Novel EPHB3 Inhibitors to Protect and Restore Function in Key Neuron Cell Types, Synapses, and Networks Impacted by Alzheimer's Disease

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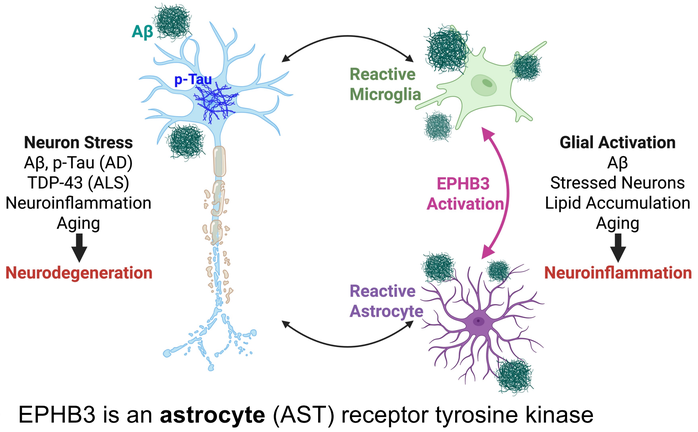
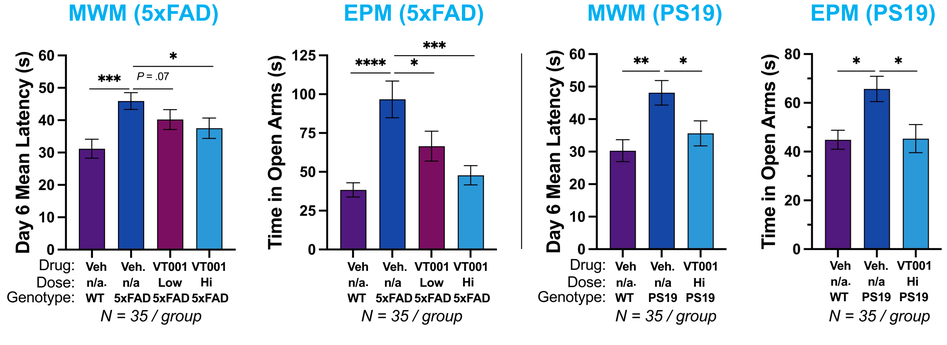
EPHB3 Controls Astrocyte-Microglia Interactions that Drive Neuroinflammation



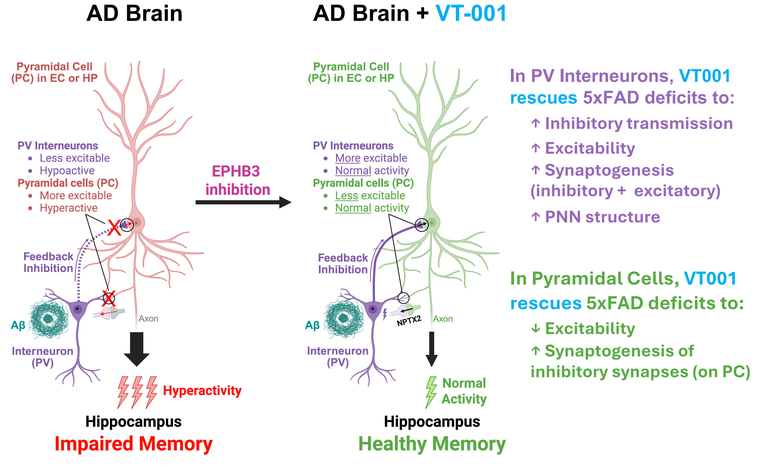
- EPHB3 is an **astrocyte** (AST) receptor tyrosine kinase
- Binds EFNB3 on **microglia** (MG): drives neuroinflammation
- Therapeutic Goal = inhibit EPHB3 to protect neurons

VT-001 is Highly Efficacious in 5xFAD (Aβ) and PS19 (tau) Mouse Models of AD Neuropathology

