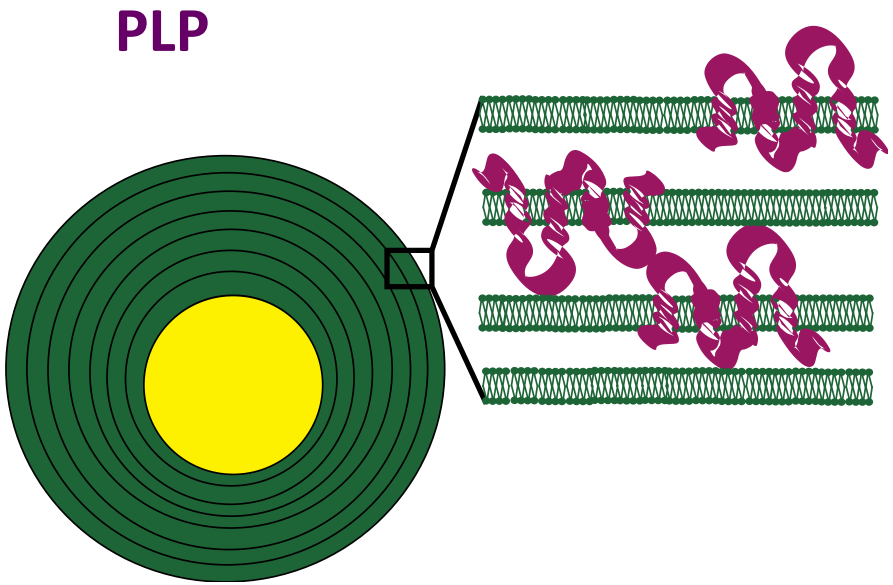
- Mild Myelin Disruption Elicits Early Alteration in Olfactory Behavior and Proliferation in the Subventricular Zone

Neurological problems in mild myelin disruption (early Multiple Sclerosis): Young adult PLP knockout mouse

Myelin is key for neuronal transmission of information



Proteolipid protein (PLP) is a structural component of the myelin sheath



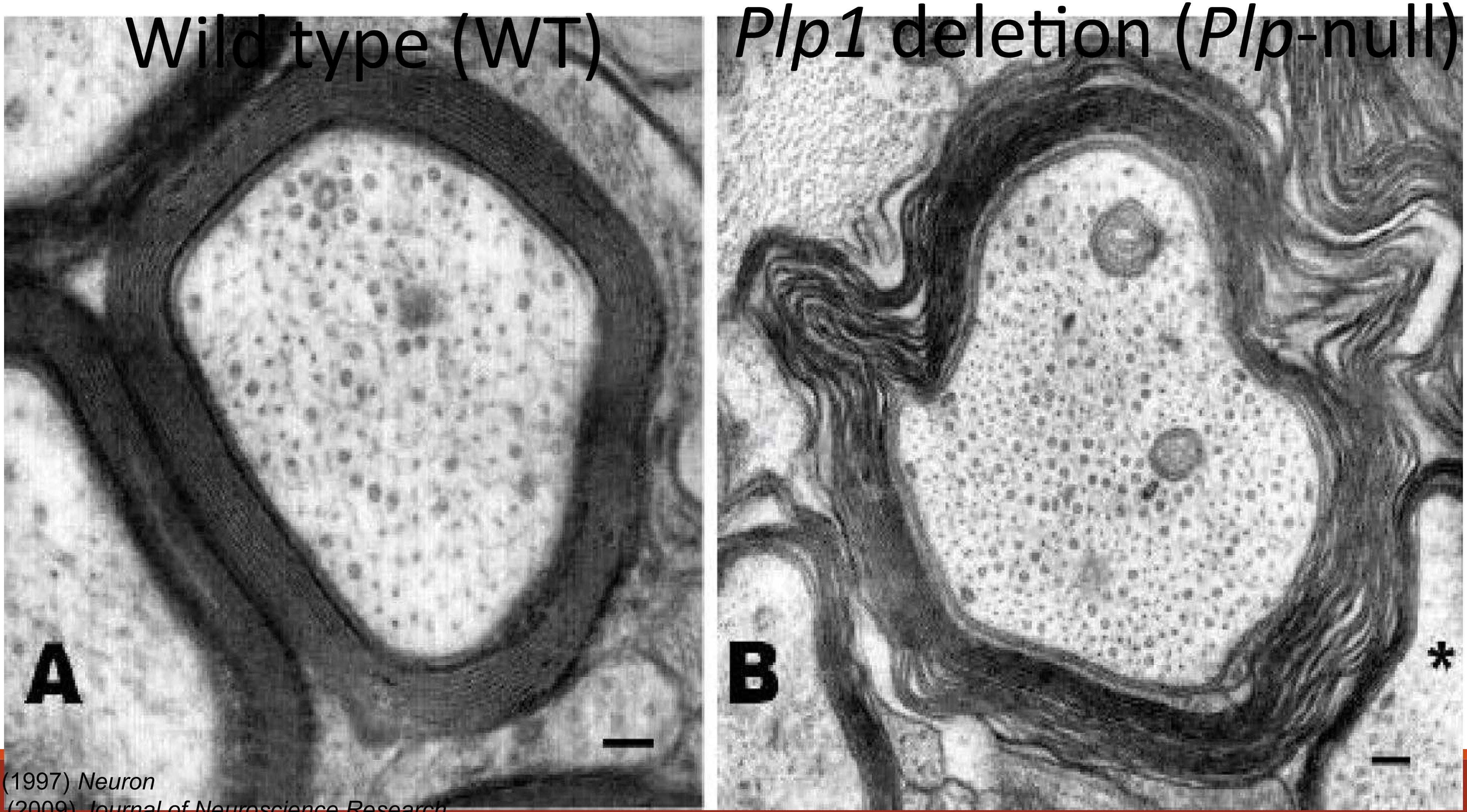
Gould et. al. eLife e34783, 2018

http://www.nih.gov

Plp point mutations or gene duplications lead to the dysmyelinating diseases, Pelizaeus-Merzbacher disease (PMD) and spastic paraplegia type 2

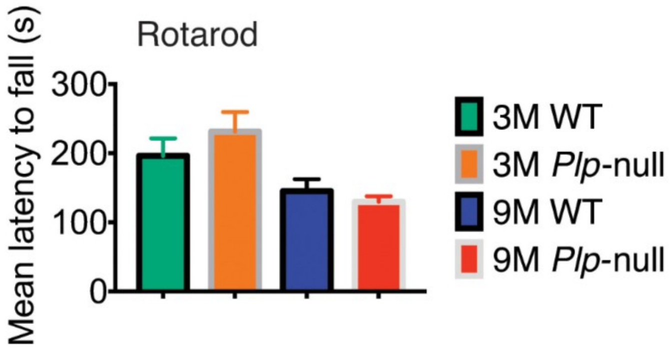
10 years old, Jacob Trossman, http://jacobsladder.ca/pmd/

Deletion of *plp1* leads to loss of myelin compaction



Klugmann, M, et al (1997) *Neuron*
Rosenbluth, J, et al (2009) *Journal of Neuroscience Research*

3 month PLP-null overstep rear paw



Gould et. al. eLife e34783, 2018

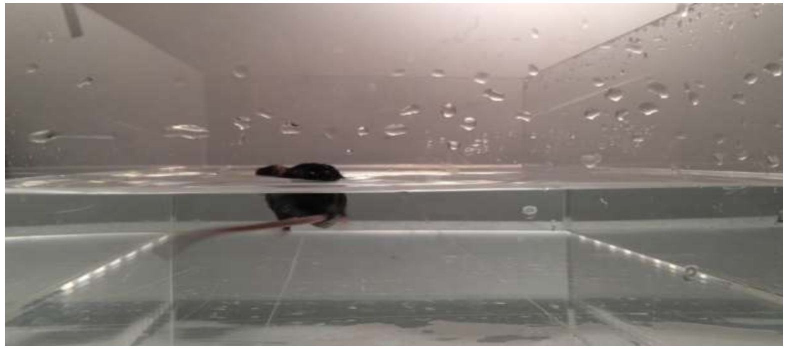
Gould et. al. eLife e34783, 2018

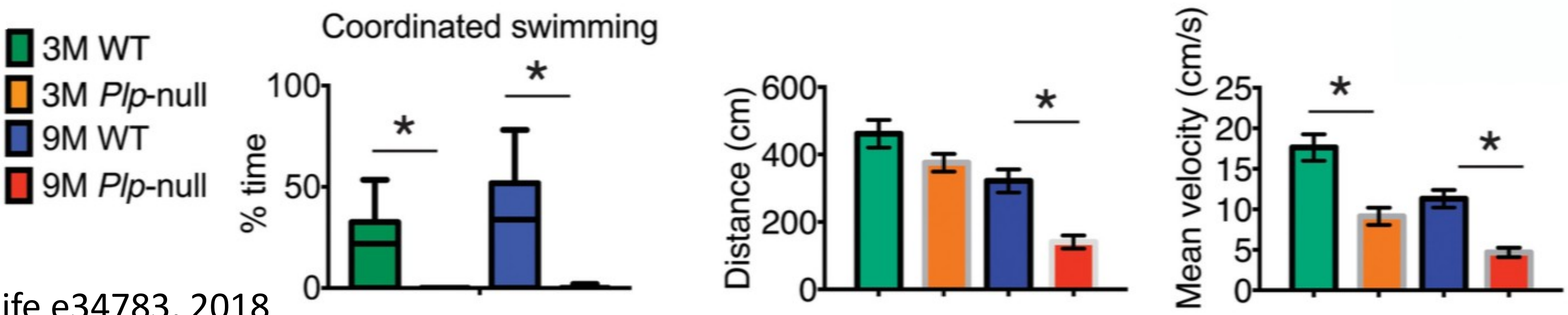
p<0.01, repeated measures ANOVA

Problems with motor coordination: swimming

WT

Plp-null

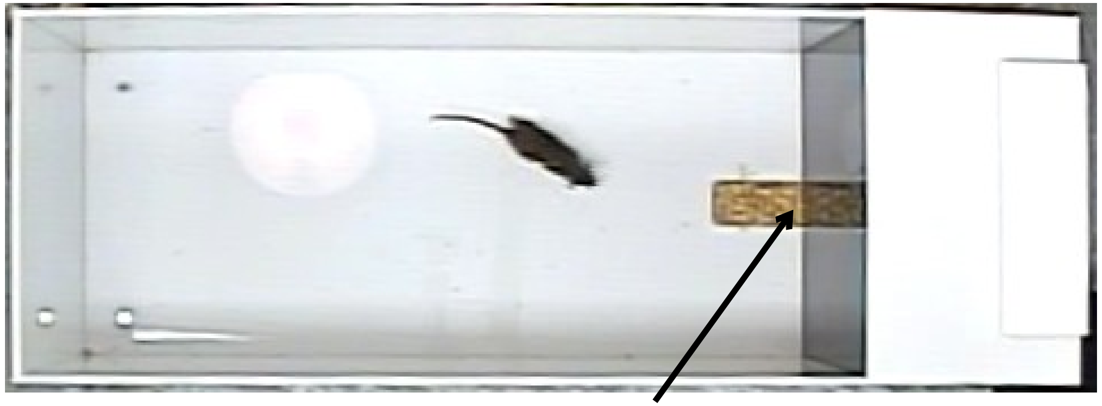




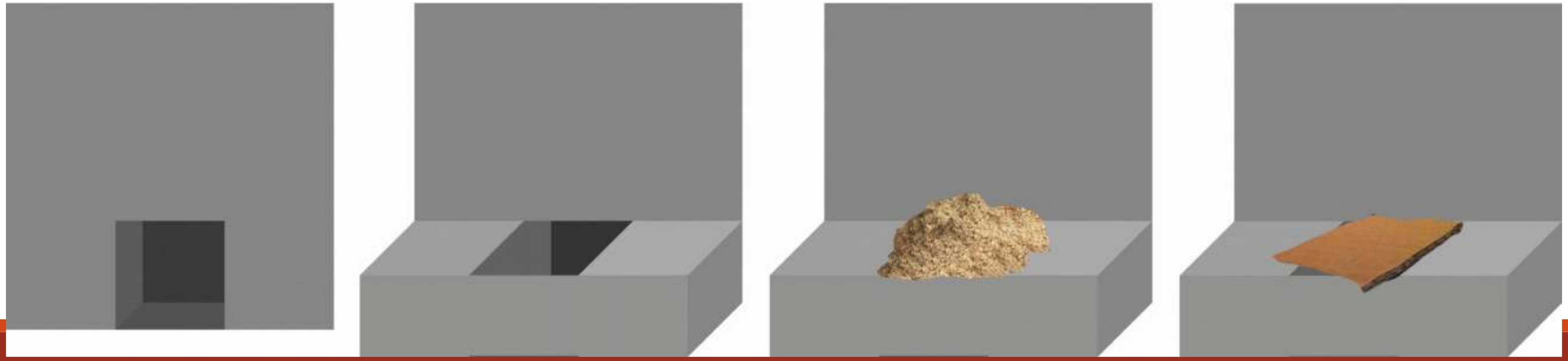
Gould et. al. eLife e34783, 2018

Puzzle box: comprehensive test of cognitive function

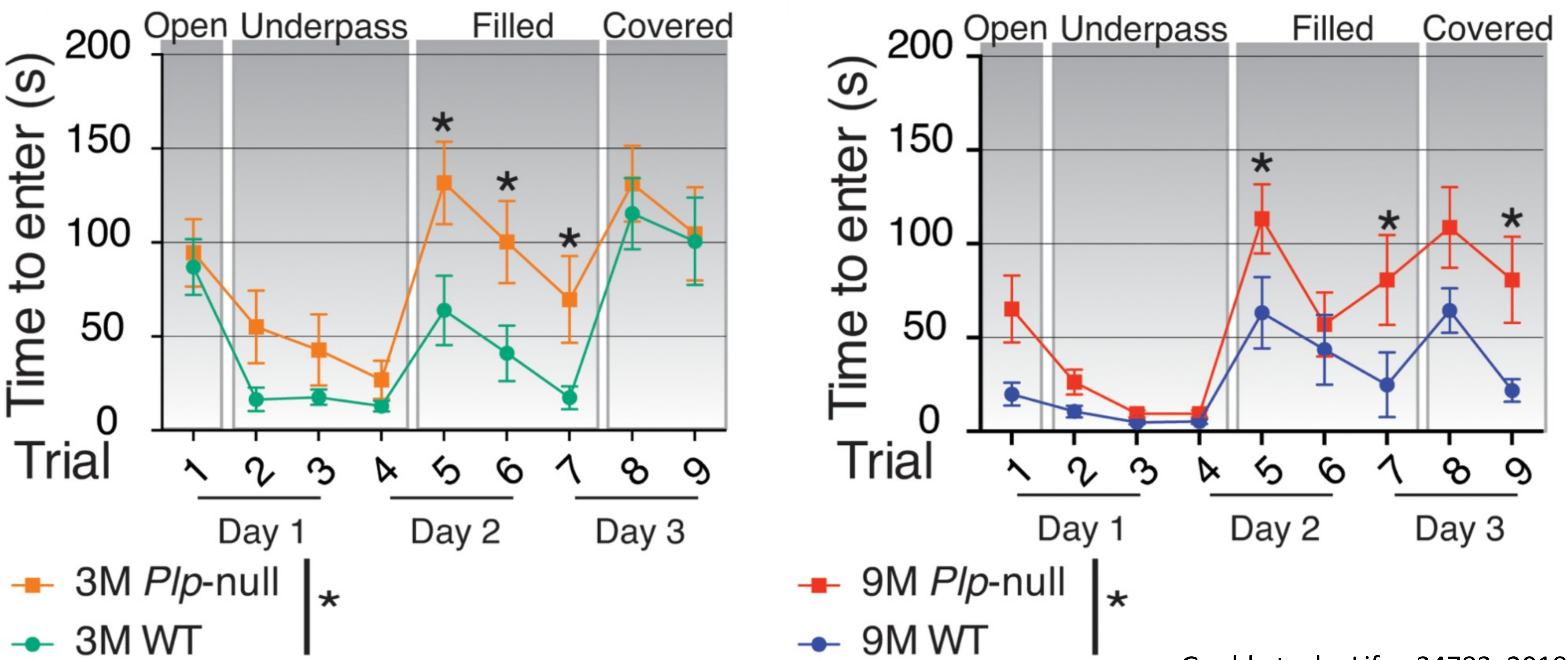
Problem-solving
Short-term memory
Long-term memory



