

Small Molecule-Guided Brain Targeted mRNA Lipid Nanoparticles for Precision Neuronal Specificity

Gal Chen, PhD

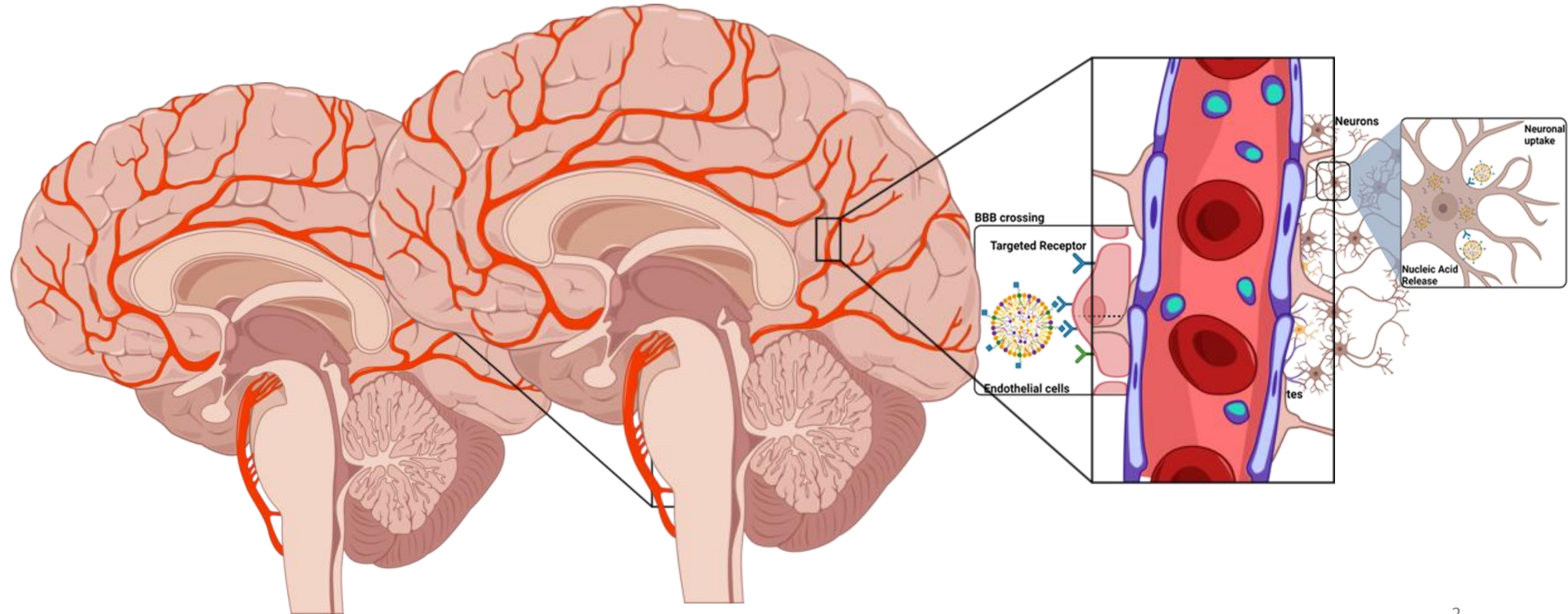
Supervised by Prof. Avi Schroeder



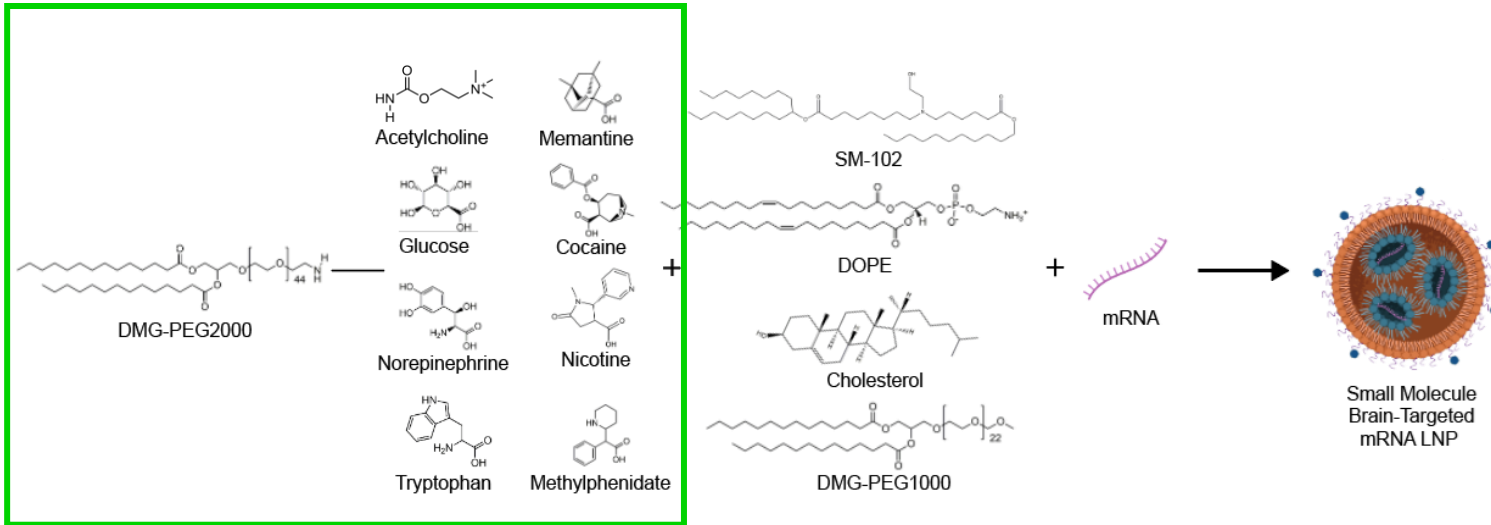
