



Development of Novel Small Molecule EPHB3 Inhibitors  
to Attenuate Synapse Deficits and Neuroinflammation  
in Alzheimer's Disease by Targeting Astrocyte-  
Mediated Mechanisms

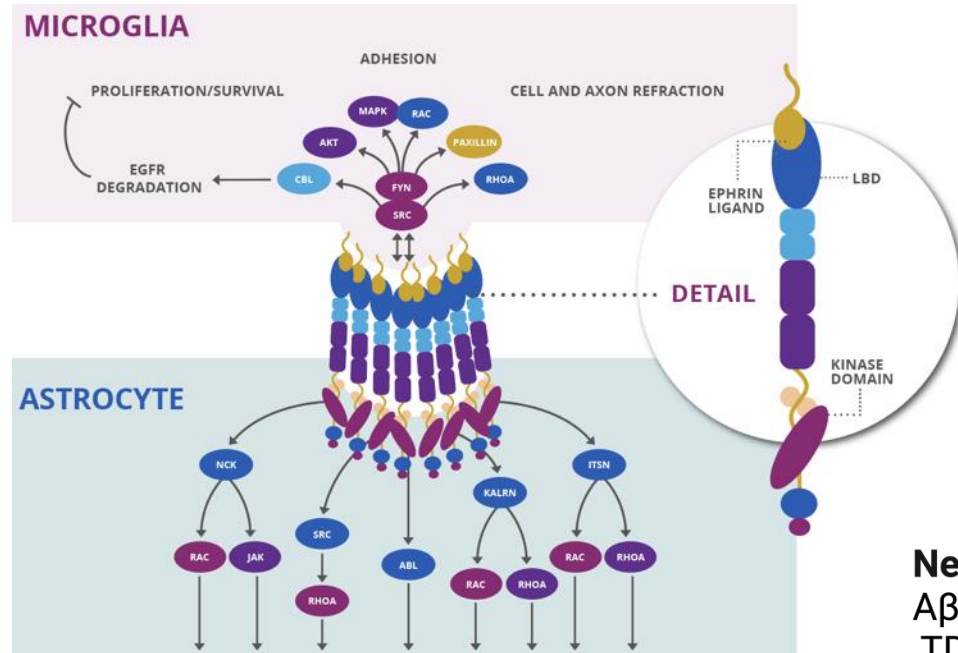
Evan Lebois, PhD



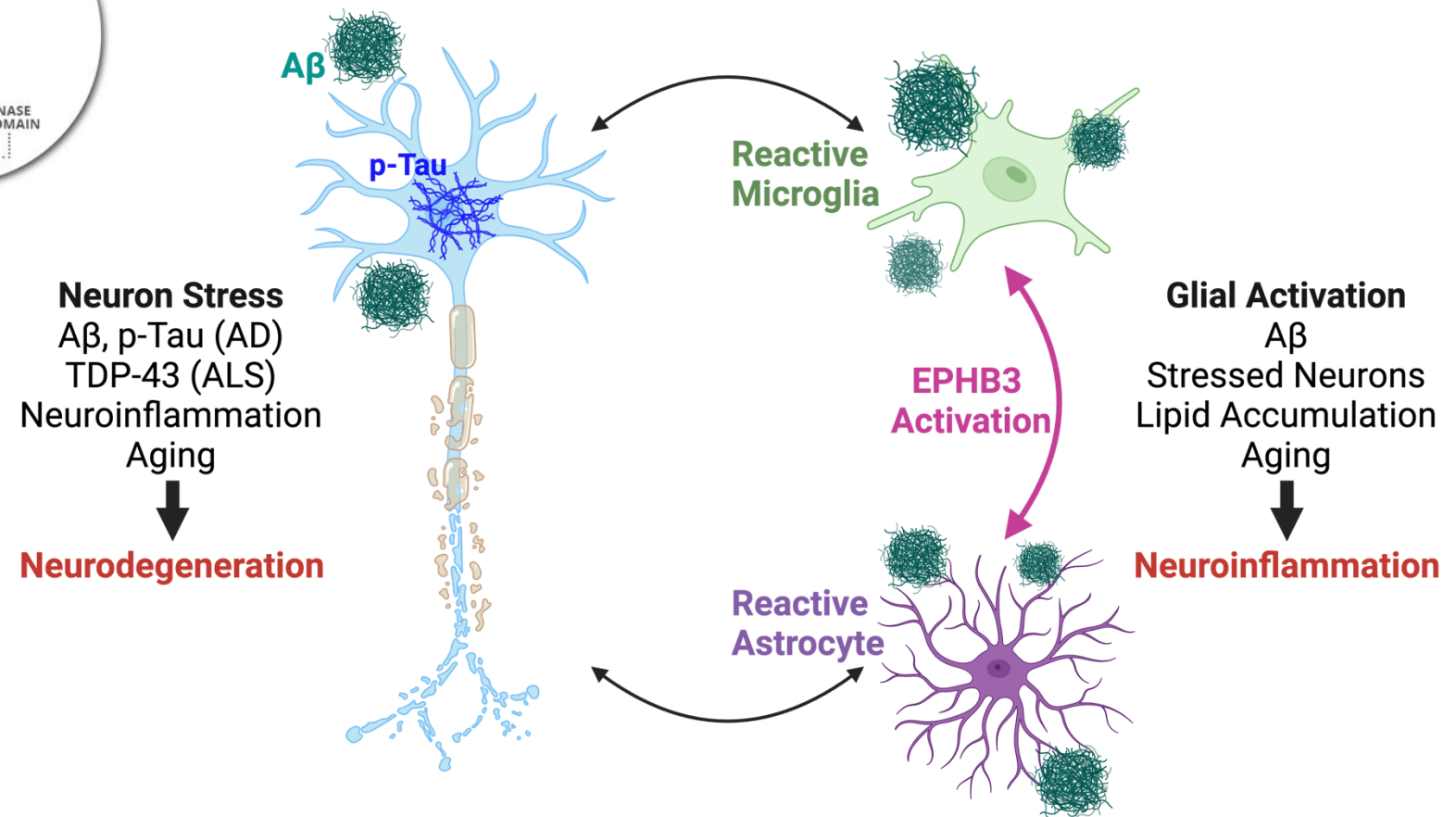
	No, Nothing to disclose
X	Yes, please specify

Company / Name	Honoraria / Expense	Consulting / Advisory Board	Funded Research	Royalties / Patent	Stock Options	Ownership / Equity Position	Employee	Other (Please specify)
Evan Lebois, Violet Therapeutics					X		X	