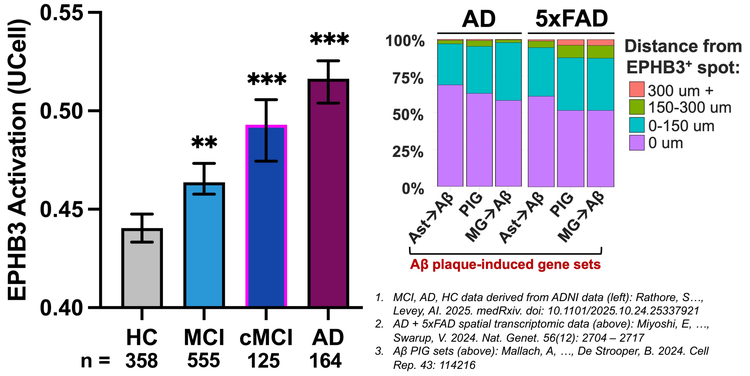


- VT-001 prevents cognitive deficits in 5xFAD (2 mo dosing: 6.5 – 8.5 m/o) and PS19 (3 mo dosing: 6 – 9 m/o) mice in the Morris water maze (MWM) and elevated plus maze (EPM) after 2 mo of dosing (6.5 – 8.5 m/o)
- **Unique efficacy profile:** highly efficacious in amyloidosis and tauopathy models

VT-001 Restores E/I Balance in AD Memory Circuits (snRNA-seq)



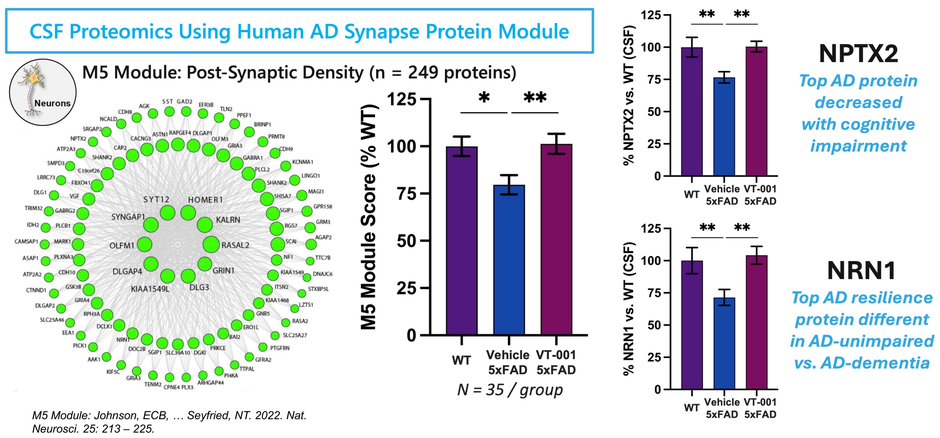
EPHB3 Activation is Increased in MCI and AD Patients and Co-Localized with AD Pathology



1. *MCI, AD, HC data derived from ADNI data (left): Rathore, S…, Levey, AI. 2025. medRxiv. doi: 10.1101/2025.10.24.25337921*
2. *AD + 5xFAD spatial transcriptomic data (above): Miyoshi, E, …, Swarup, V. 2024. Nat. Genet. 56(12): 2704 – 2717*
3. *Aβ PIG sets (above): Mallach, A, …, De Strooper, B. 2024. Cell Rep. 43: 114216*

- EPHB3 activation is increased in CSF of MCI, AD patients
- MCI patients who convert to AD dementia in a 3-year time frame (cMCI; magenta border) are enriched for EPHB3 activation vs. MCI
- High co-localization of EPHB3 activation and Aβ plaques in human AD brain and 5xFAD mice by spatial transcriptomics

VT-001 Rescues Proteomic Signatures of AD Synapse Pathology in 5xFAD CSF



M5 Module: Johnson, ECB, … Seyfried, NT. 2022. Nat. Neurosci. 25: 213 – 225.