PHILADELPHIA, PA
JULY 13-18, 2025

**NEXT-GENERATION
DELIVERY INNOVATIONS**

Facilitating Brain Gene Delivery Using BBB-Interacting Small Molecules

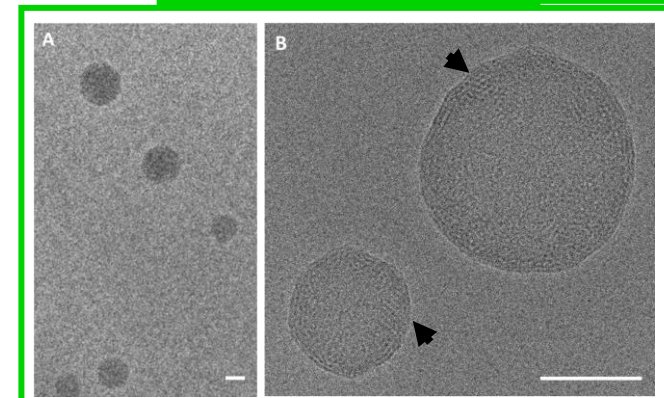
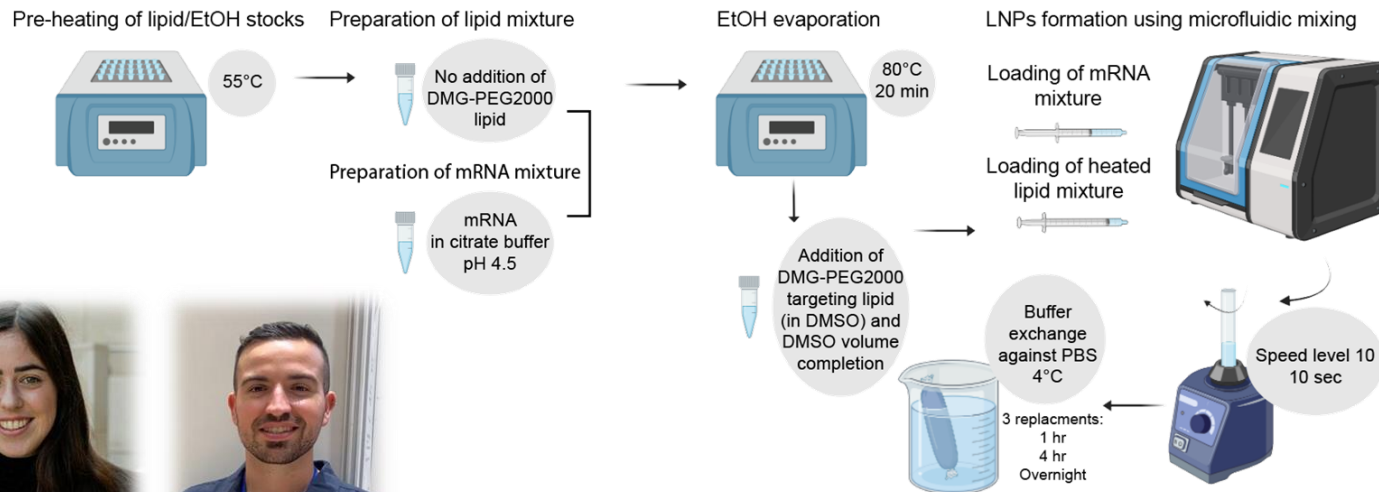