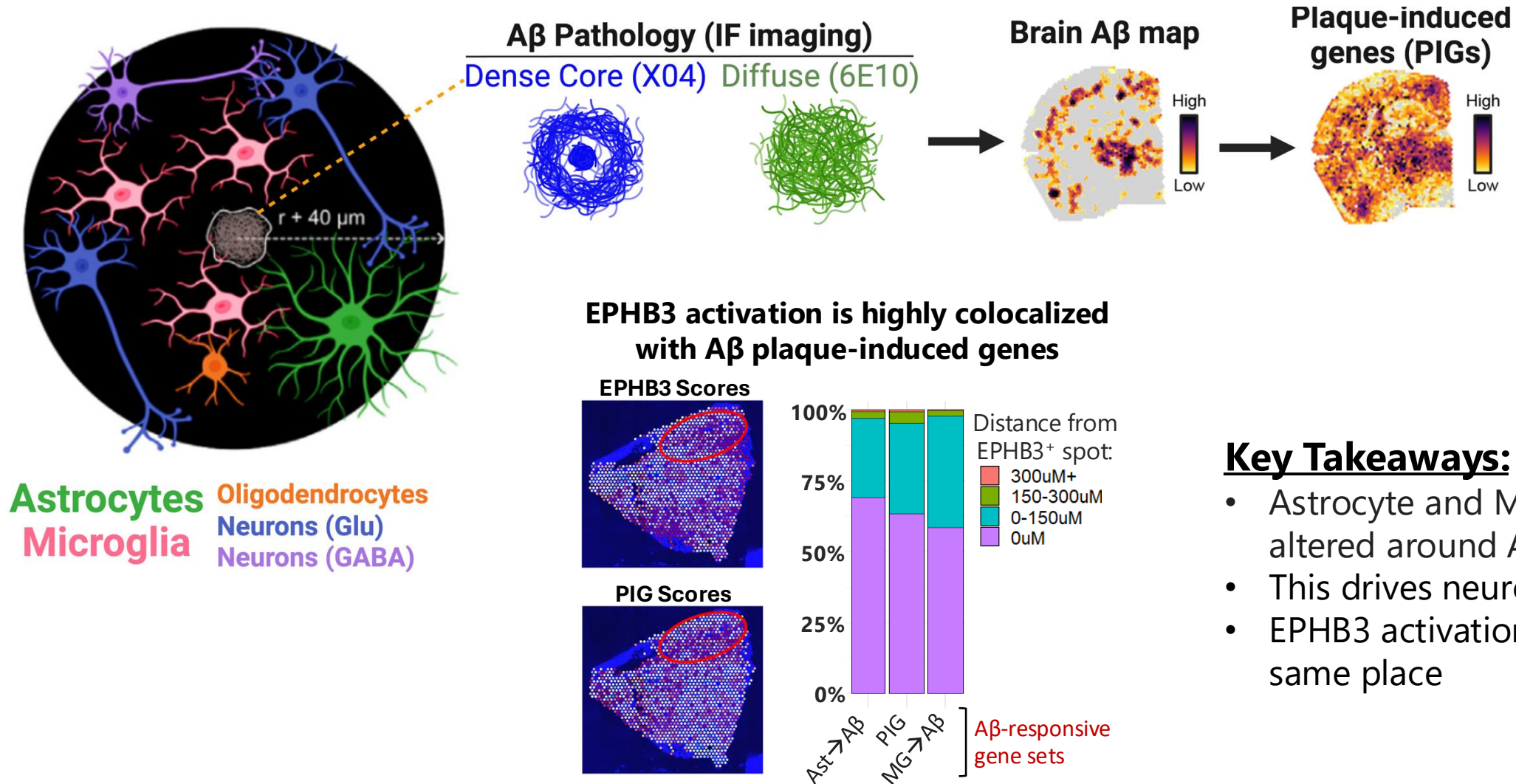
# EPHB3 is an astrocyte receptor tyrosine kinase that facilitates interactions with microglia to drive neuroinflammation



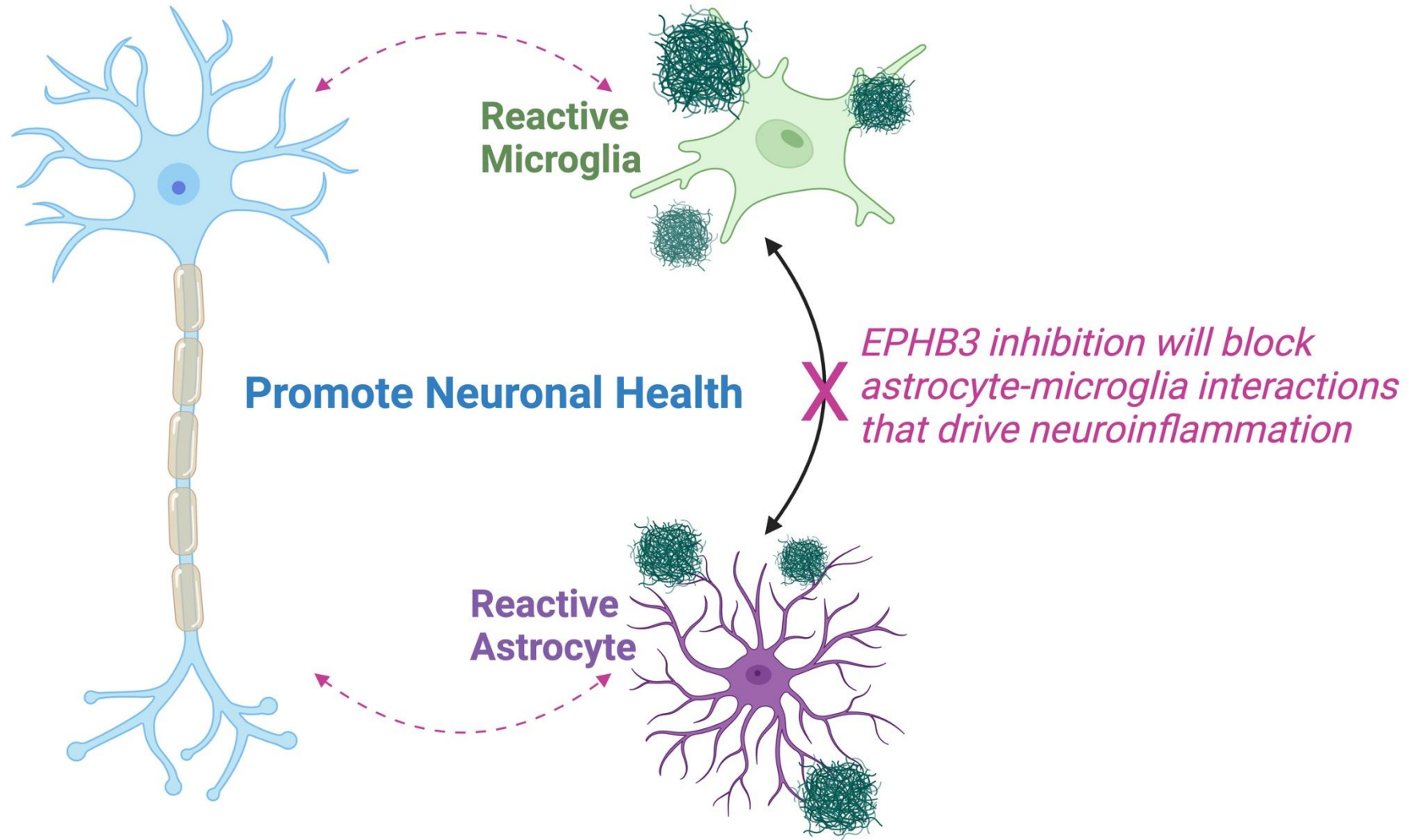
**Classical role:** neuronal development and axon guidance  
**New role:** regulator of astrocyte-microglial interactions