Entry to goal box

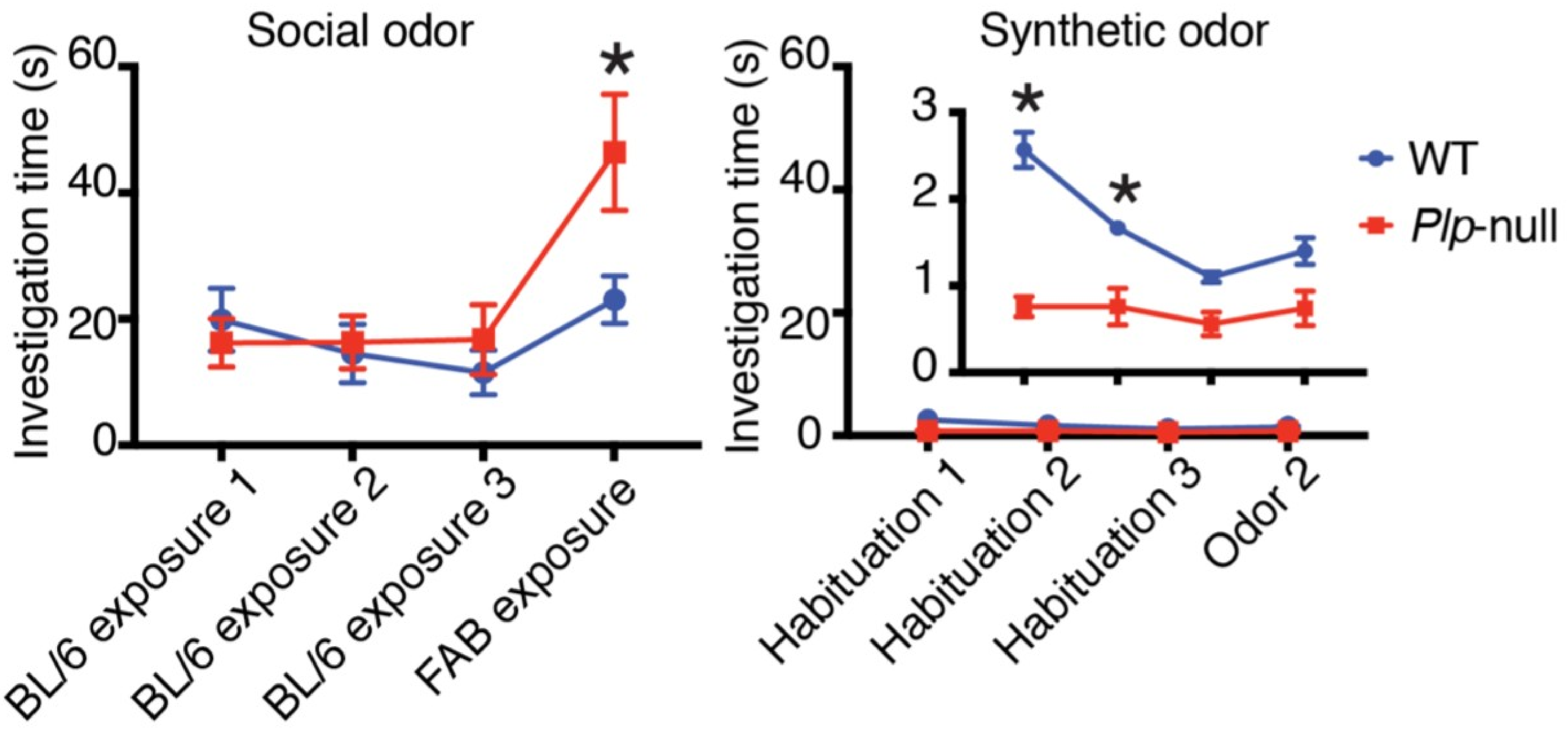


Cognitive function is impaired in PLP-null mice



Gould et. al. eLife e34783, 2018

Plp1-null mice exhibit increased exploration of novel social odors



Gould et. al. eLife e34783, 2018

The distance traveled, velocity and behavioral outcome did not differ in a subset of behavioral tasks

	3 months			9 months		
Behavioral performance	WT (n = 10) Mean(SEM)	Null (n = 10) Mean(SEM)	p value	WT (n = 10) Mean(SEM)	Null (n = 10) Mean(SEM)	p value
Open field (duration in center (s))	217.0 (15.52)	203.71 (0.336)	0.88	**238.5** (25.14)	**158.6** (22.99)	**0.02**
Zero maze (duration open arms (s))	**92.65** (17.03)	**175.7** (21.24)	**0.03**	126.5 (27.41)	117.2 (26.09)	0.95
Y maze (% successful alternations)	61.48 (2.484)	61.56 (2.220)	0.99	57.33 (2.888)	57.32 (3.726)	0.99
Y maze (arm entries)	32.18 (2.529)	35.91 (2.387)	0.60	32.20 (3.608)	29.80 (3.252)	0.82
Marble burying (marbles buried)	**4.800** (1.052)	**1.600** (0.400)	**<0.01**	**3.900** (0.862)	**0.200** (0.133)	**<0.01**
Marble burying (time digging (s))	8.428 (2.245)	2.994 (0.666)	0.25	**13.24** (3.767)	**4.294** (2.390)	**0.04**
Locomotion						
Rotarod (mean latency to fall (s))	196.2 (25.08)	231.2 (28.41)	0.88	145.1 (17.38)	129.7 (7.948)	0.94
Distance traveled (cm)						
Open field	5255 (466.3)	4589 (229.8)	0.39	4510 (444.4)	5205 (322.4)	0.36
Zero maze	9532 (3170)	10831 (2210)	0.92	6148 (1708)	7030 (1694)	0.96
Y maze	27693 (1346)	15484 (11985)	0.68	3931 (734.5)	3812 (324.3)	0.99
Marble burying	3729 (379.1)	2931 (361.1)	0.16	5591 (204.0)	5566 (289.0)	0.99
Habituation				845.2 (78.3)	1041 (56.31)	0.06
Velocity (cm/s)						
Open field	9.551 (0.673)	9.287 (0.336)	0.94	7.518 (0.741)	8.667 (0.537)	0.32
Zero maze	16.34 (6.224)	10.25 (2.864)	0.52	11.79 (2.815)	18.38 (3.695)	0.46
Y maze	65.95 (43.50)	33.15 (24.92)	0.61	8.987 (1.585)	8.198 (0.885)	0.99
Marble burying	6.766 (0.407)	6.188 (0.595)	0.61	9.323 (0.339)	9.298 (0.482)	0.99
Habituation				7.859 (0.619)	6.986 (1.028)	0.47

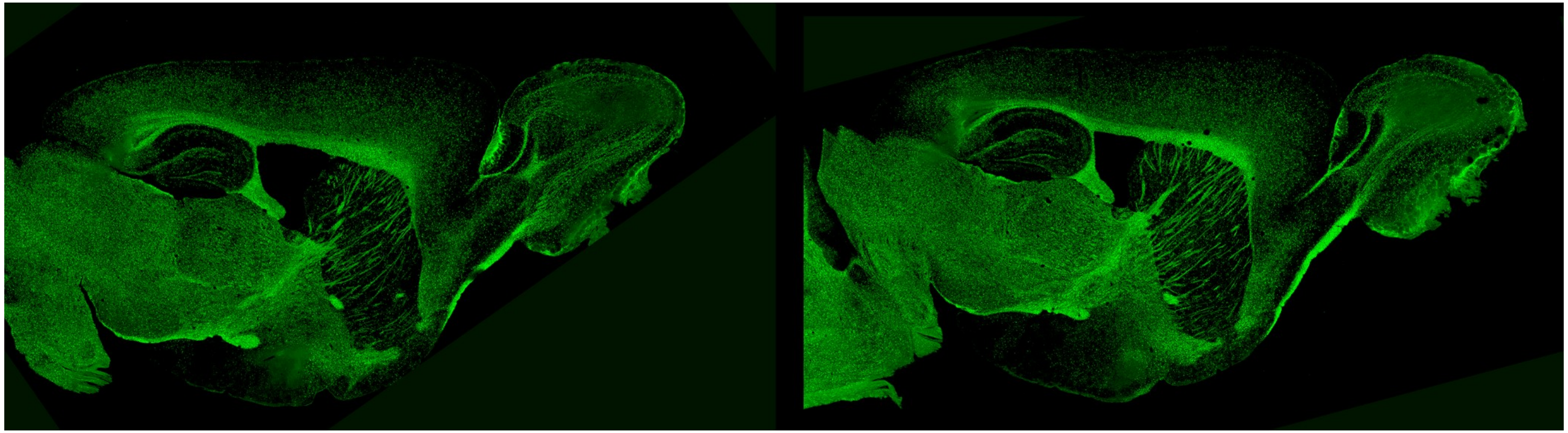
Gould et. al. eLife e34783, 2018

Region-wide analysis of oligodendrocytes (OL)

**Klugmann et al. (1997)

	Progressive		Late
Cellular	**< 2 months**	**2 -16 months**	**> 16 months**
Oligodendrocytes	Normal appearance	Are there region-specific differences?	Demyelination (spinal cord)

WT PLP-null

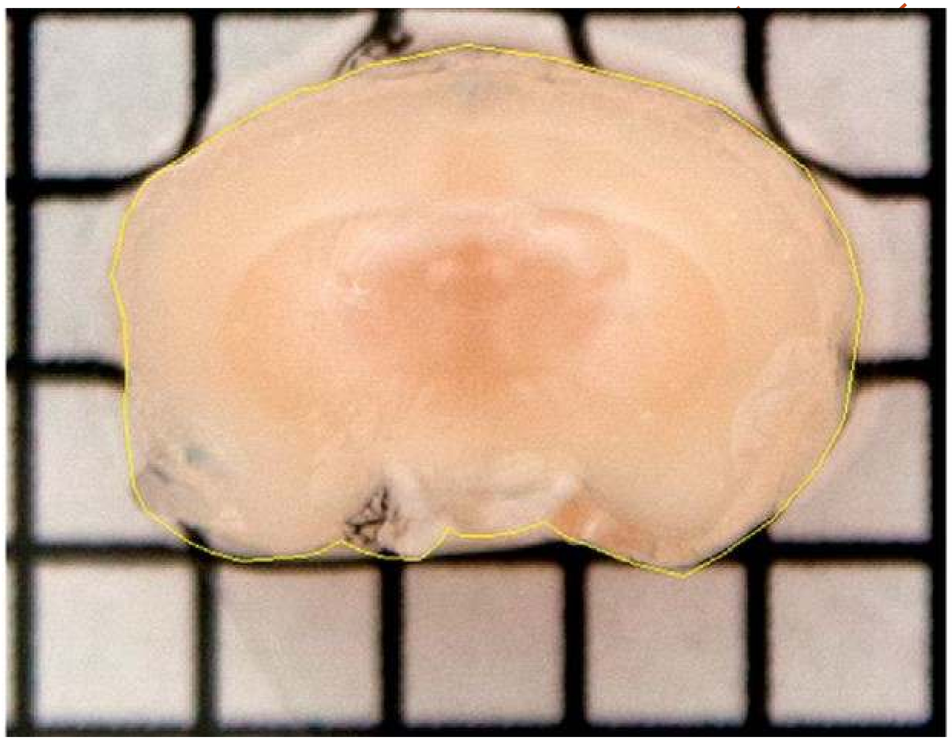


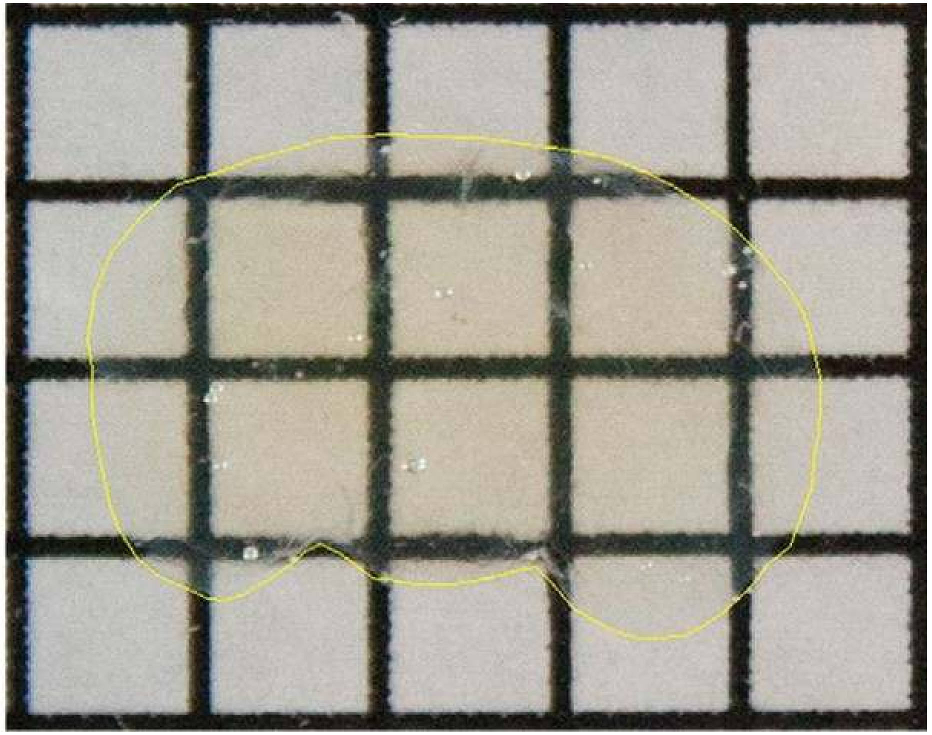
PLP-eGFP labels majority of mature oligodendrocytes in adult mouse

Established in 2002 by Mallon et al.