Synthesis of a Brain-Targeted (BT) LNP Series



Targeting moiety	LNP size [nm]	Zeta potential [mV]	PDI	EE [%]
Untargeted	109.8±20.9	-6.7±2.5	0.14±0.05	91.7±2.1
Glucose	155.1±21.1	-9.6±2.2	0.08±0.04	91.7±1.1
Methylphenidate	141.9±10.9	-8.6±1.1	0.18±0.05	87.6±8.0
Memantine	153.9±19.7	-11.8±2.9	0.31±0.05	89.2±5.1
Acetylcholine	149.1±10.6	-13.4±1.6	0.13±0.05	90.1±5.7
Cocaine	148.6±15.1	-12.2±2.1	0.19±0.08	90.7±3.8
Tryptophan	147.7±9.2	-12.6±0.3	0.12±0.08	89.3±2.5
Nicotine	161±13.5	-10.7±4.1	0.10±0.05	93.7±1.9
Norepinephrine	155.5±17.6	-13.6±5.7	0.18±0.07	89.2±3.1

Adjusting microfluidic mixing method for the production of LNPs with Small-Molecule functionalized PEG-lipids



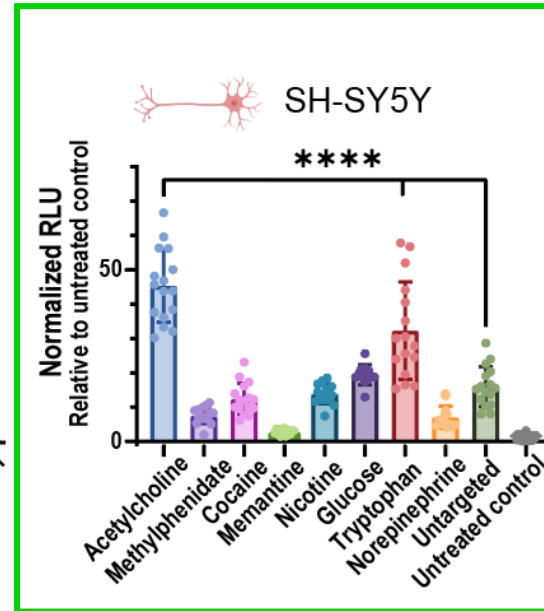
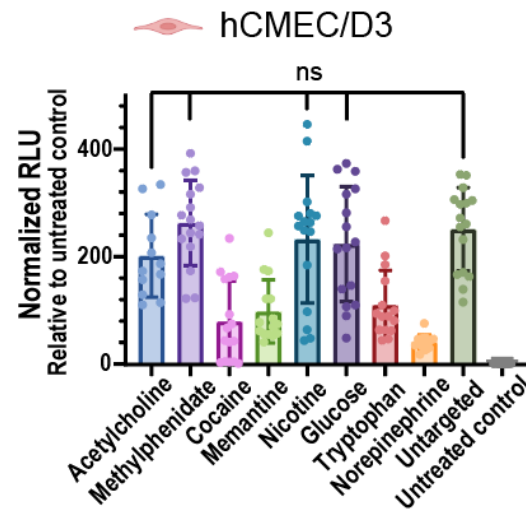
Representative Cryo-TEM images of BT-LNPs
Scale bar=100nm