# EPHB3 activation is highly co-localized and correlated with A $\beta$ plaque-induced gene (PIG) response in AD, revealed by spatial transcriptomics

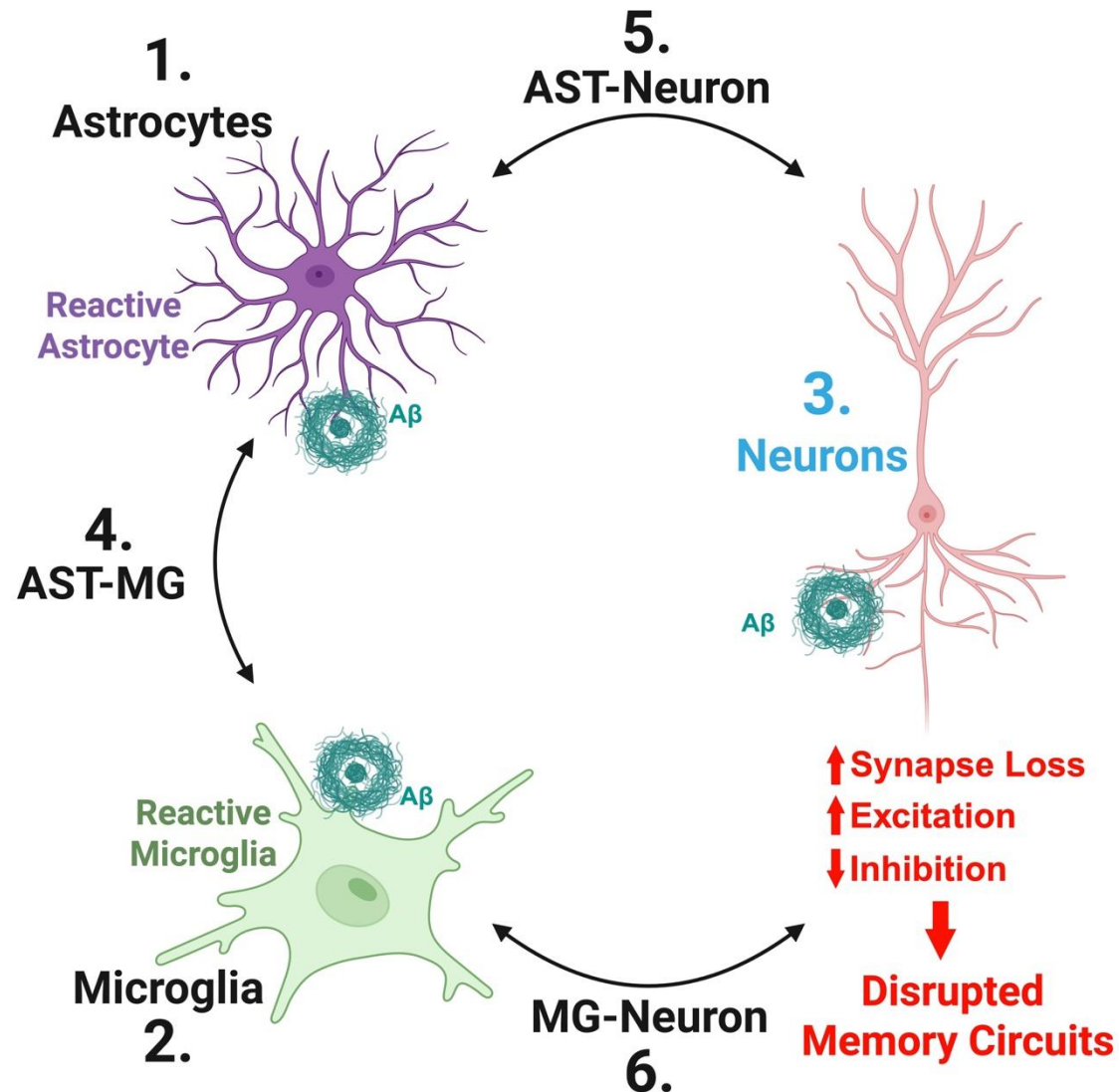


# Therapeutic Hypothesis: EPHB3 inhibition will promote neuron health by blocking astrocyte-microglia interactions that drive neuroinflammation



# VT-001 improves memory in 5xFAD mice by eliciting neuroprotective effects across key domains of glial and neuronal biology

## A $\beta$ impairs key glial and neuronal biology



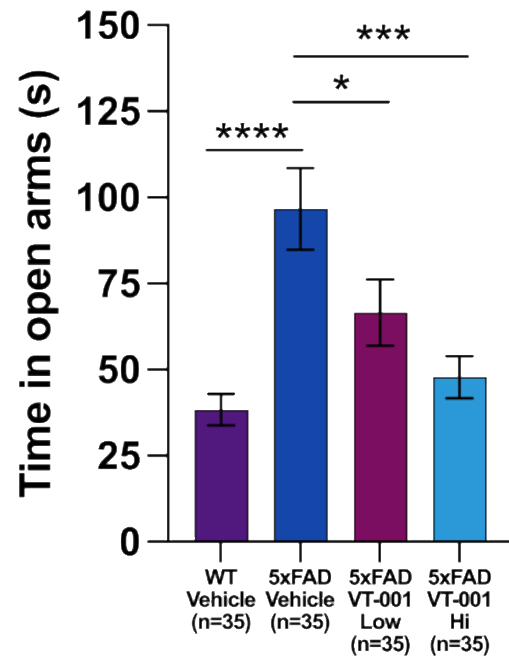
## VT-001 rescues deficits in:

- 1. Astrocytes (AST)**
  - ✓ ↓ A $\beta$  PIG expression (snRNA-seq)
  - ✓ ↓ complement + Type I IFN (snRNA-seq + proteomics)
- 2. Microglia (MG)**
  - ✓ ↓ A $\beta$  PIG expression (snRNA-seq)
  - ✓ ↓ complement + Type I IFN (snRNA-seq + proteomics)
- 3. Neurons**
  - ✓ ↑ Inhibition (PV; snRNA-seq + CSF proteomics)
  - ✓ ↓ Excitation (snRNA-seq + CSF proteomics)
  - ✓ Rescues multiple AD synapse modules (CSF proteomics)
  - ✓ Rescues neuron interactions across key memory circuits relevant to AD (snRNA-seq; NeuronChat)
- 4. AST-MG Interaction**
  - ✓ ↓ AST-MG A $\beta$  PIG expression (snRNA-seq)
- 5+6 AST-Neuron and MG-Neuron interaction**
  - Ongoing (L/R analysis)

# VT-001 rescues cognitive deficits in vivo and AD synapse protein deficits in the CSF of 5xFAD mice

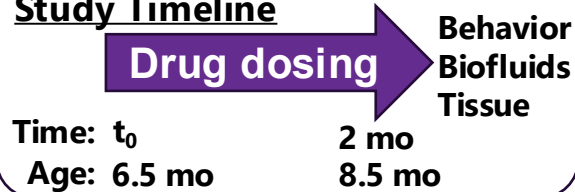
## Behavioral efficacy

### Elevated plus maze



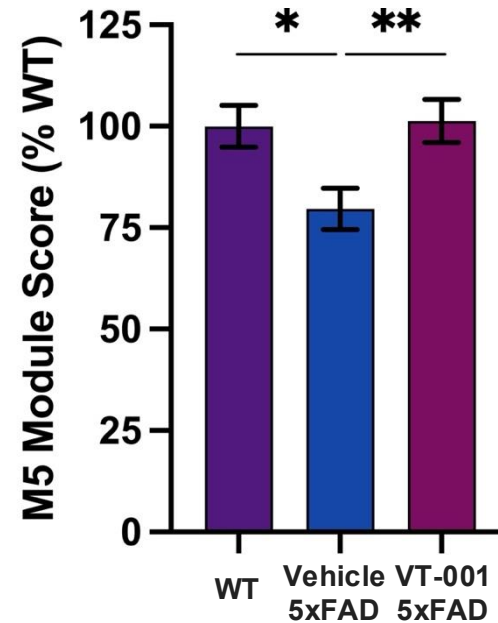
N = 35/group

### Study Timeline

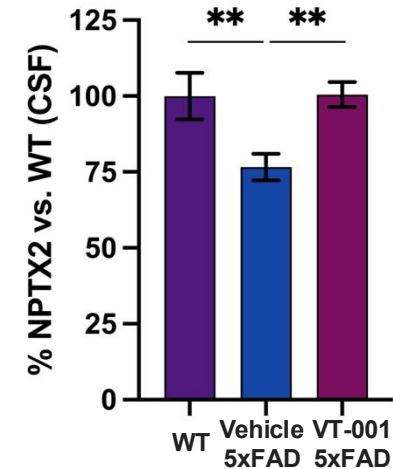


## CSF Proteomics Using Human AD Synaptic Protein Module

### M5 Module: Post-Synaptic

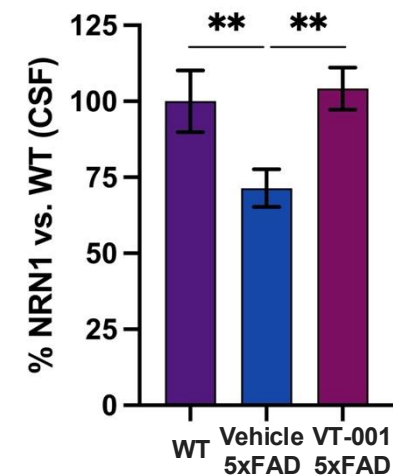


### NPTX2



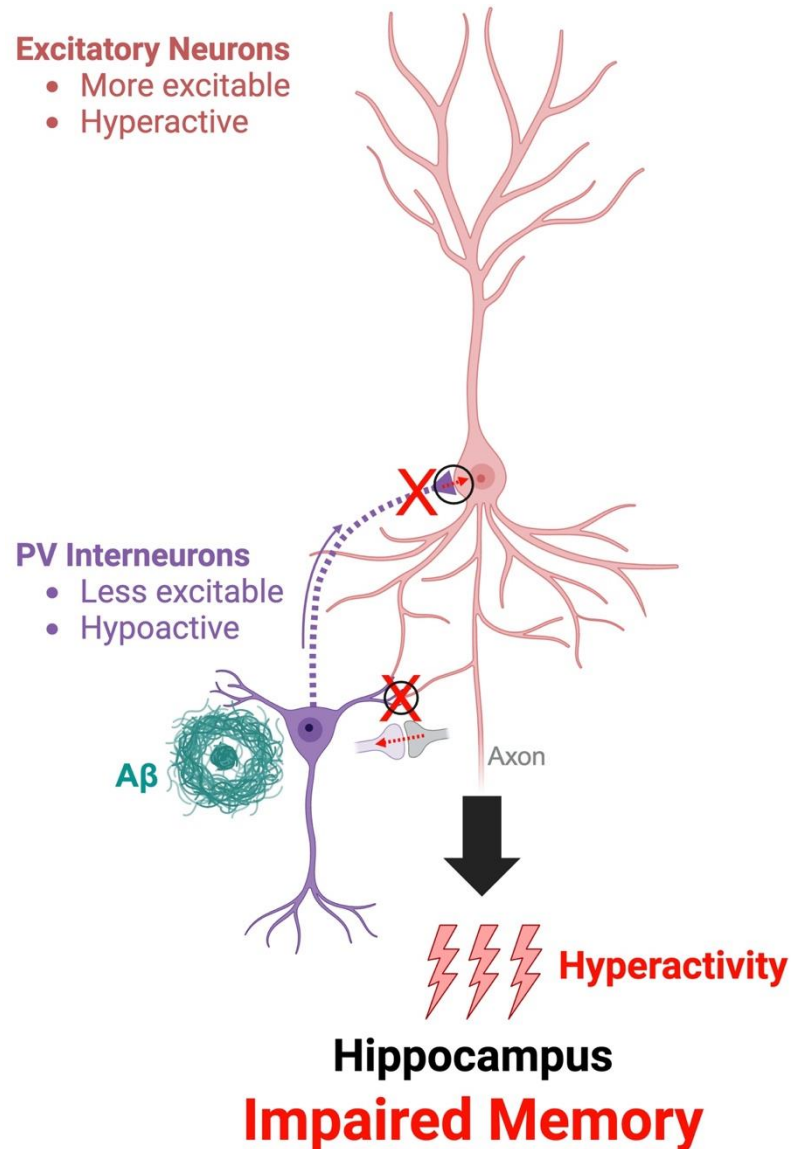
Top AD protein decreased with cognitive impairment