30 µm section tiled image

Clear tissue enables high volume imaging

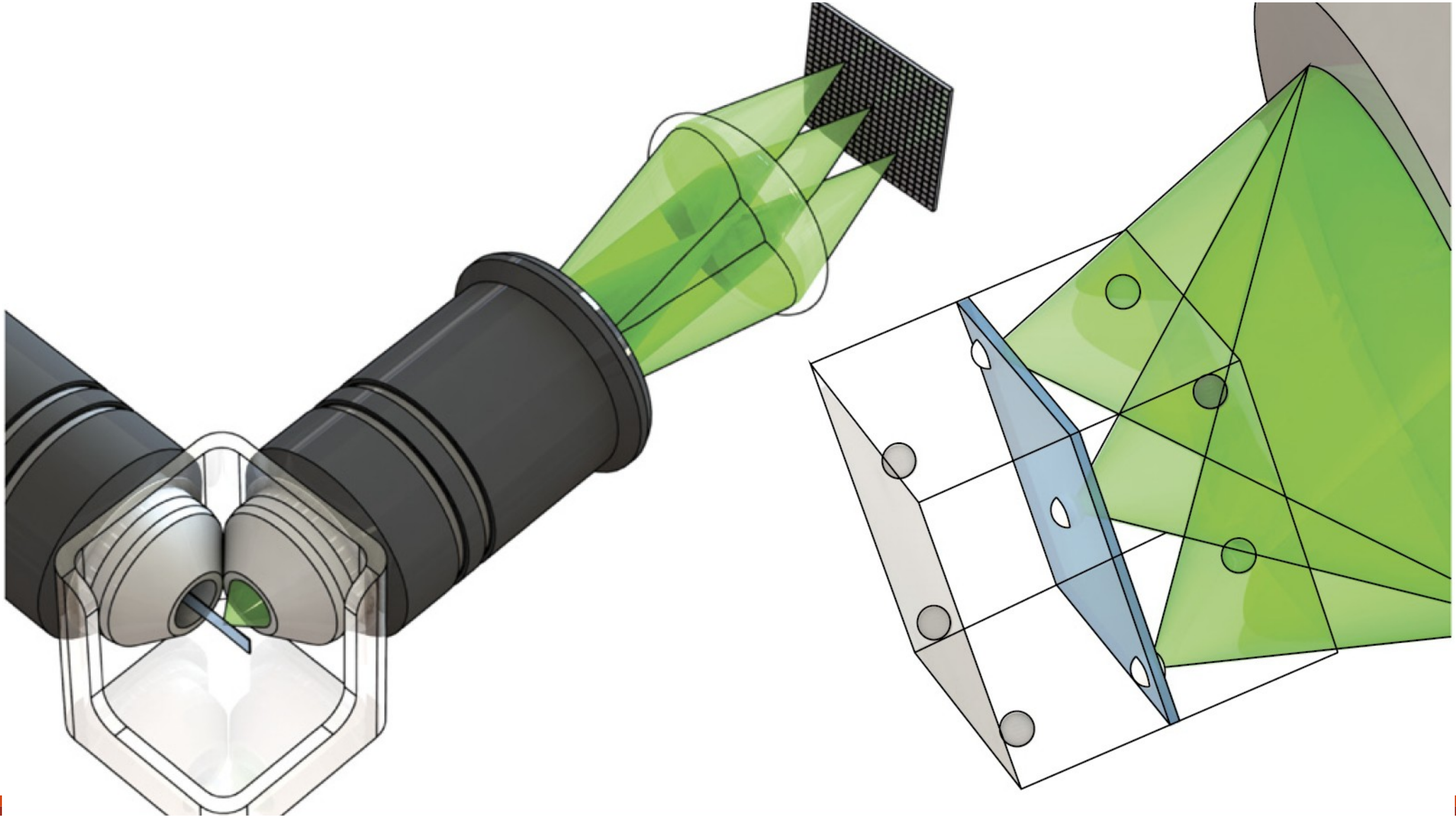




Epp et al 2014

Light sheet fluorescence microscopy

University of Colorado
Denver | Anschutz Medical Campus



Power and Huisken (2017) *Nature Methods*

Light sheet fluorescence microscopy

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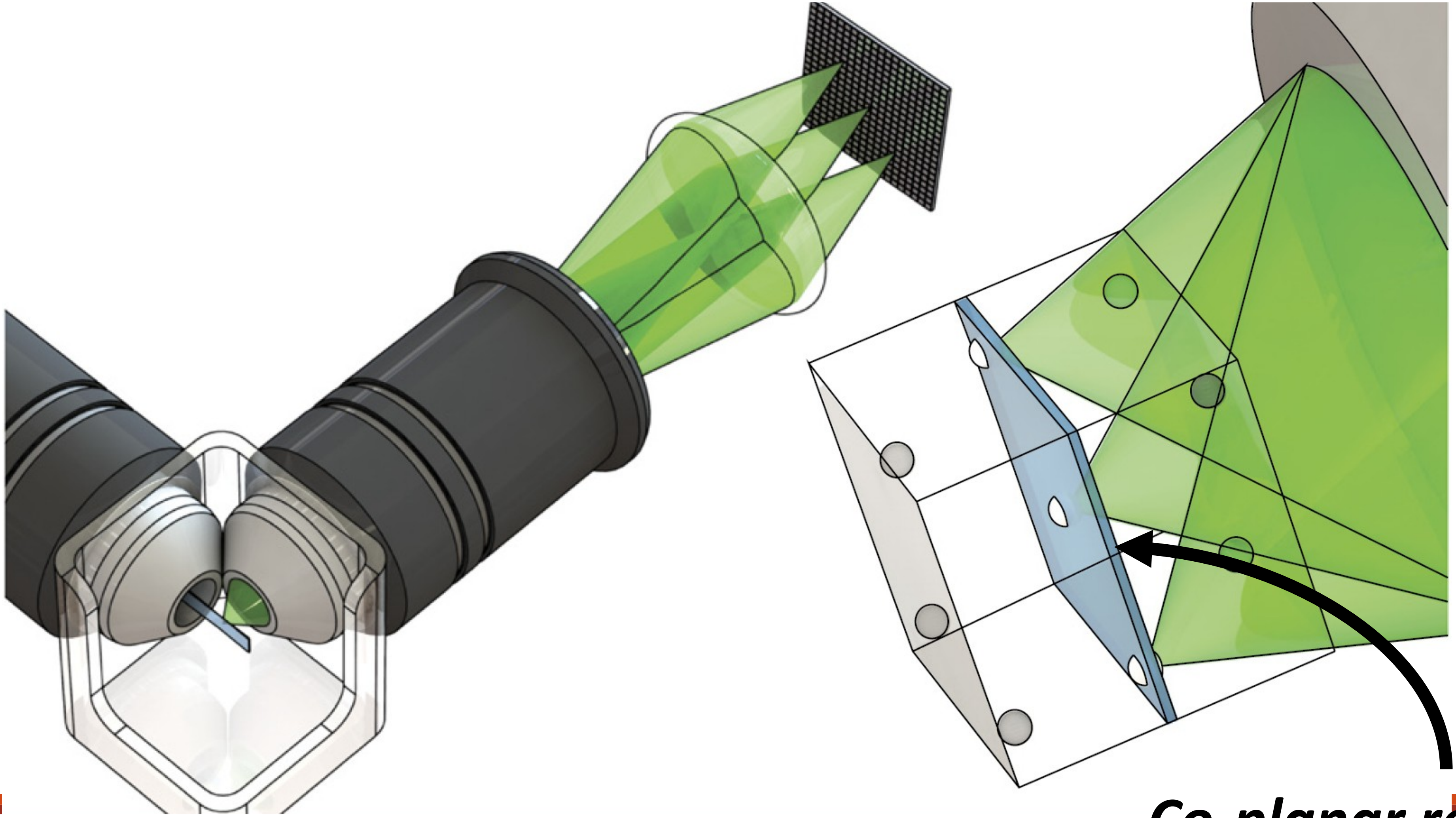
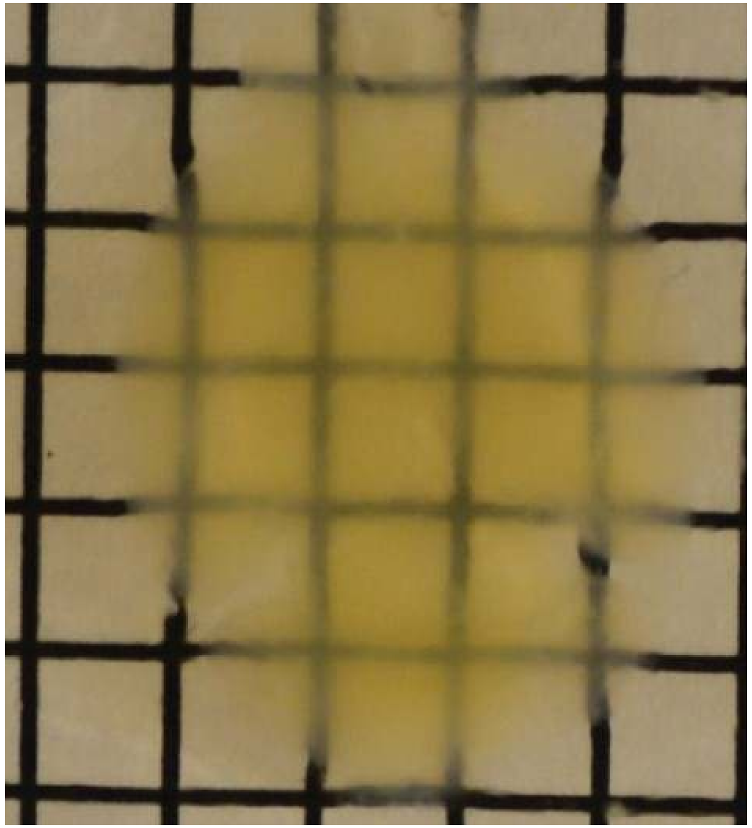
Denver | Anschutz Medical Campus



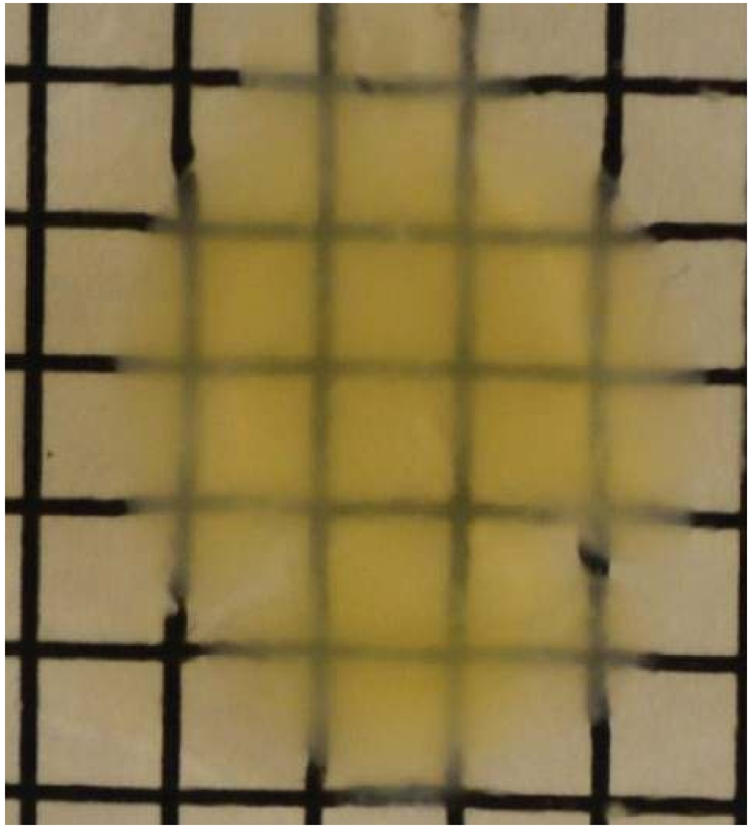
Co-planar requirement

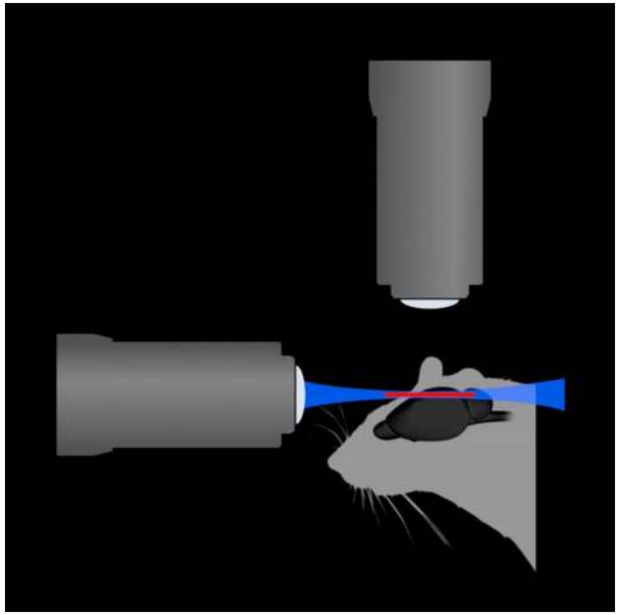
Heterogenous RI breaks co-planarity in light sheet fluorescence microscopy



Chung, K, et al (2013) *Nature*
Yang, B, et al (2014) *Cell*
Ryan, D. et al (2017) *Nature Communications*

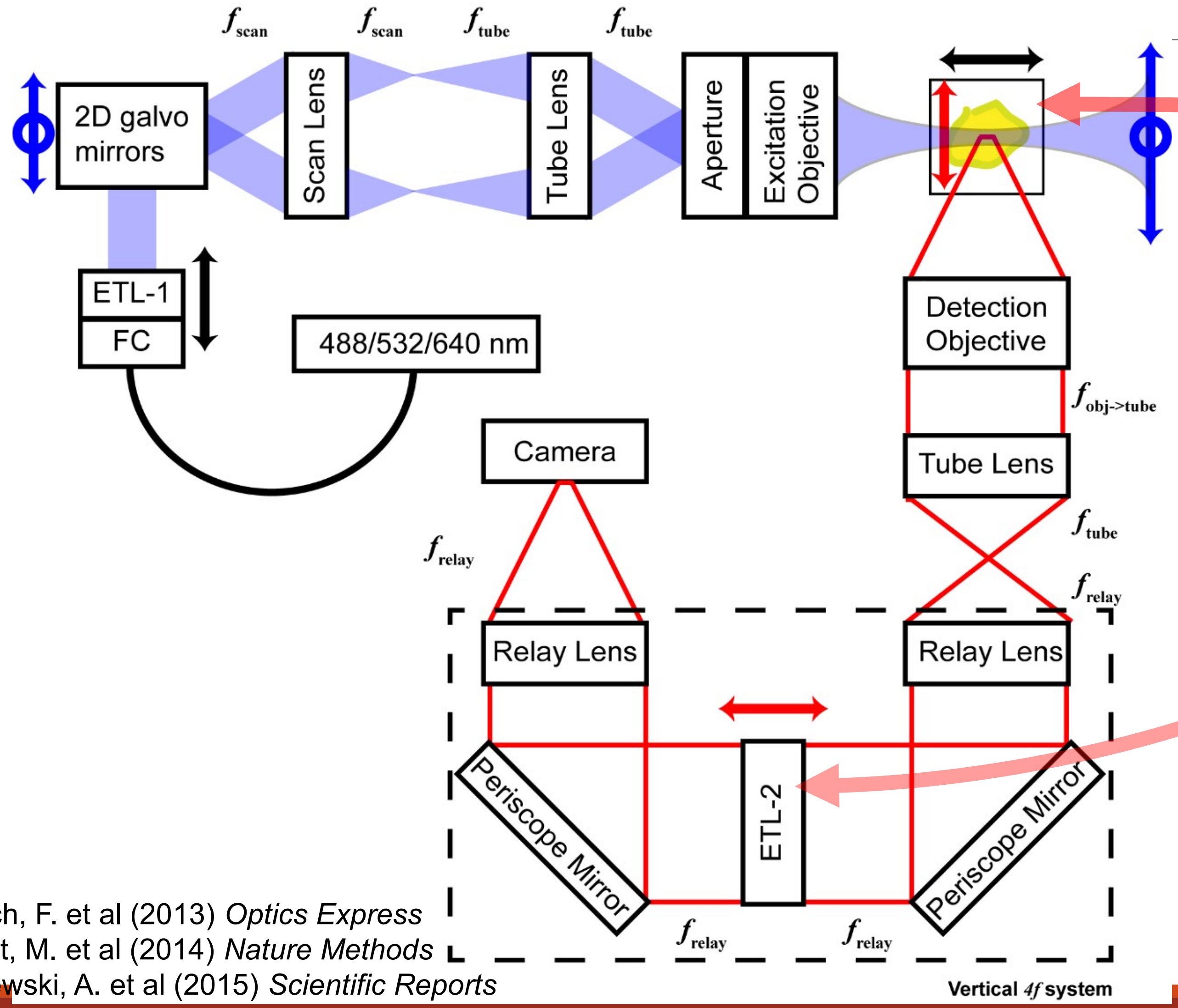
Heterogenous RI breaks co-planarity in light sheet fluorescence microscopy



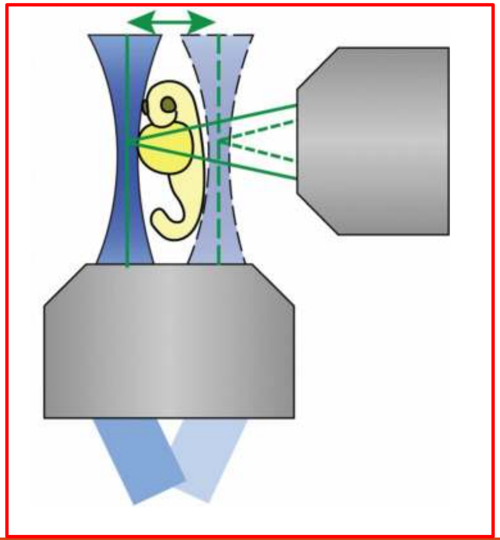


Chung, K, et al (2013) *Nature*
Yang, B, et al (2014) *Cell*
Ryan, D. et al (2017) *Nature Communications*

Inertia-free volumetric imaging



$$\Delta z_{ETL-2} = -\frac{1}{M_{DO}^{2}}\left[\frac{n \cdot f_{L1}^{2}}{f_{ETL-2}}\right]$$

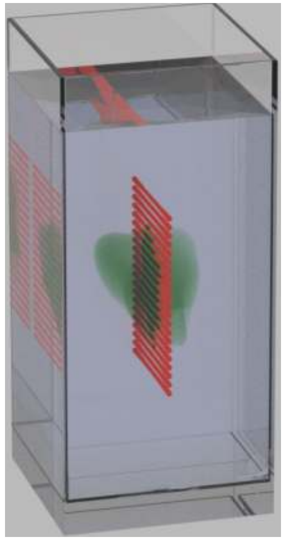


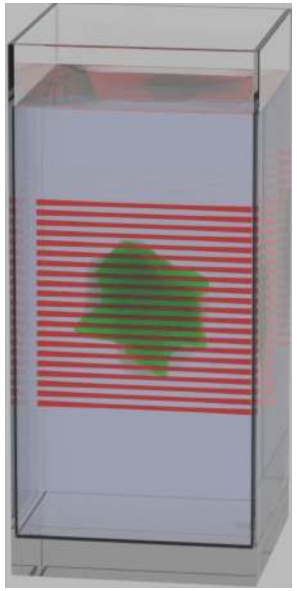
Inertia free imaging

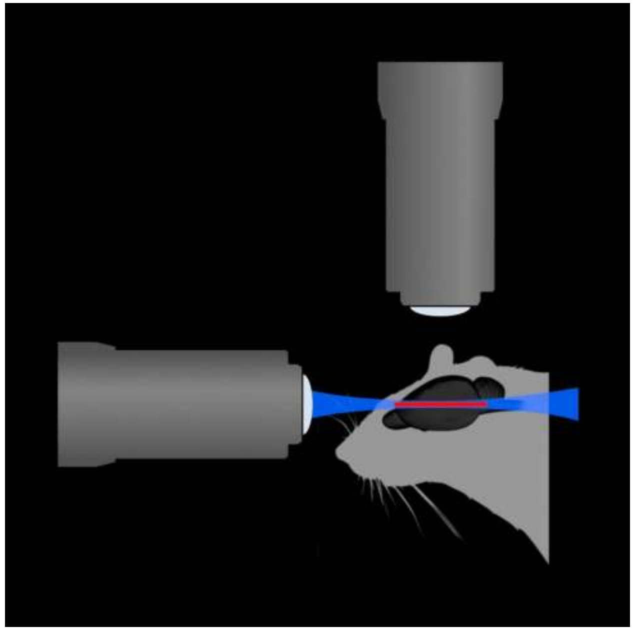
Fahrbach, F. et al (2013) *Optics Express*
Mickoleit, M. et al (2014) *Nature Methods*
Chmielewski, A. et al (2015) *Scientific Reports*
Ryan, D. et al (2017) *Nature Communications*