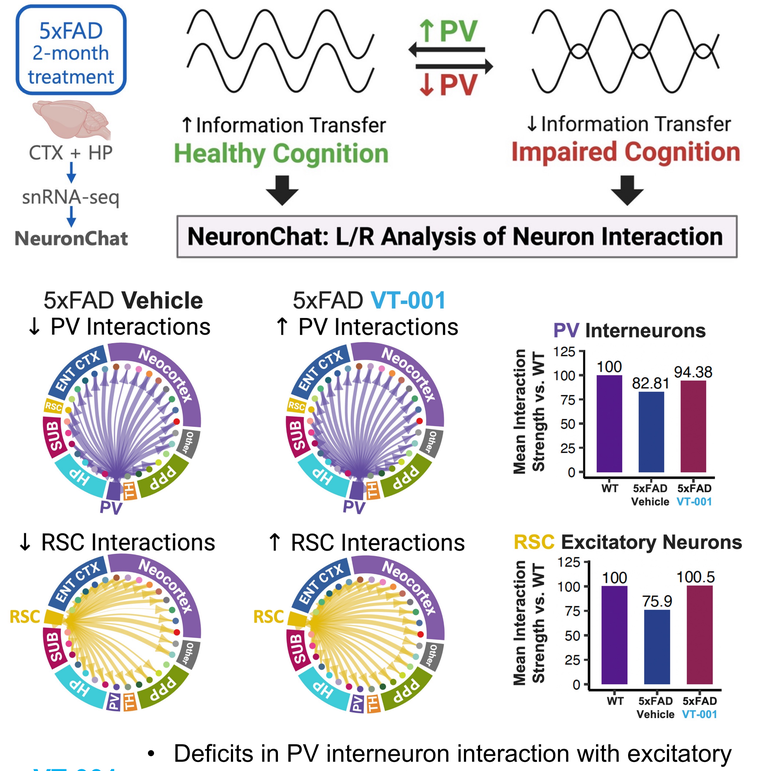
NPTX2
Top AD protein decreased with cognitive impairment

NRN1
Top AD resilience protein different in AD-unimpaired vs. AD-dementia

VT-001 Rescues

- Synapse protein deficits in 5xFAD CSF: human AD protein modules used
- NPTX2 and NRN1, two key AD synapse proteins, to WT levels

VT-001 Restores Neuron-Neuron Interactions Across Key Memory Circuits Disrupted in AD



VT-001 Rescues

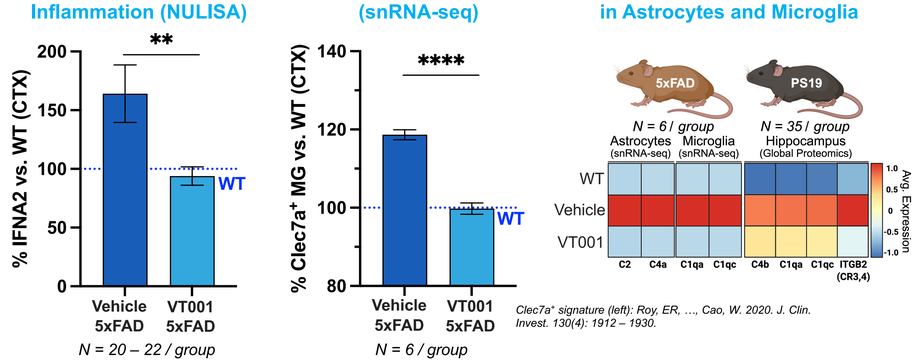
- Deficits in PV interneuron interaction with excitatory neuron networks (top)
- Interactions in excitatory neuron networks critical for memory (e.g. retrosplenial cortex; bottom)

VT-001: a Novel Small Molecule EPHB3 Inhibitor

Parameter	Selected Assay/Target	VT-001
Properties	CNS MPO Score	5
	Kinetic solubility (7.4, uM)	192
	MW, cLogD	< 410, < 1
	PPB % unbound (m, r, d, c, h)	64, 71, 65, 61, 51
Biochemical	EPHB3 (IC50, uM)	0.048
	Carna NanoBRET (196 kinases)	1/240 (EPHB3)
Kpuu	Rat	40 %
In Vitro Tox.	CYPs	< 50 at 10 uM
	hERG	37 % at 10 uM
	SafetyScreen44	1/44 (3 uM AChE)

- Very potent
- Highly selective
- CNS-permeable
- Active in acute (LPS) and chronic (EAE) neuroinflammation assays (not shown)

VT-001 Blocks Type-I IFN Mechanisms that Drive AD Synapse Loss



Clec7a+ signature (left): Roy, ER, …, Cao, W. 2020. J. Clin. Invest. 130(4): 1912 – 1930.

VT-001 Decreases

- Type-I IFN protein levels (IFNA2) in 5xFAD neocortex
- Clec7a+ microglia (Type-I IFN driver) in 5xFAD neocortex
- Complement components and receptors in 5xFAD and PS19 brain

Ongoing and Future Directions

- Neurohistology in 5xFAD and PS19 to quantify synapse density
- Electrophysiology in 5xFAD and PS19 to assess synapse function

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