### NRN1



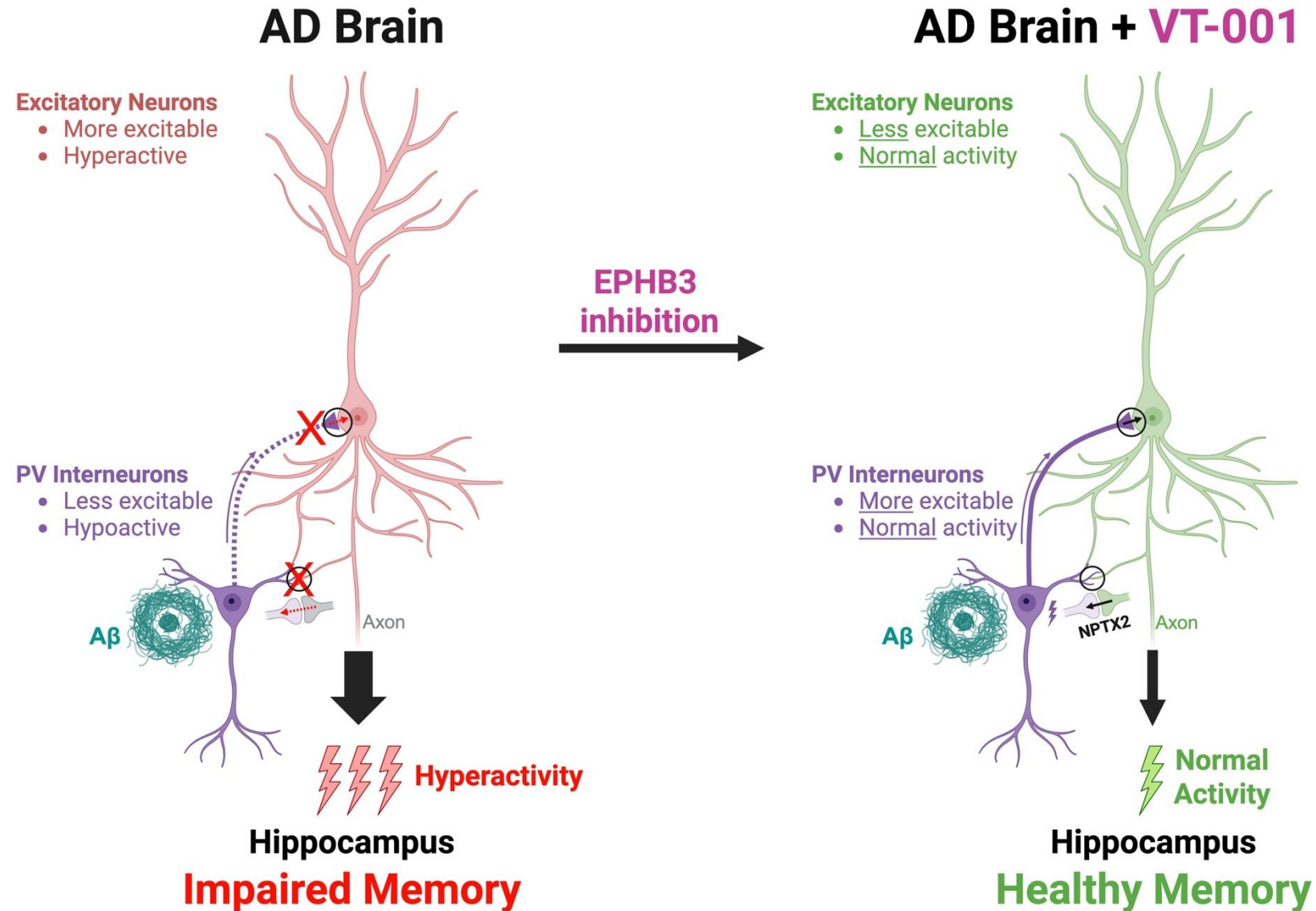
Top AD resilience protein different in AD-unimpaired vs. AD-dementia

# Memory impairments in early AD are driven by a loss of inhibition mediated by PV interneurons (IN) in key memory circuits



- Retrosplenial cortex (RSC), entorhinal cortex (EC), hippocampus (HP) critical for memory and severely compromised in AD
  - ✓ Among earliest regions to show neuron loss and dysfunction in AD
- AD patients and mice show PV-IN loss and neuron hyperactivity in RSC-EC-HP system with disrupted gamma rhythms
  - ✓ Aβ drives PV-IN deficits and neuron disinhibition
  - ✓ significantly impairs cognition
- AD patients show ↓NPTX2 CSF levels
  - ✓ Most strongly correlated protein with AD cognitive deficits
  - ✓ **NPTX2 increases excitatory drive onto PV-IN**
  - ✓ **NPTX2 loss = molecular correlate of neuron disinhibition by PV-IN**