Patent issued April 8th 2018

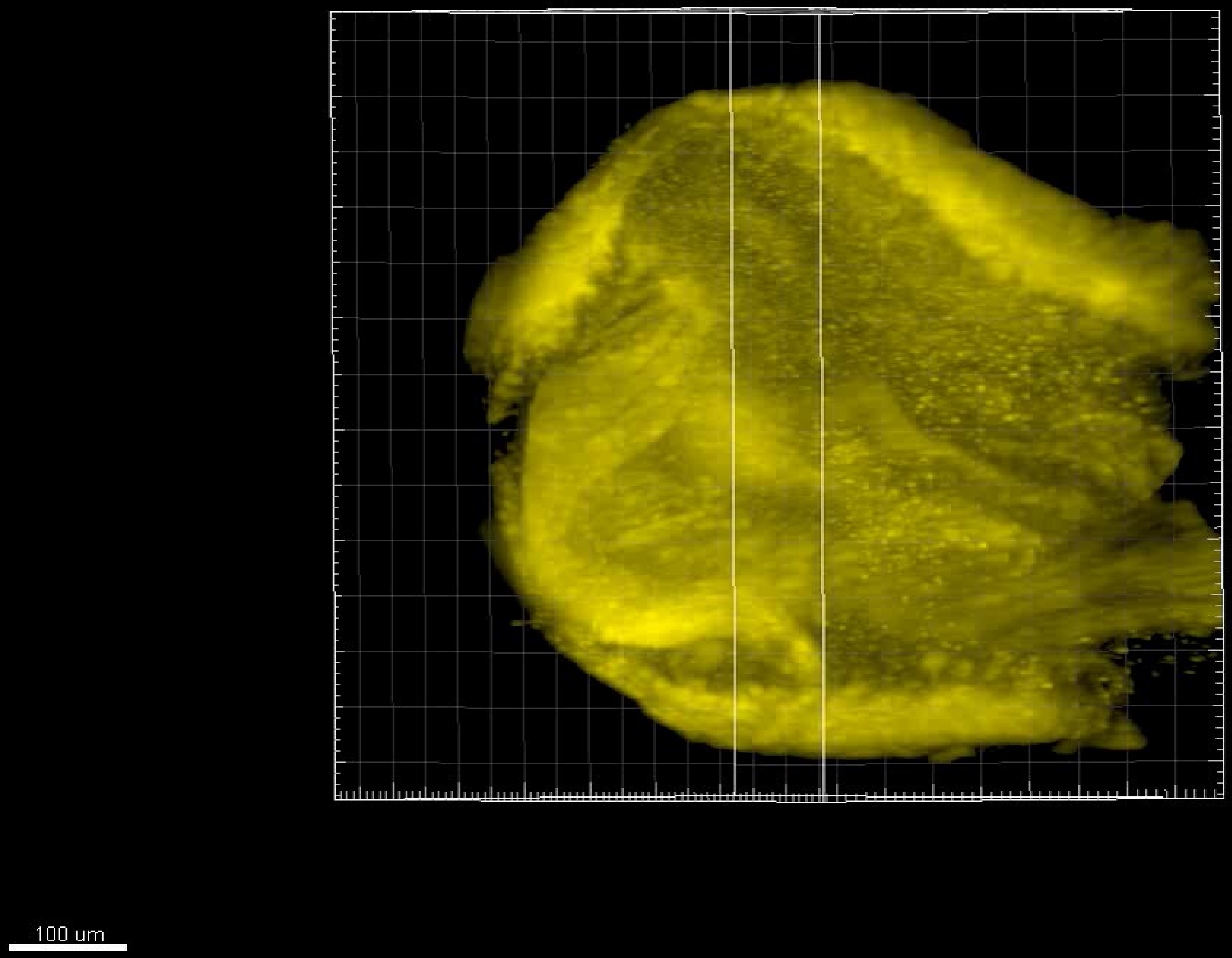
Computer vision to maintain co-planarity





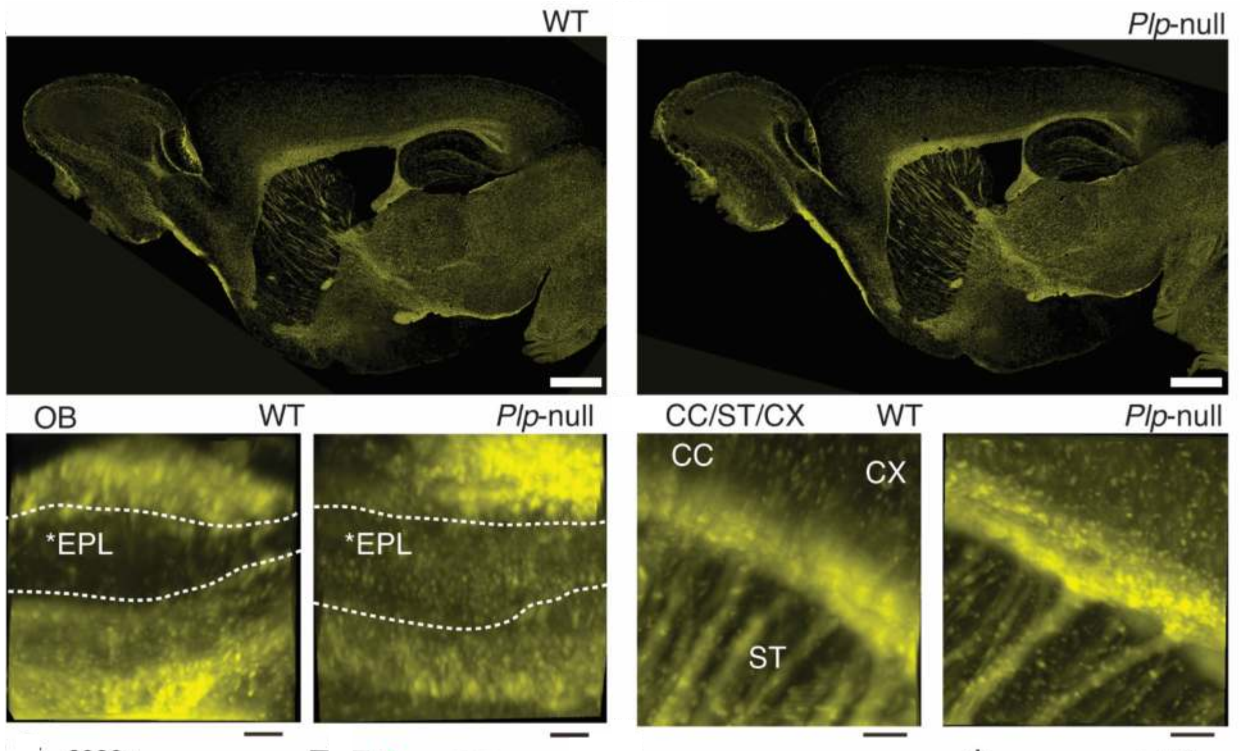


Light sheet imaging oligodendrocytes in



Adaptive light sheet microscopy of PLP-eGFP. Ryan et al. Nature Comms. 8:612, 2017

Loss of *plpl1* alters regional production of oligodendrocyte cells



Gould, E. et al (2018) *eLife*

University of Colorado
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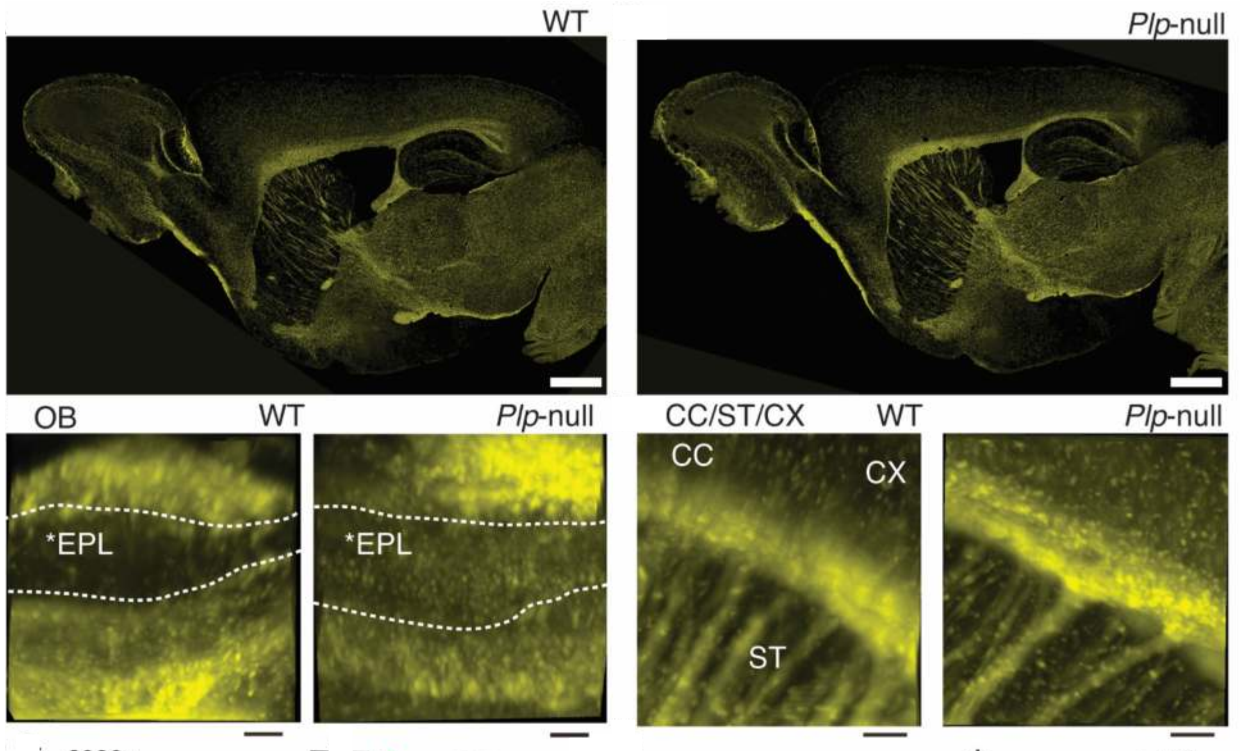
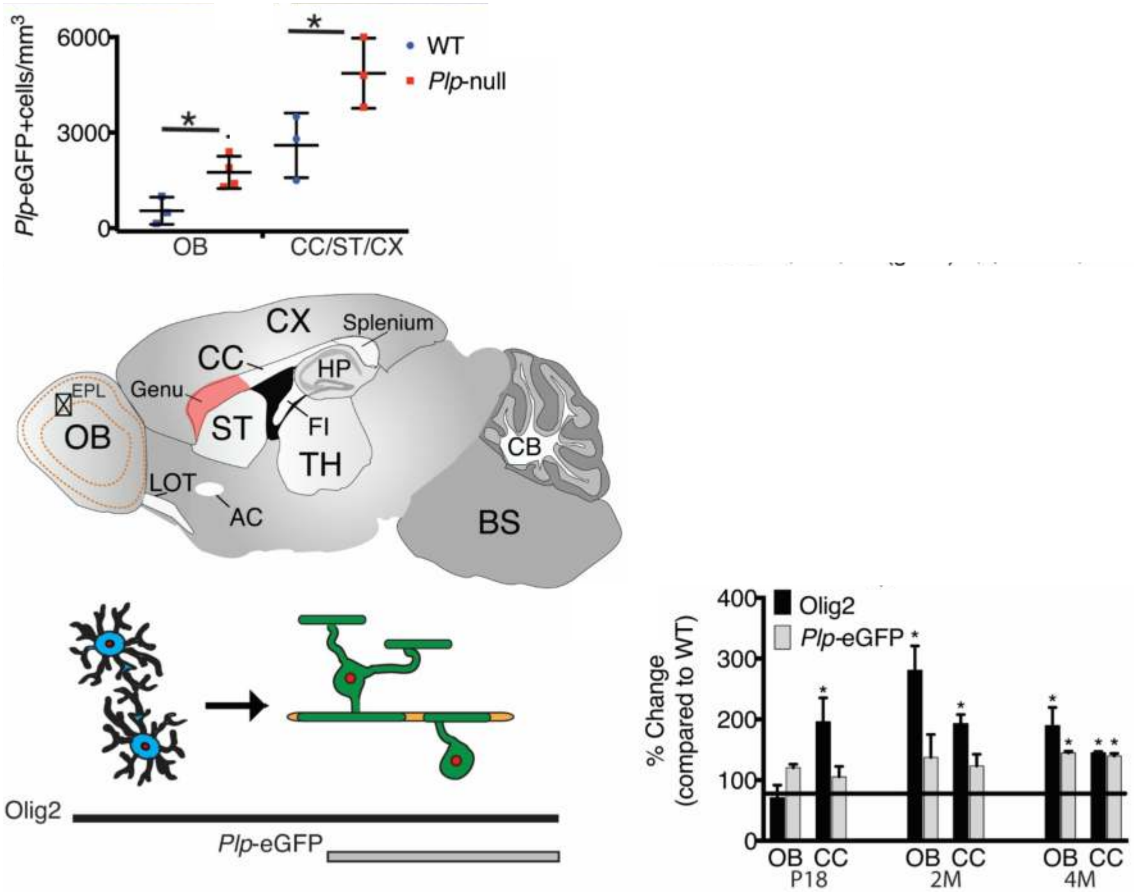
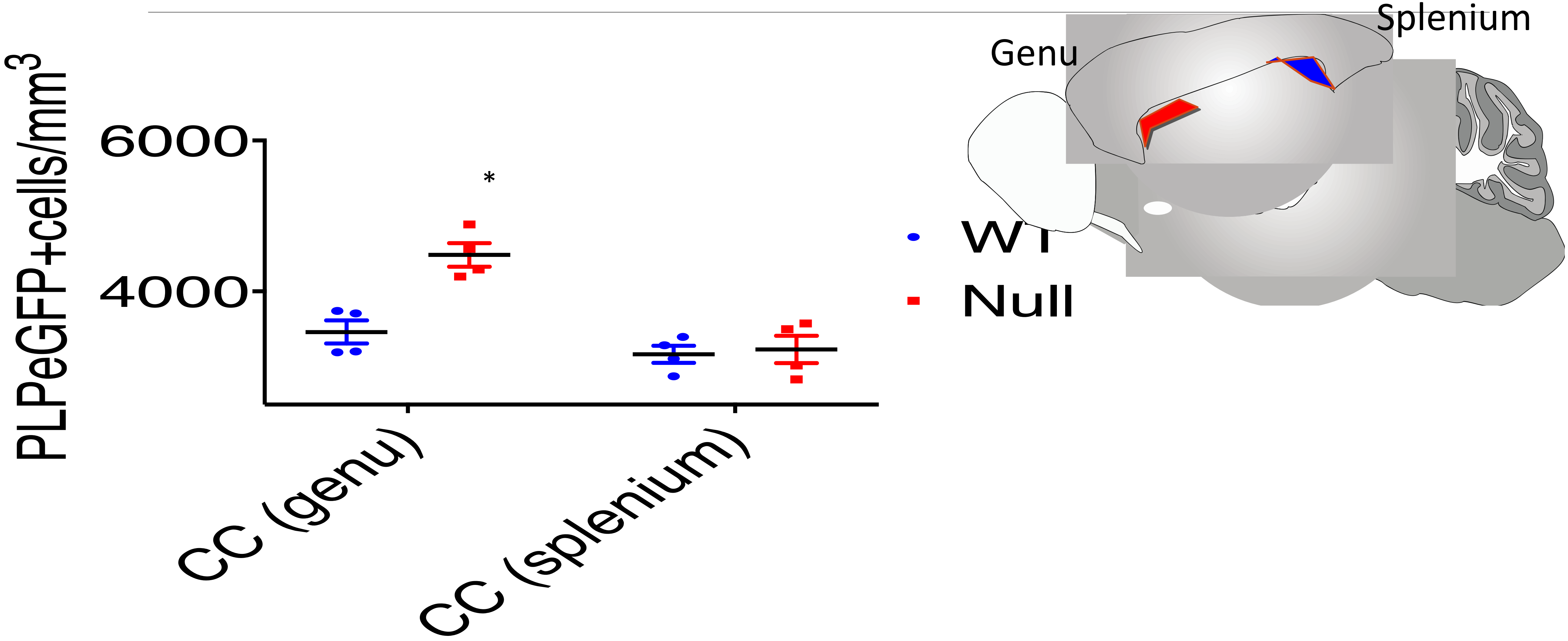
Loss of *plpl1* alters regional production of oligodendrocyte cells