In collaboration with Dr. Inbal Abutbul-Ionita and Prof. Dganit Danino

Dr. Mor Sela Haim Kadosh

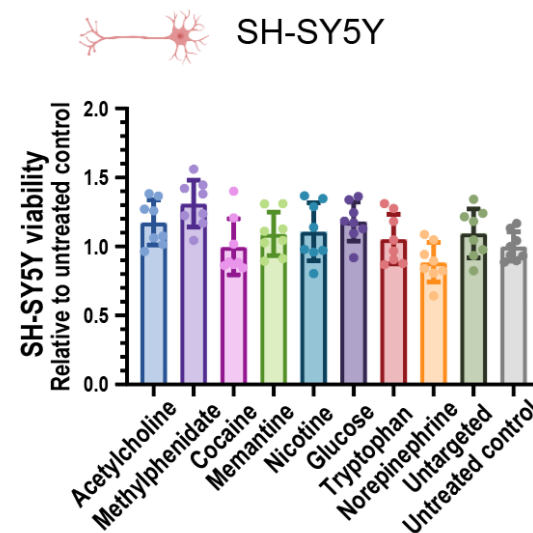
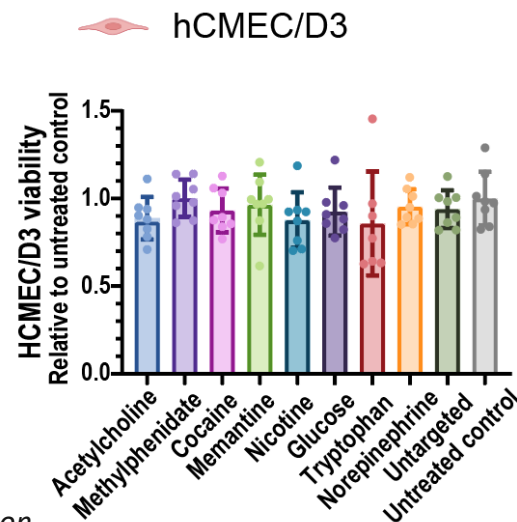
BT LNPs Transfection Efficiency and Cytotoxicity

200ng FLuc mRNA per LNP treatment [16hrs]
Luminescence signal was referred to as formulation transfection efficiency



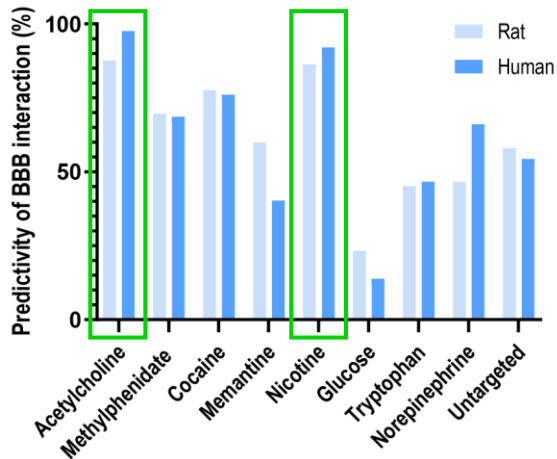
Acetylcholine and tryptophan LNPs showed the best transfection efficiency in neuronal-like cells