# VT-001 restores memory by restoring healthy excitatory/inhibitory (E/I) balance in memory circuits compromised by AD



# VT-001 engages multiple key mechanisms in PV interneurons to restore inhibitory tone in memory circuits

5xFAD  
2-month  
treatment



CTX + HP



snRNA-seq

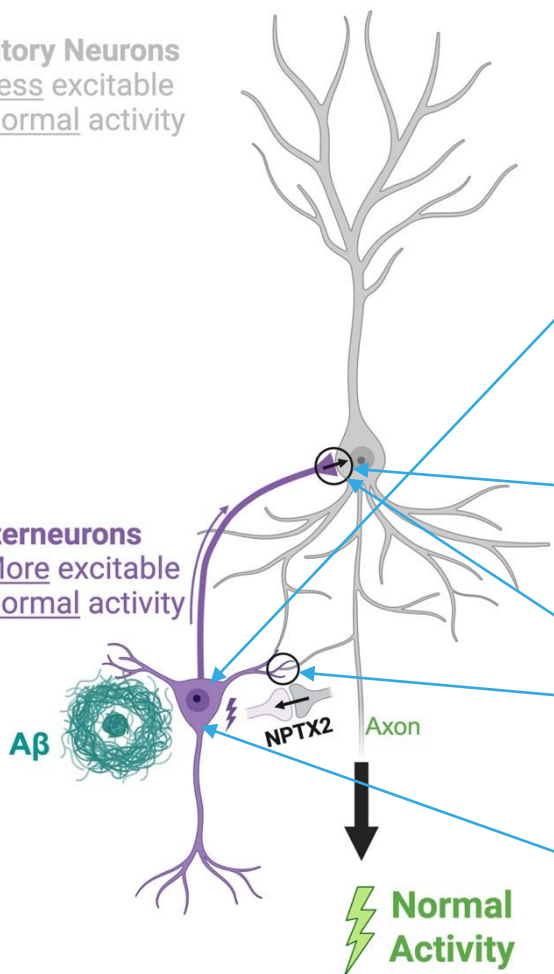
## AD Brain + VT-001

### Excitatory Neurons

- Less excitable
- Normal activity

### PV Interneurons

- More excitable
- Normal activity



Hippocampus

Healthy Memory

Changes significantly ↓ in 5xFAD vehicle and ↑ by VT-001 (snRNA-seq)

### ↑ Excitability of PV interneurons

- **KCNC1**: K<sup>+</sup> channel, enables high-frequency firing
- **GRIP1**: AMPAR membrane anchoring at excitatory synapses
- **FRMPd4**: key PSD protein at excitatory synapses
- **NPTX2** (protein): ↑ excitatory drive onto PV interneurons

### ↑ Inhibitory neurotransmission

- **GABRA1**: mediates fast inhibitory transmission
- **EPHA7**: mediates gephyrin clustering to maintain inhibitory synapses

### ↑ Synaptogenesis of inhibitory and excitatory synapses

- **CLSTN2,3**: ↑ formation and function of inhibitory and excitatory synapses

### ↑ Peri-neuronal net (PNN) structure critical for PV interneuron function and NPTX2 binding

- **CSPG5**: major structural protein of PNNs

# VT-001 normalizes excitability of excitatory (Glu) neurons

## AD Brain + VT-001

Changes significantly ↓ in 5xFAD vehicle and ↑ by VT-001 (snRNA-seq)

5xFAD  
2-month  
treatment



CTX + HP

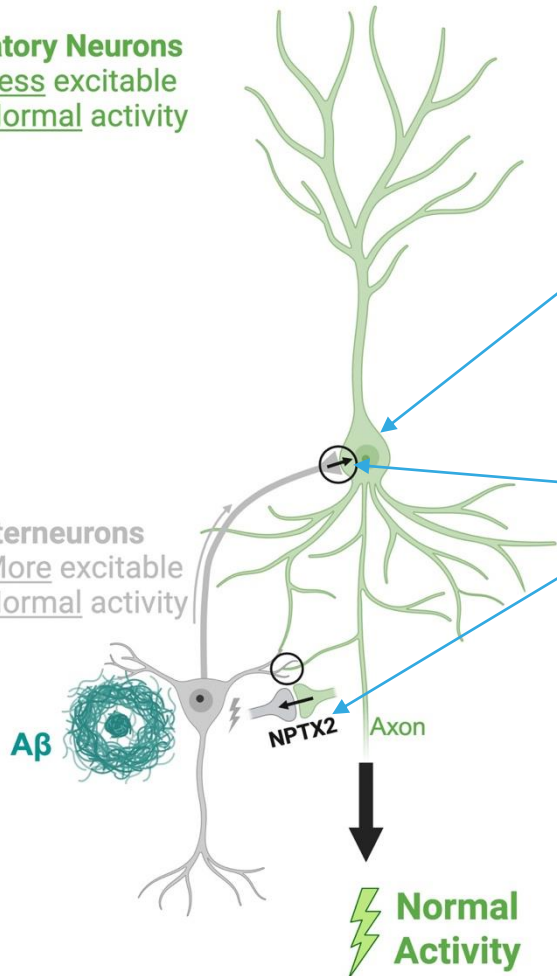
↓  
snRNA-seq

### Excitatory Neurons

- Less excitable
- Normal activity

### PV Interneurons

- More excitable
- Normal activity



### ↓ Excitability of excitatory (Glu) neurons

- **KCNC2**: K<sup>+</sup> channel, hyperpolarizing, enables high-frequency firing
- **KCNQ3**: K<sup>+</sup> channel that regulates neuronal excitability
- **KCNH7**: K<sup>+</sup> channel that regulates neuronal excitability
- **KCNT2**: Na<sup>+</sup>-activated K<sup>+</sup> channel that regulates neuronal excitability

### ↑ Synptogenesis of inhibitory synapses onto excitatory neurons and strengthening of PC-PV synapses

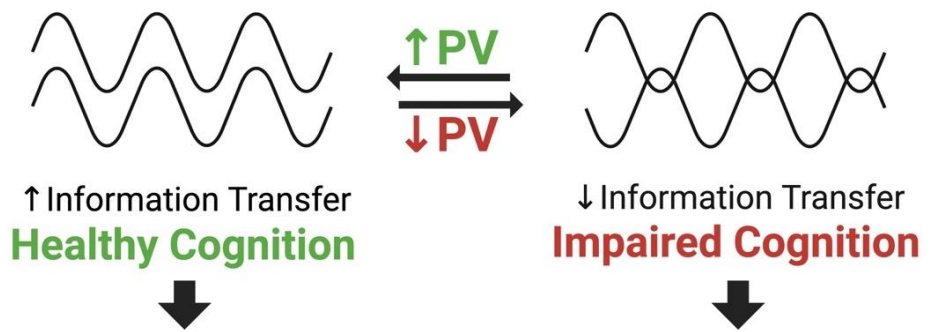
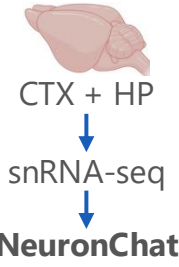
- **NPAS4**
  - ✓ drives new inhibitory synapses development on excitatory neurons
  - ✓ critical regulator of E/I balance
  - ✓ NPTX2: key NPAS4-regulated gene that strengthens PC-PV synapses

Hippocampus

Healthy Memory

# 5xFAD mice show broadly disrupted PV interneuron interaction with excitatory neurons and disrupted excitatory neuron networks