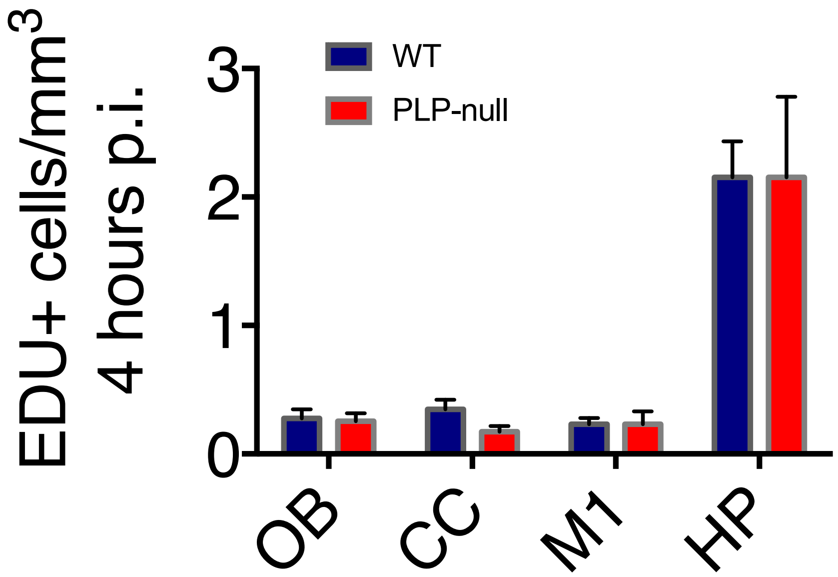
Gould, E. et al (2018) *eLife*

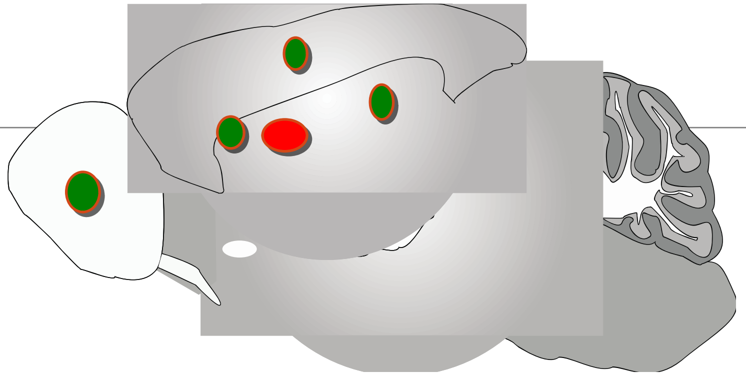
increase in OL is region-specific increase in the CC of the 6 month PLP-null mice

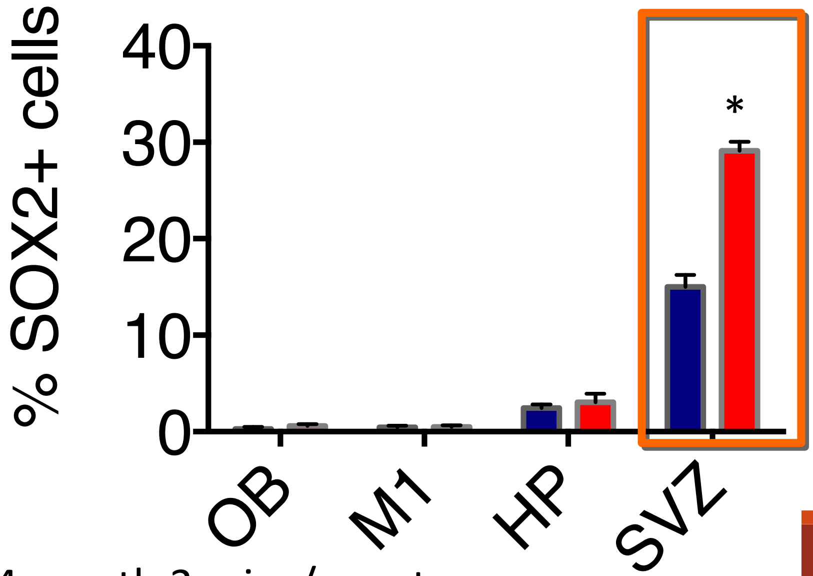


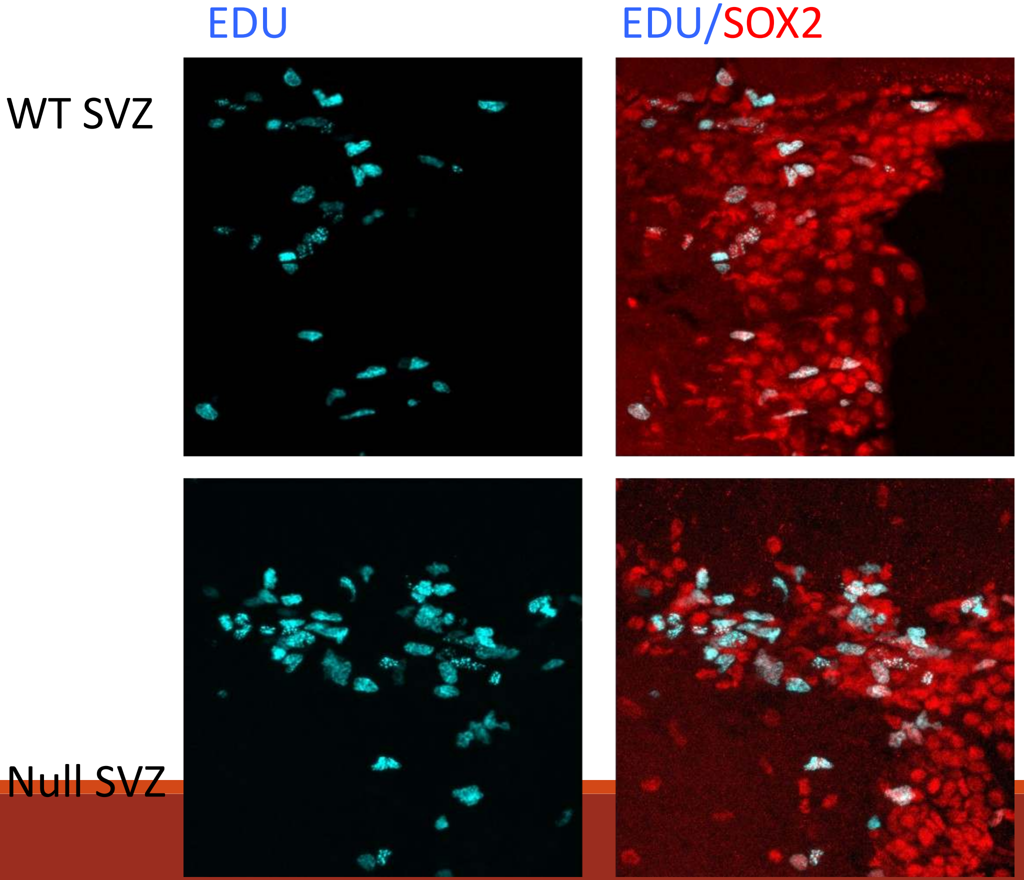
Gould et. al. eLife e34783, 2018

Proliferative response in SVZ









4 month 3 mice/genotype

Summary of EDU experiments:

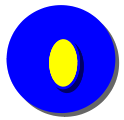
Increase in OL in CC and OB of young adult PLP-null mice

Accumulation of new OL

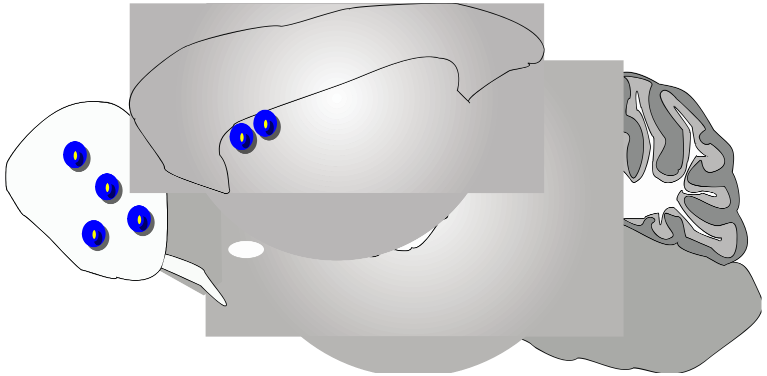
1 2 3

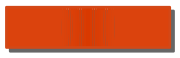
Day

3 weeks

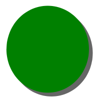


EDU+ OL

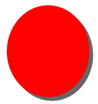




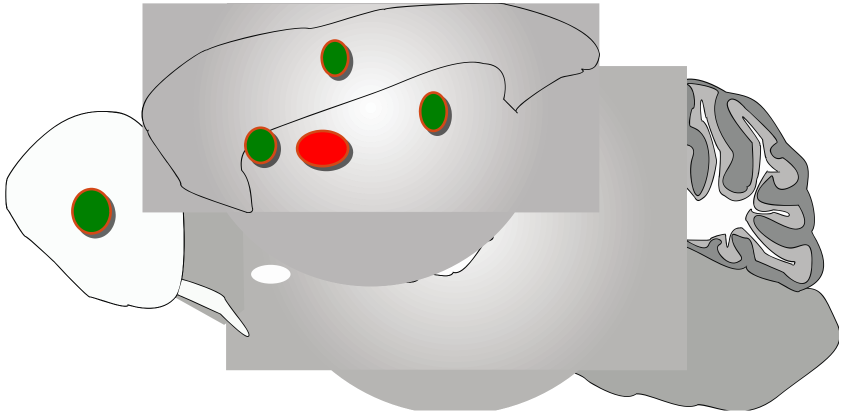
4 hours



Local OPC- no change

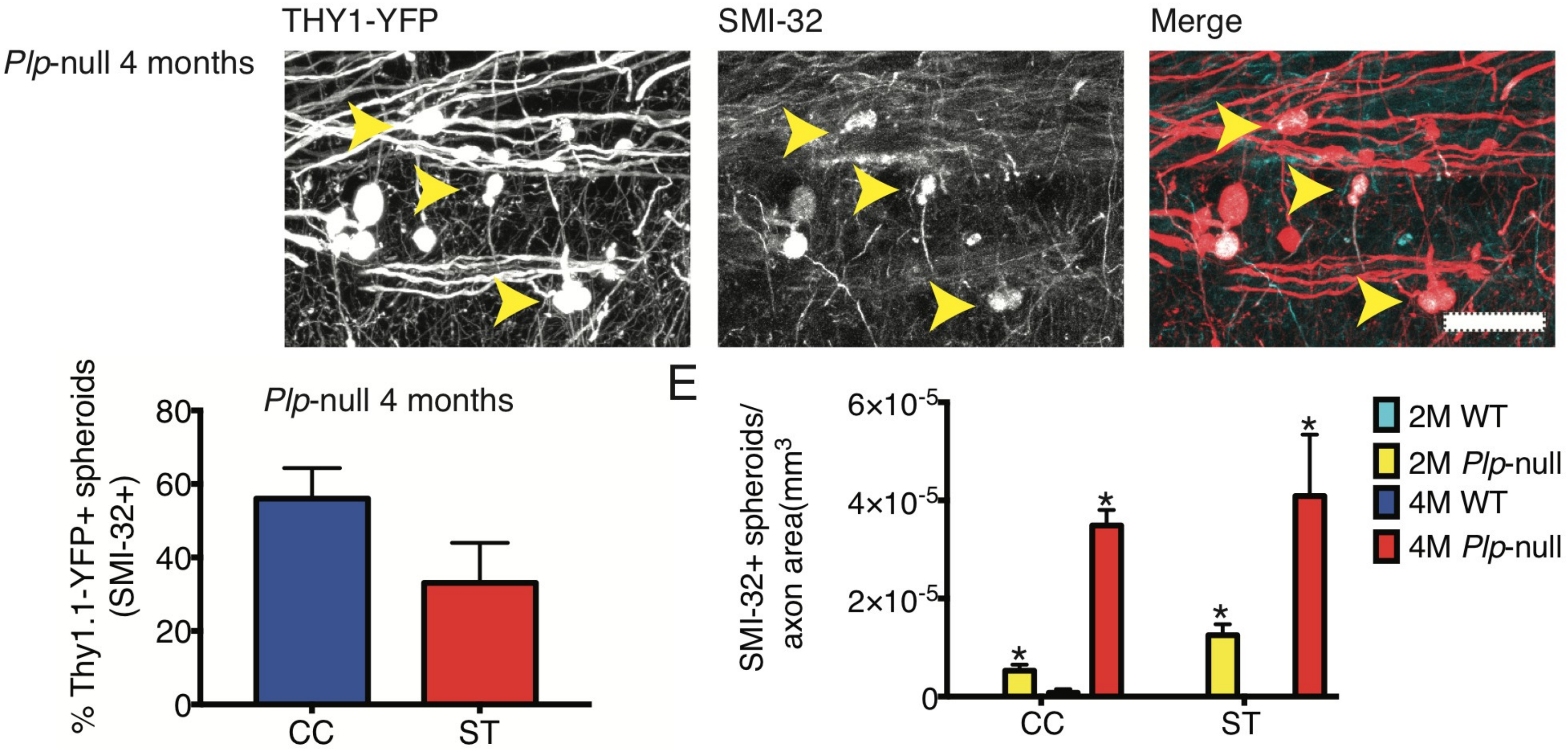


Subventricular zone (SVZ)- proliferation increased



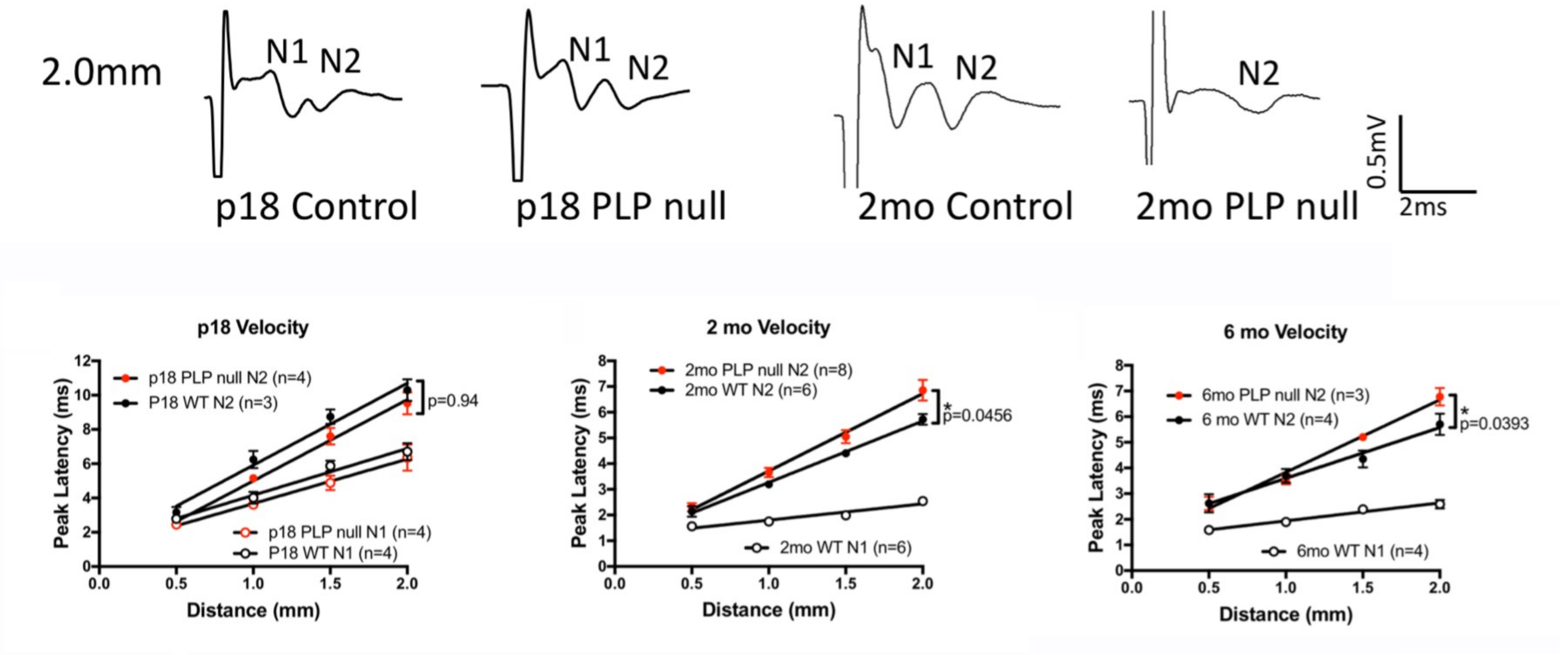
Gould et. al. eLife e34783, 2018

Cortical axon disruption in *Plp*-null mice evidenced by the presence of axonal spheroids

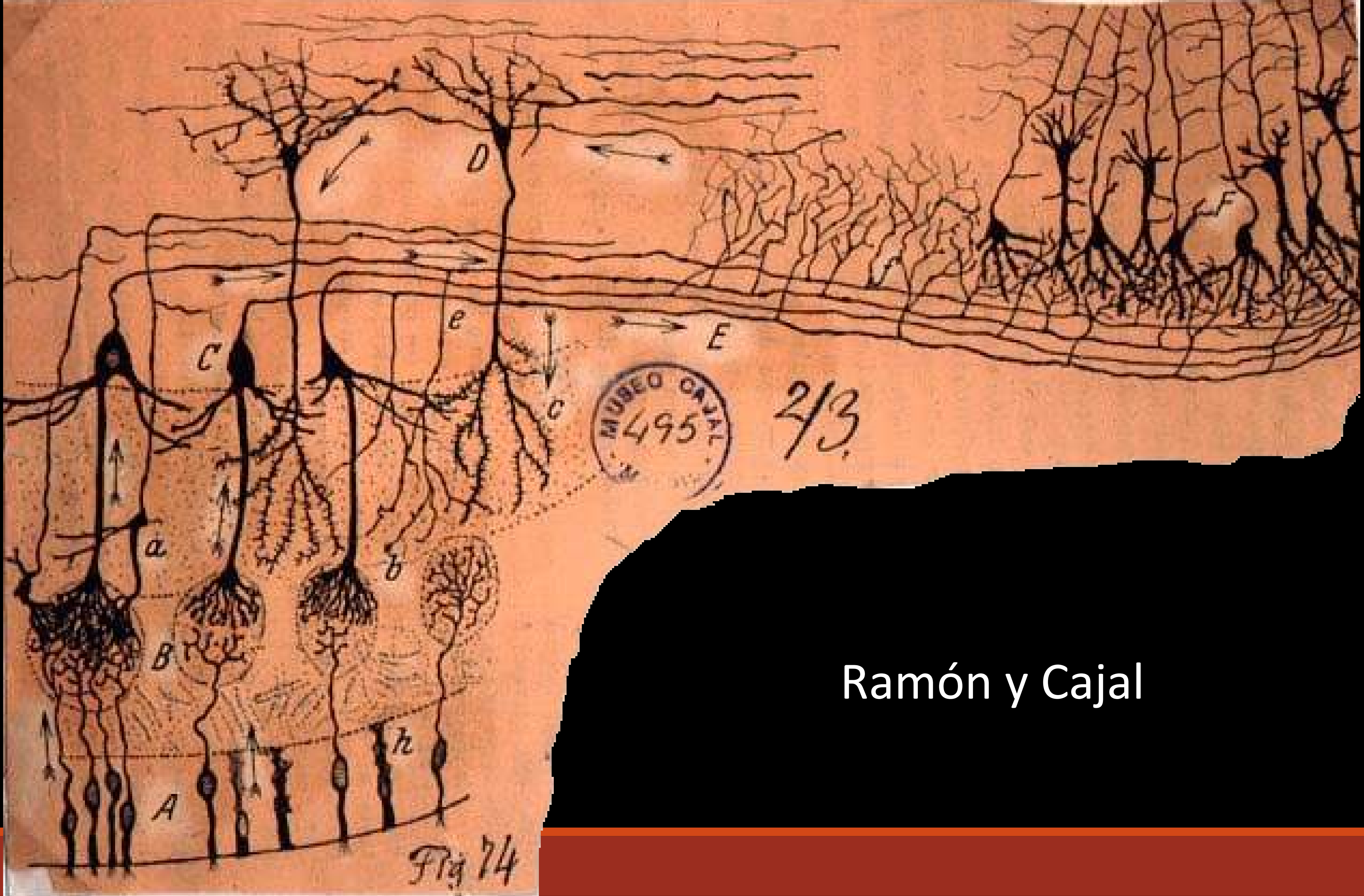


Gould et. al. eLife e34783, 2018

Mild demyelination elicits a decrease in the velocity of action potential transmission in the corpus callosum

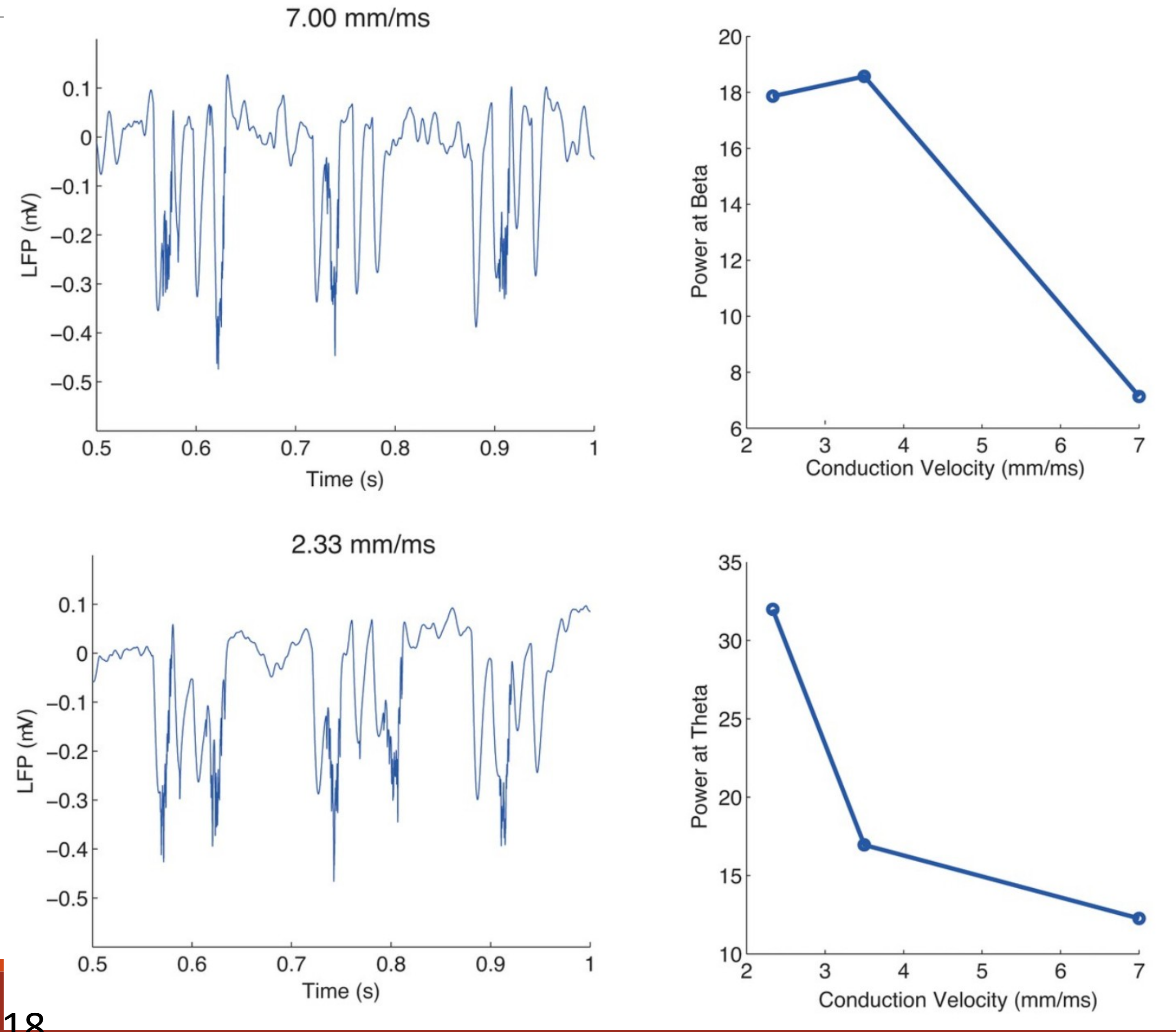


Gould et. al. eLife e34783, 2018



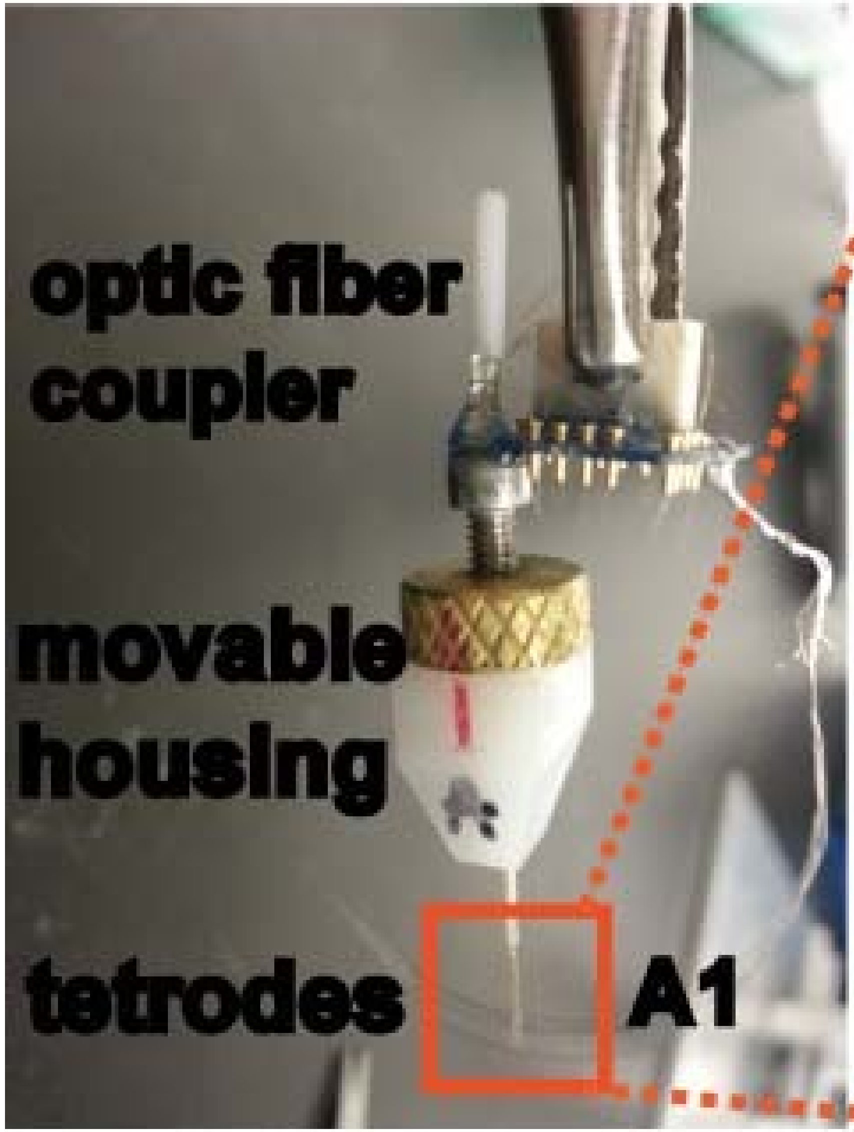
Ramón y Cajal

Modeling predicts that a decrease in axonal conduction velocity should increase neuronal oscillations in piriform cortex

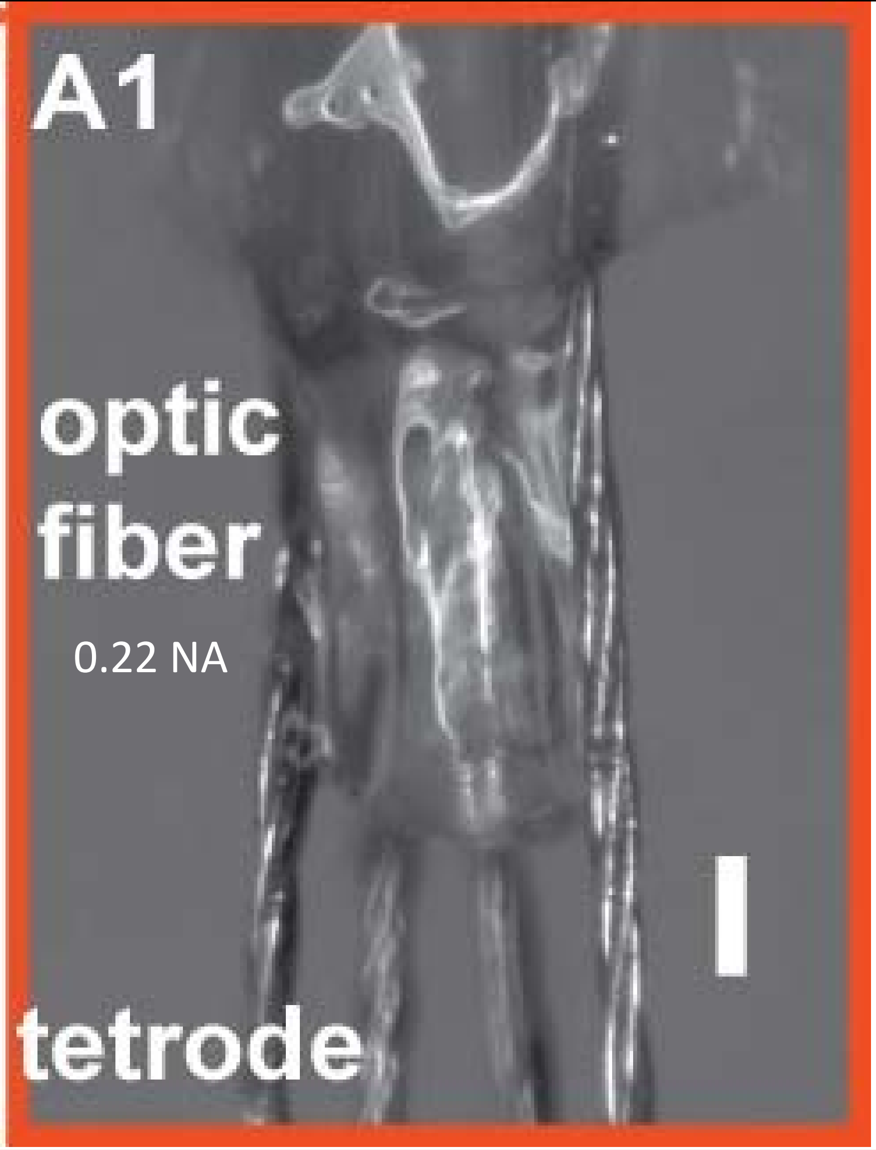


Gould et. al. eLife e34783, 2018

Optetrode



optic fiber
coupler
movable
housing
tetrodes
A1



A1
optic
fiber
0.22 NA
tetrode

Go-no Go Associative Learning Task

Optetrode implantation

↓

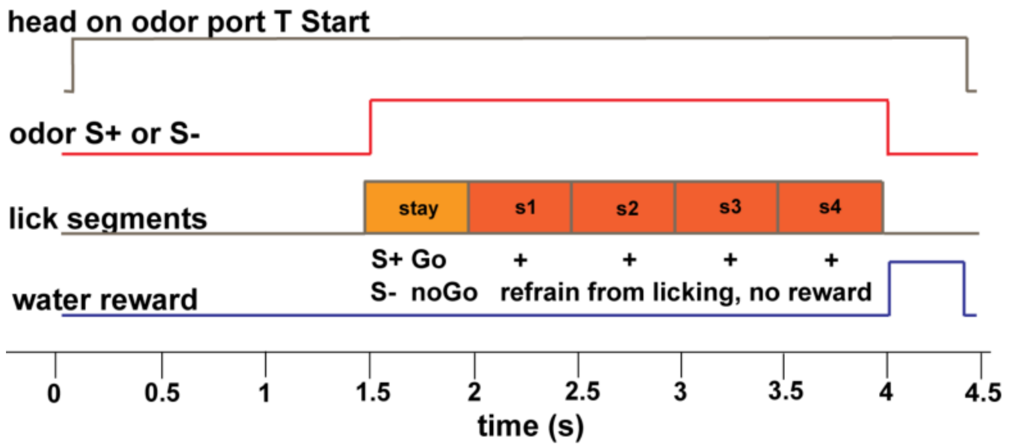
Electrophysiological recordings of neurons during an associative learning task (i.e. go-no go)