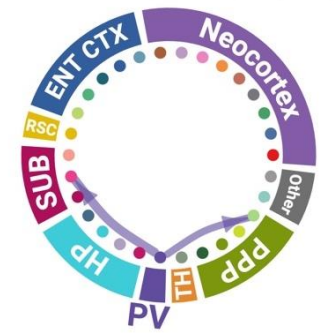
5xFAD  
2-month  
treatment



**NeuronChat: L/R Analysis of Neuron Interaction**

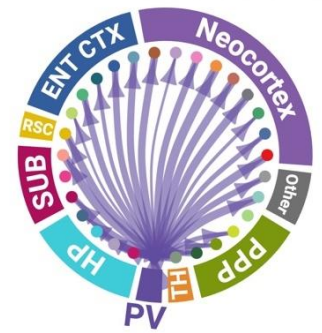
↑ PV Interactions

5xFAD  
Vehicle



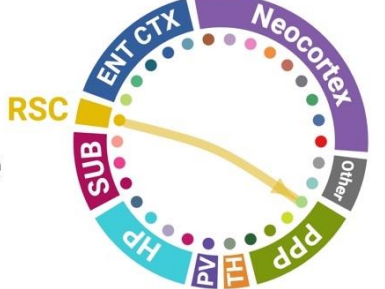
↓ PV Interactions

**Striking loss of PV IN interaction with excitatory neurons across brain areas vital for memory**



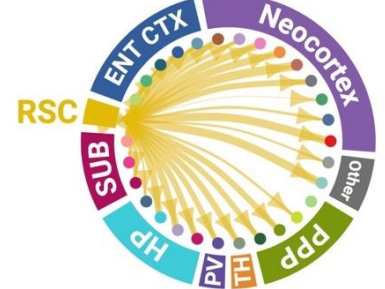
↑ RSC Interactions

5xFAD  
Vehicle



↓ RSC Interactions

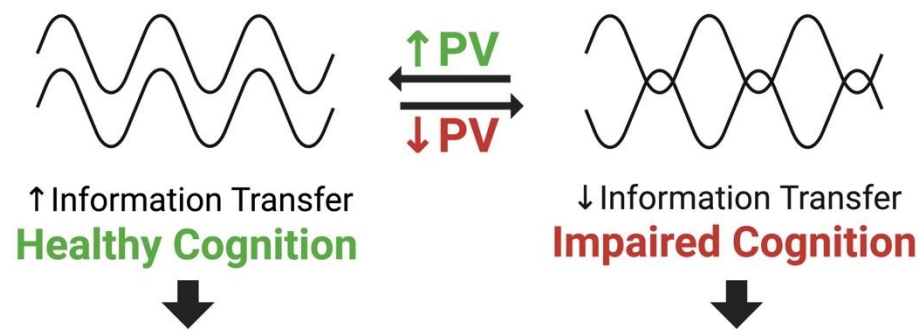
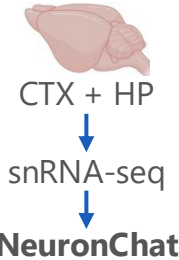
**Profound disruption of excitatory neuron networks central to AD memory impairments: retrosplenial cortex (RSC)**



- To transmit information between brain regions neurons need to be synchronized
- PV interneurons (IN) powerfully synchronize cognitive networks: essential for healthy cognition
- Losing PV IN function profoundly disrupts the ability of cognitive networks to talk to one another
- *Retrosplenial cortex (RSC) network disruption strongly predicts MCI → AD conversion: **due to ↓PV IN function***

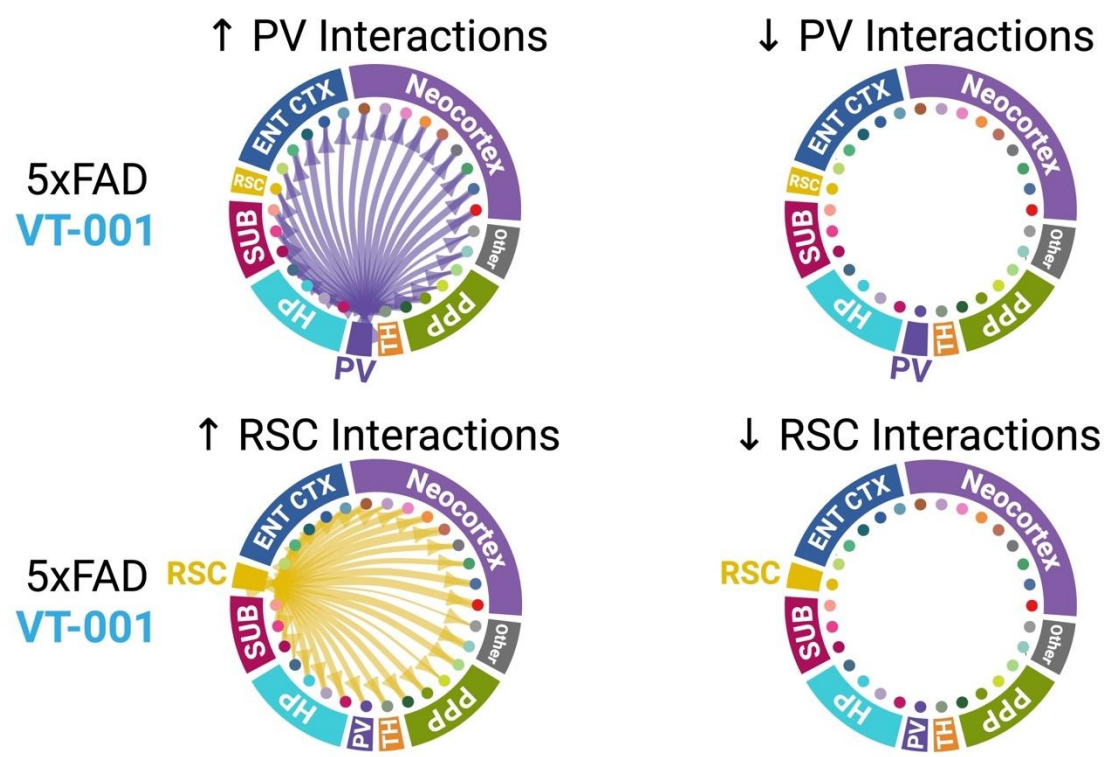
# VT-001 rescues PV interneuron deficits and restores interactions in key excitatory neuron networks critical for memory

5xFAD  
2-month  
treatment



- To transmit information between brain regions neurons need to be synchronized
- **PV** interneurons (IN) powerfully synchronize cognitive networks: essential for healthy cognition
- Losing PV IN function profoundly disrupts the ability of cognitive networks to talk to one another
- *Retrosplenial cortex (RSC) network disruption strongly predicts MCI → AD conversion: due to ↓PV IN function*