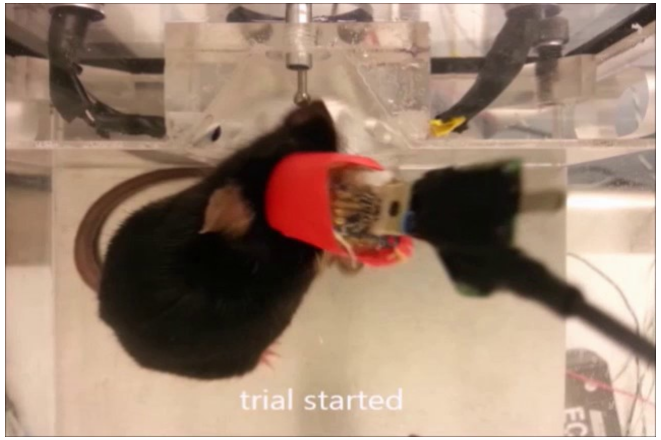
↓

Light delivery protocol

↓

Off-line spike sorting analysis single and multi-unit identification





Synchronous neuronal activity in piriform cortex

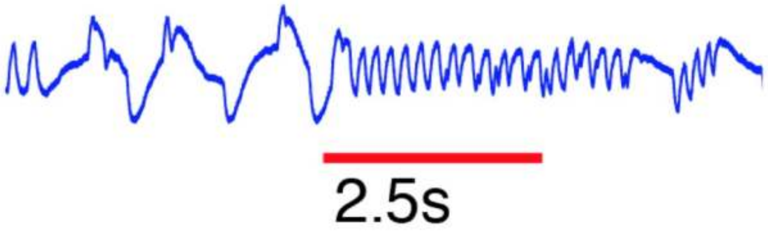
Myelin disruption leads to inefficient conduction

Alters timing within neuronal populations

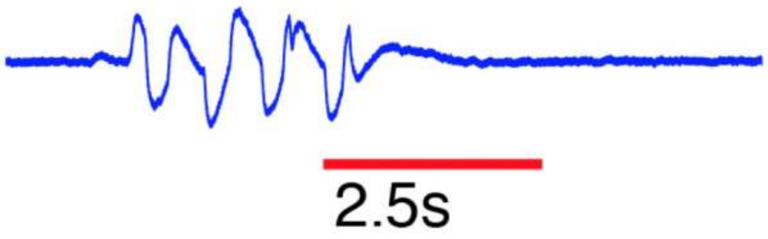
Impairs synchronous neuronal activity

Alters oscillations

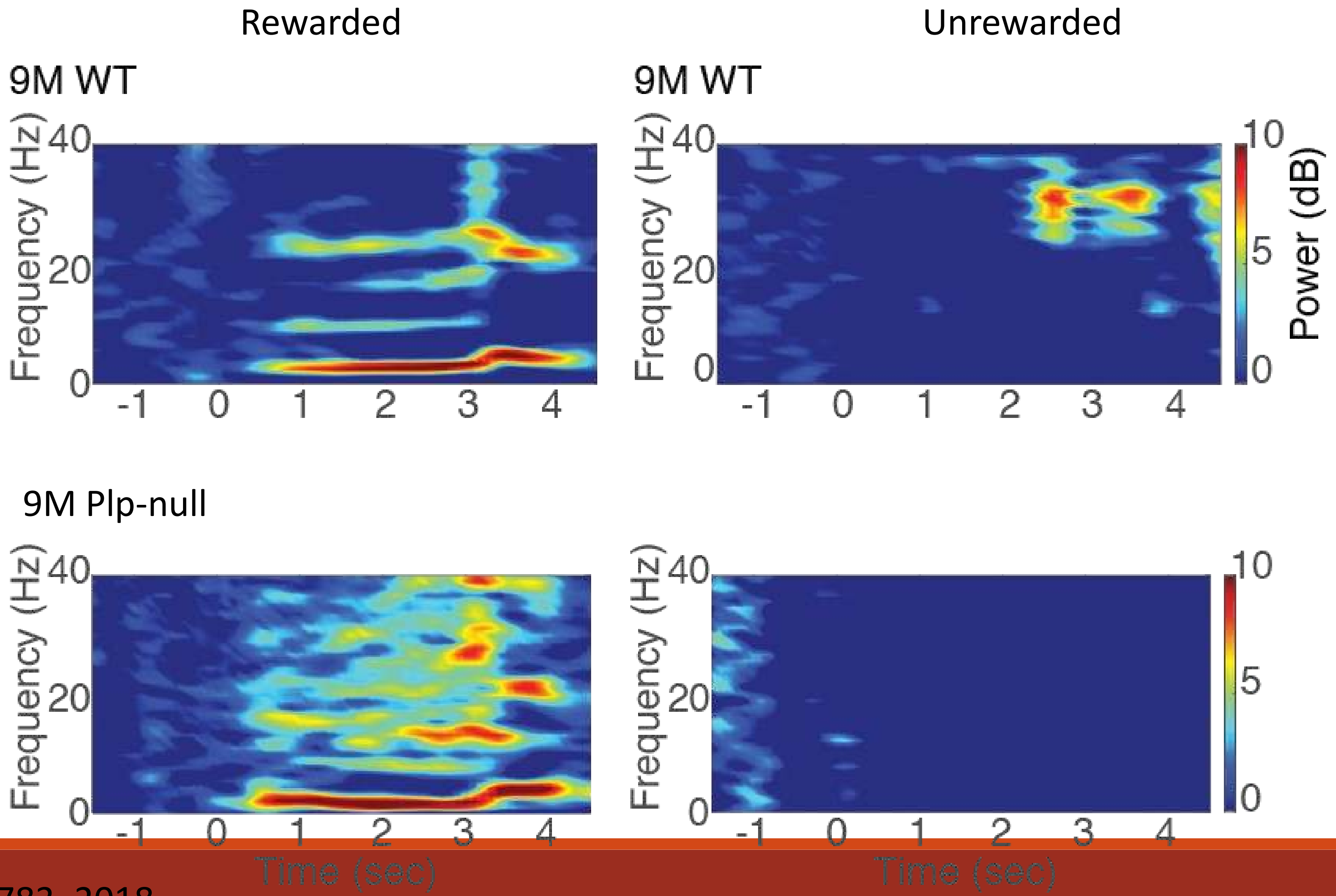
Rewarded



Unrewarded

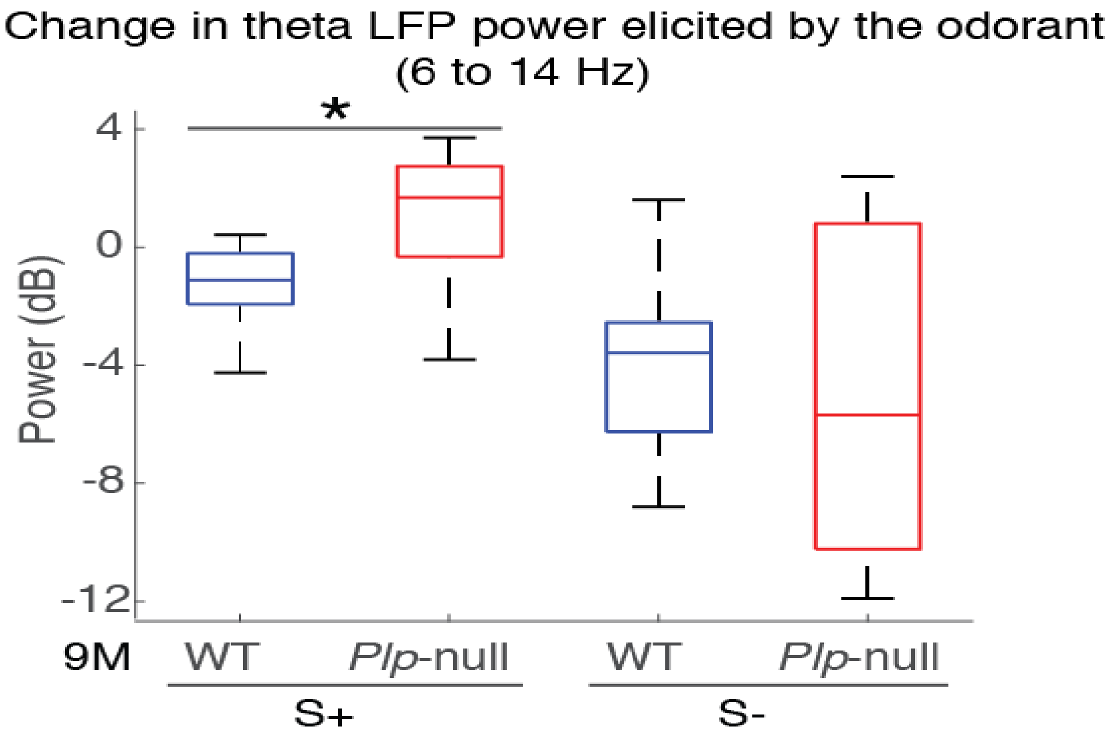


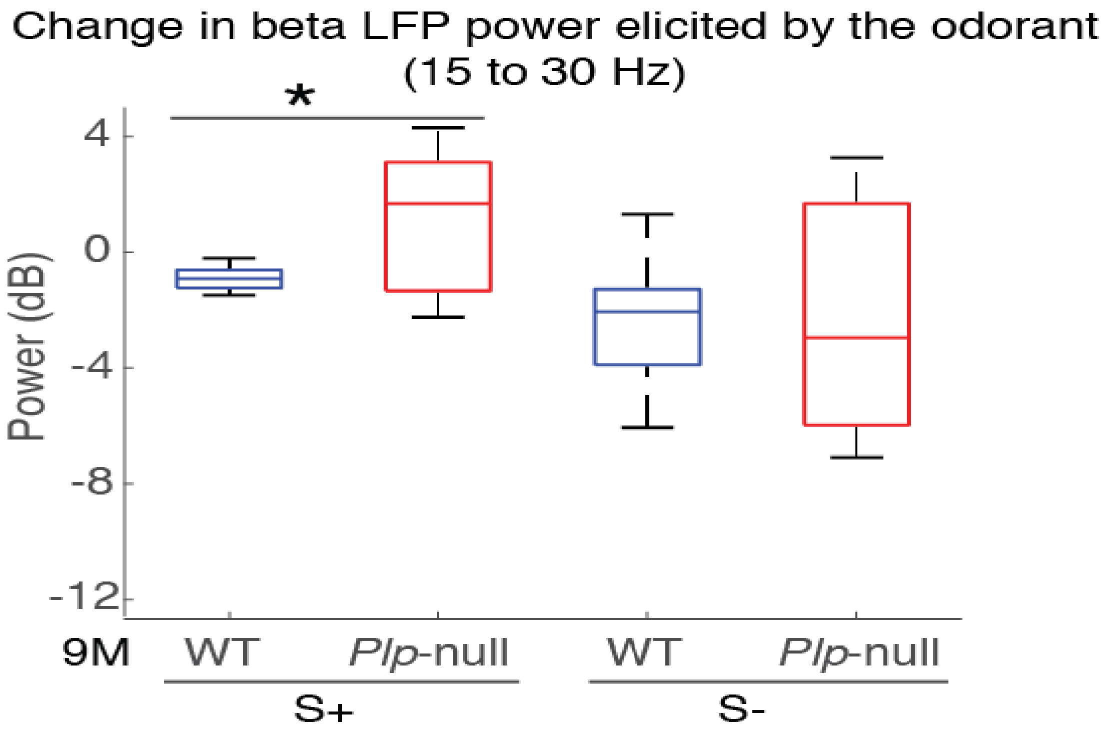
Oscillations in piriform cortex



Gould et. al. eLife e34783, 2018

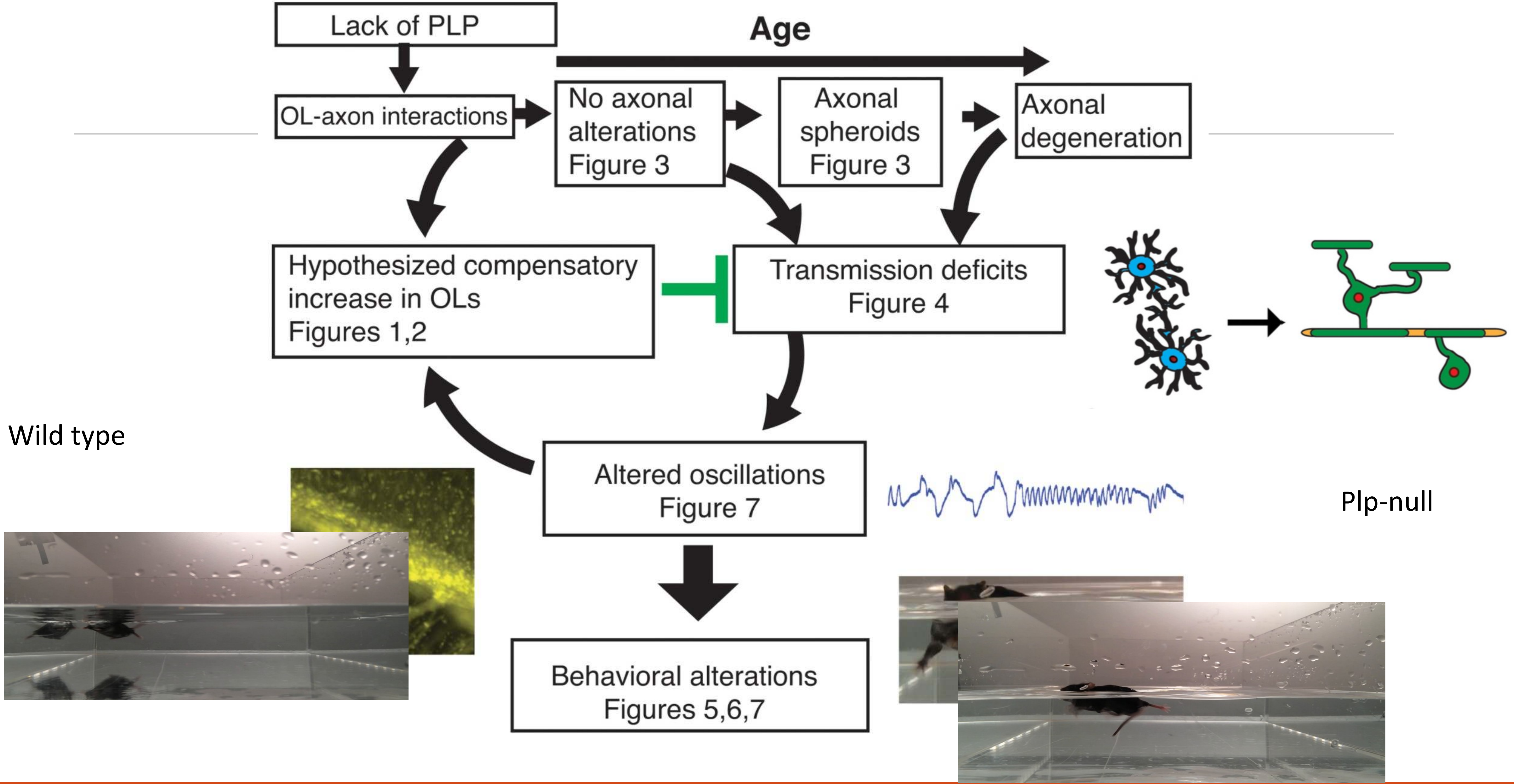
Myelin disruption can be a primary cause of altered oscillations