**NeuronChat: L/R Analysis of Neuron Interaction**

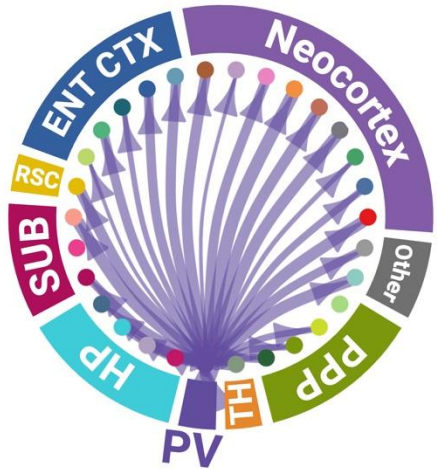


**VT-001 rescues widespread loss of PV IN interaction with excitatory neurons**

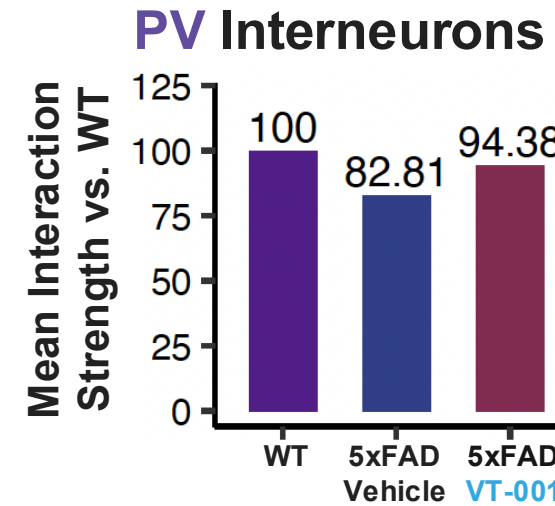
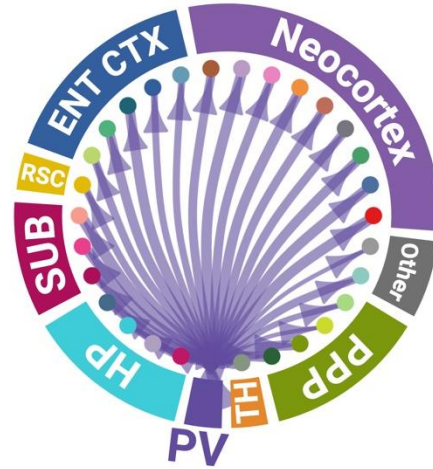
**Restored interactions in key excitatory neuron networks critical for memory (RSC)**

# VT-001 rescues PV interneuron deficits and restores interactions in key excitatory neuron networks critical for memory

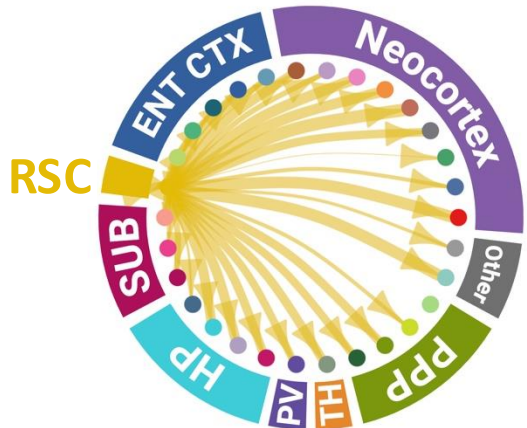
**5xFAD Vehicle**  
↓ PV Interactions



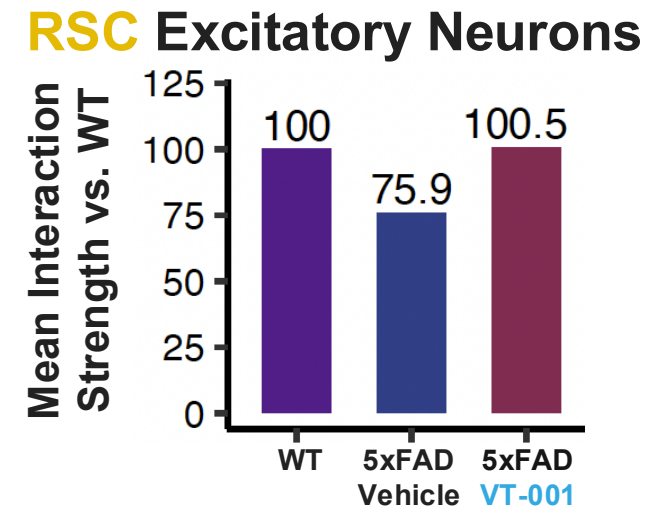
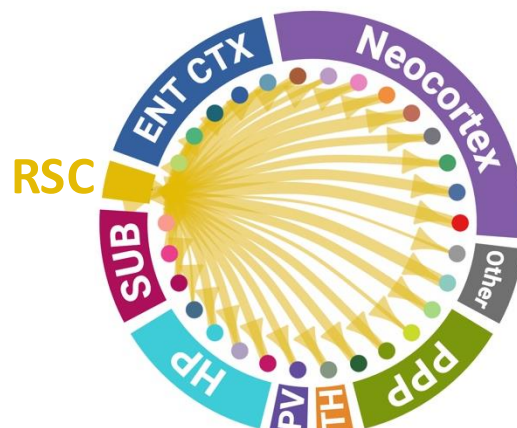
**5xFAD VT-001**  
↑ PV Interactions



↓ RSC Interactions



↑ RSC Interactions



# Conclusions and next steps

**Overall Conclusion:** VT-001 improves memory in 5xFAD mice by normalizing the excitability of PV interneurons and excitatory neurons to restore neuronal interactions across key networks central to AD memory impairments

- ✓ In 5xFAD mice VT-001 significantly rescues:
  - cognitive deficits (EPM, MWM)
  - human AD synaptic biomarker deficits strongly linked to cognitive impairment (CSF proteomics)
  - Intrinsic excitability deficits in PV interneurons and excitatory neurons (snRNA-seq)
  - Neuron interaction deficits between PV interneurons and excitatory neurons across brain regions (NeuronChat)
  - Neuron interaction deficits in key excitatory neuron networks that support memory (RSC, EC, HP; NeuronChat)

## **Next steps in 5xFAD mice treated with VT-001:**

1. Neurohistology: quantify excitatory synapse density and NPTX2 levels on PV interneurons
2. Ex vivo HP electrophysiology: excitability changes in PV interneurons and excitatory neurons
3. In vivo electrophysiology: neural dynamics in RSC-EC-HP system

# Thank you



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Founder of ImmunArray, Alma Bio, AnToIRx



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### Our Investors:

Dementia Discovery Fund

Mass General Brigham Ventures

Ono Ventures

UTEC