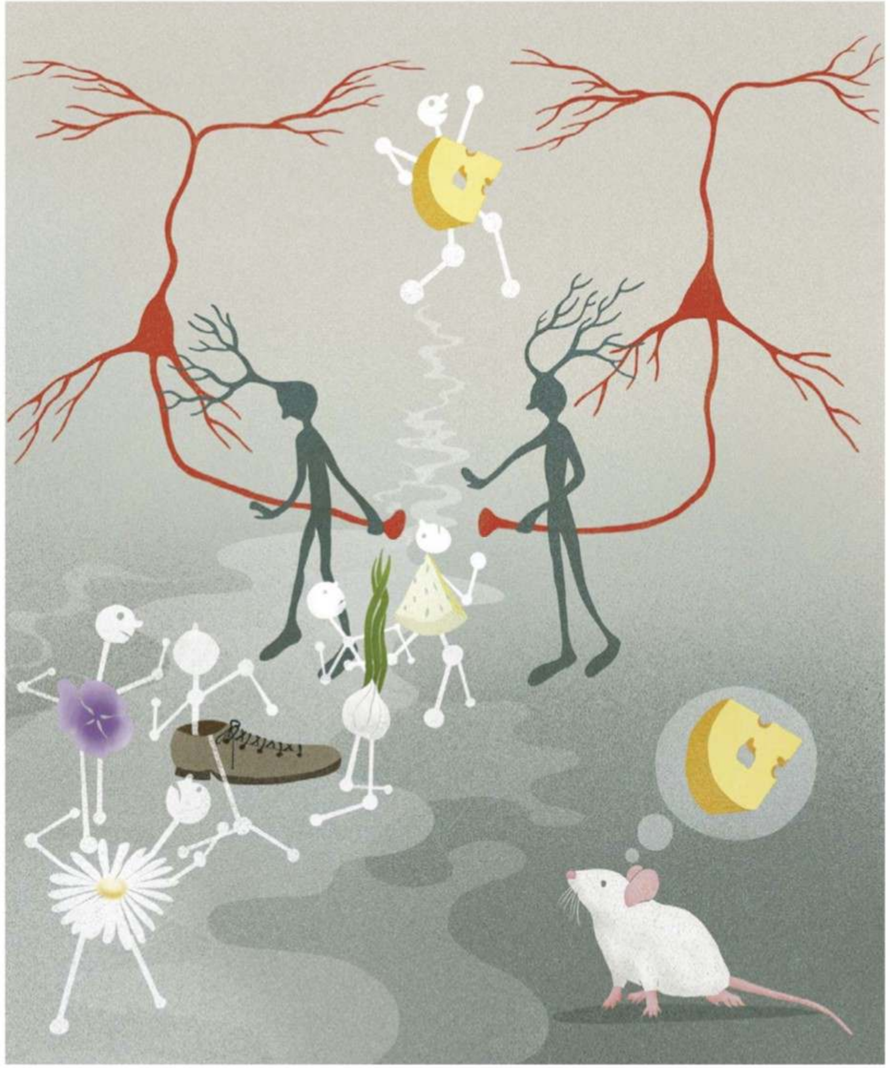
Gould et. al. eLife e34783, 2018

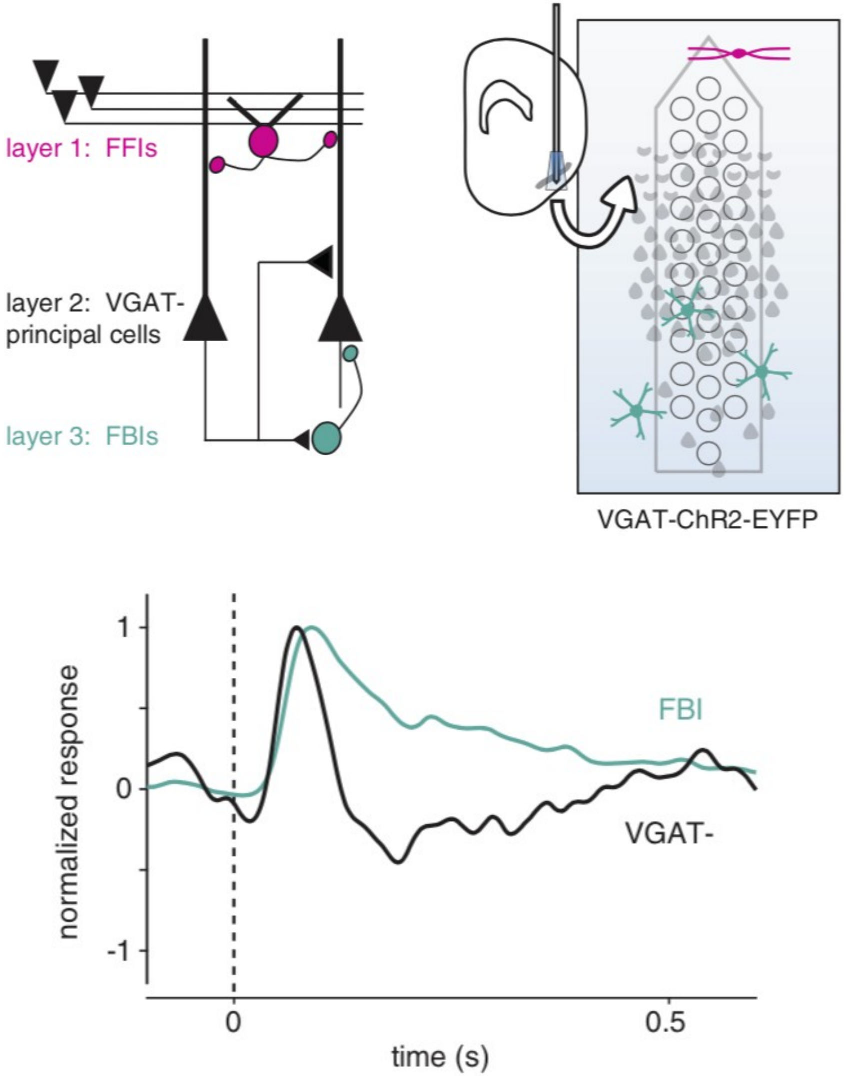
Mann-Whitney U test. Data not normally distributed



Gould et. al. eLife e34783, 2018

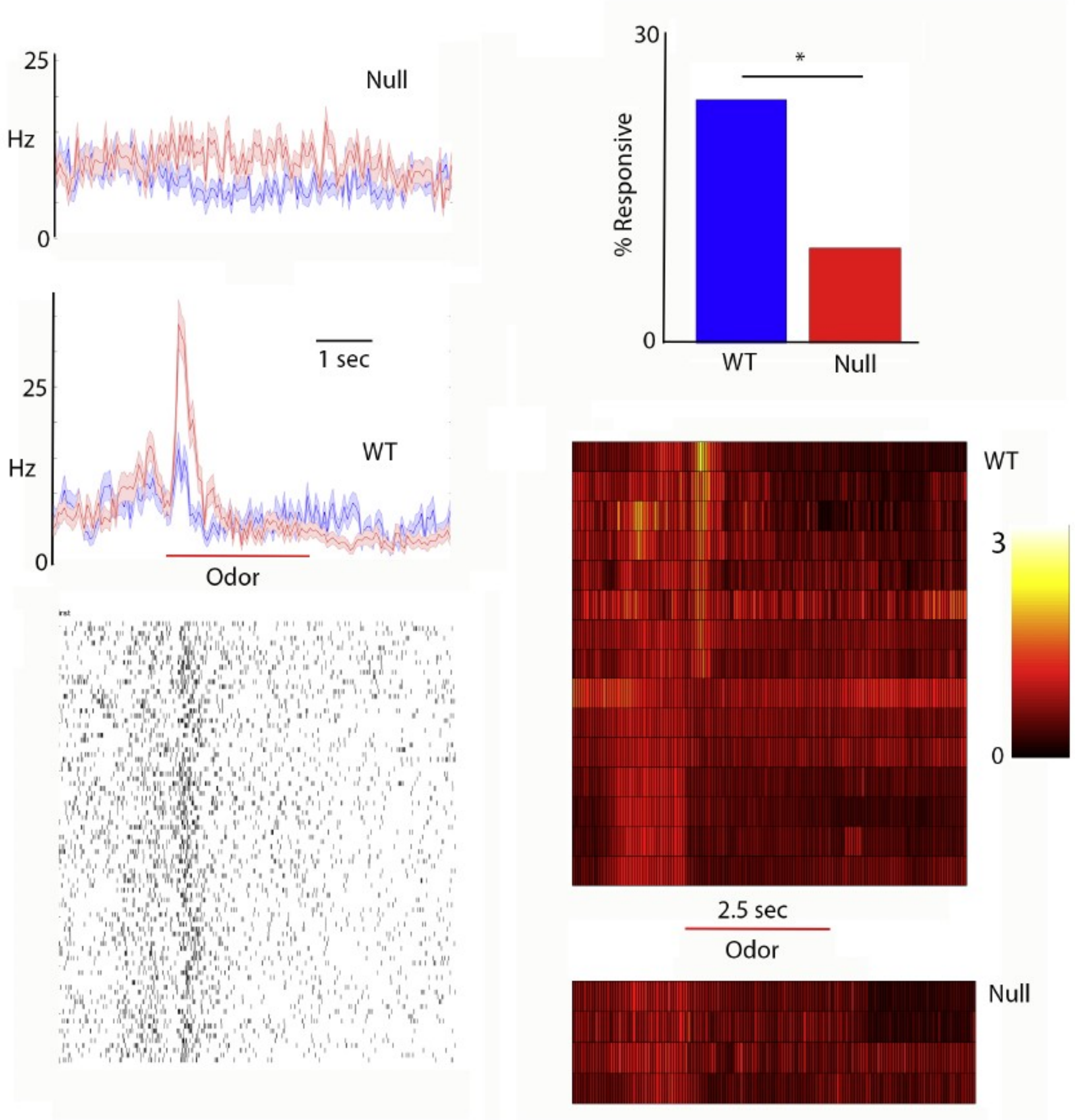
In piriform cortex feedback inhibition shortens the response to the odorant ensuring correct decoding of odor identity



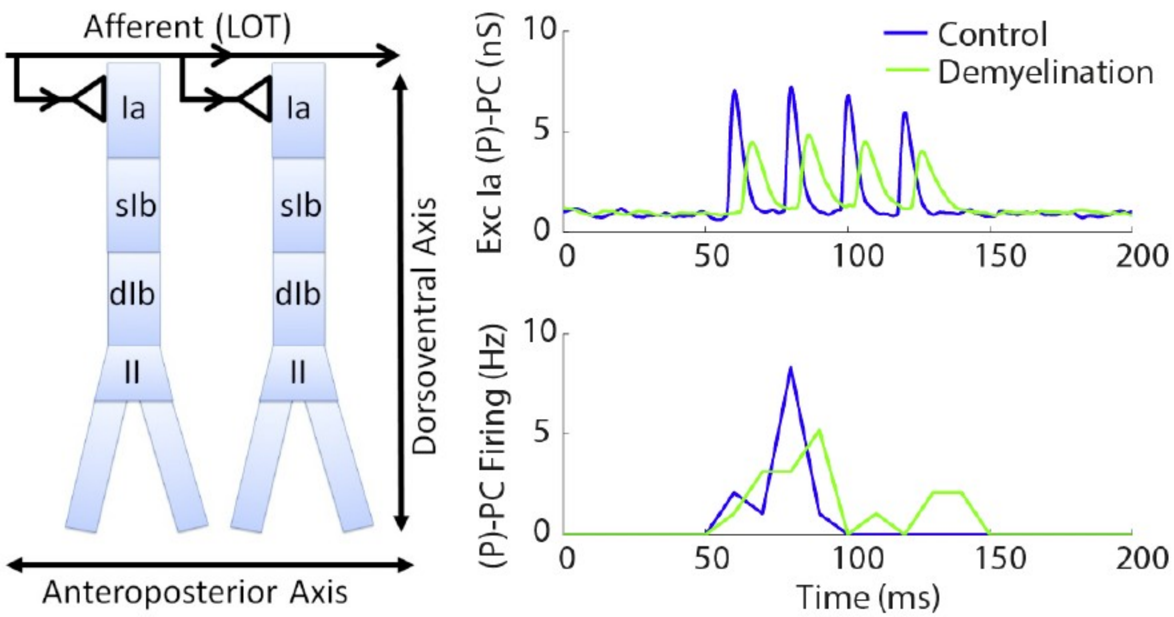


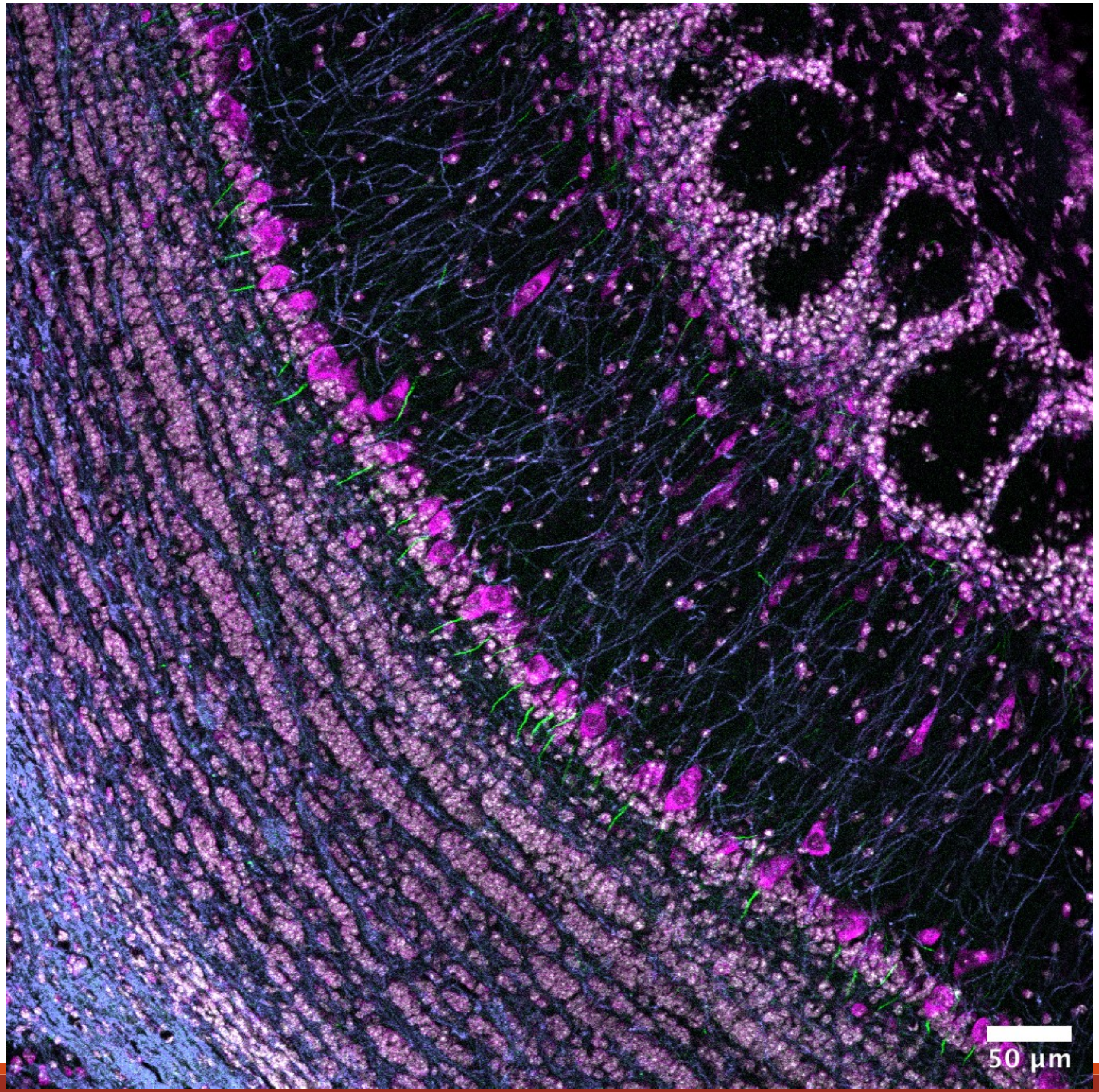
Bolding et al., Science 361, eaat6904 (2018)

Odorant responses are severely diminished in the piriform cortex of *Plp*-null mice



In collaboration with Fabio Simoes de Souza we intend to model the effect of demyelination on olfactory circuit function



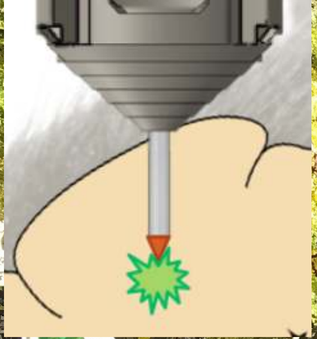


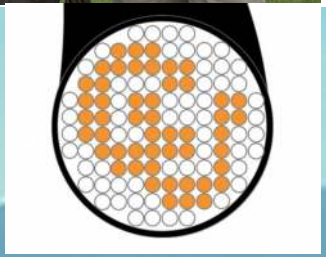
Mild myelin disruption

In the young adult mouse mild myelin dysfunction leads to:

- Select deficits in higher-order processes (motor coordination, problem-solving, motivation, sensory perception)
- Triggering of production of oligodendrocyte progenitors by the subventricular zone
- Transfer of new oligodendrocyte progenitors to the olfactory bulb and the genu of the corpus callosum
- Decreased action potential conduction velocity
- Increased oscillations

These findings raise the question whether the SVZ production of new oligodendrocytes and the accompanying increase in oscillatory power is a protective response that reduces the behavioral effects of the loss of PLP in myelin





Our CU neurophotonics group is hiring a postdoctoral fellow with interest in interacting with engineers to beautiful Colorado!