Cytotoxicity assay using PrestoBlue



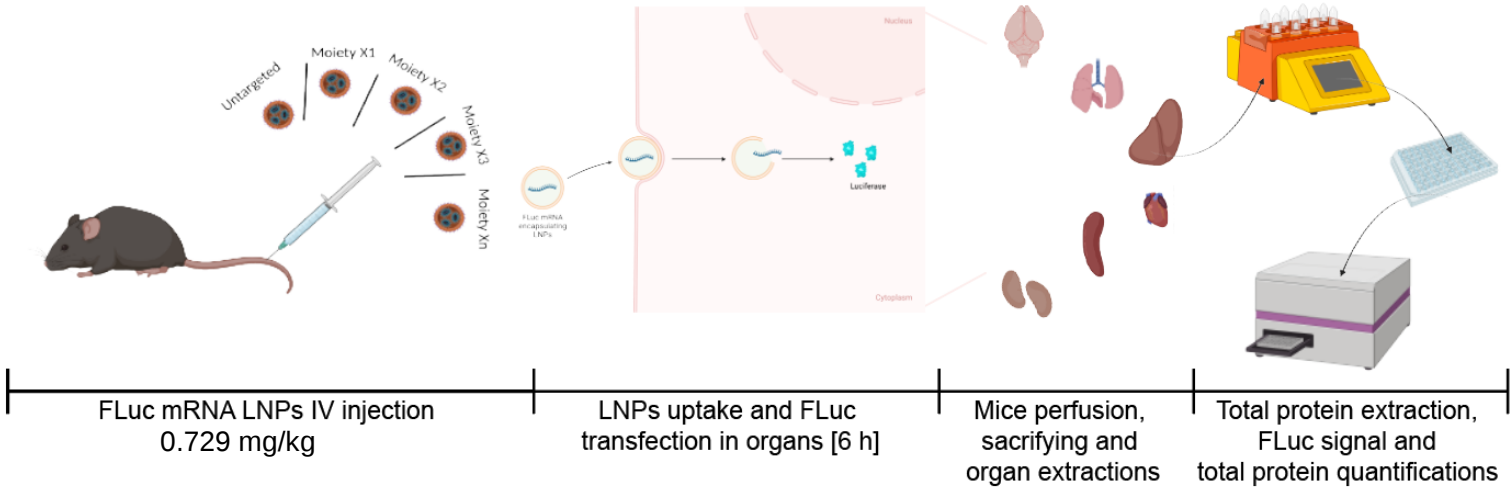
BT LNPs Alter Transfection Efficiency in the Brain

A graph neural network-based model for molecular activity prediction in human tissues – utilized for predicting the BBB interaction potential of molecular targeting candidates

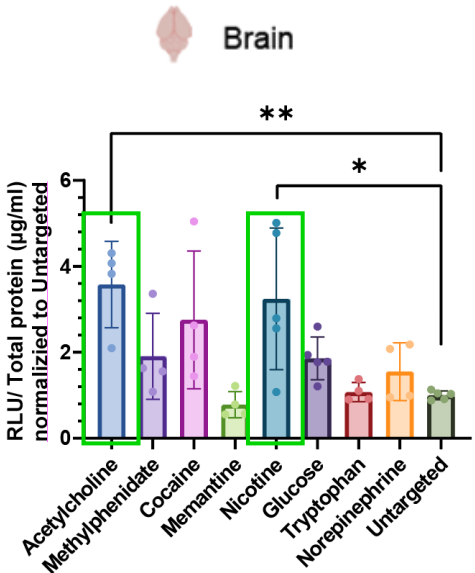


AUC=0.82

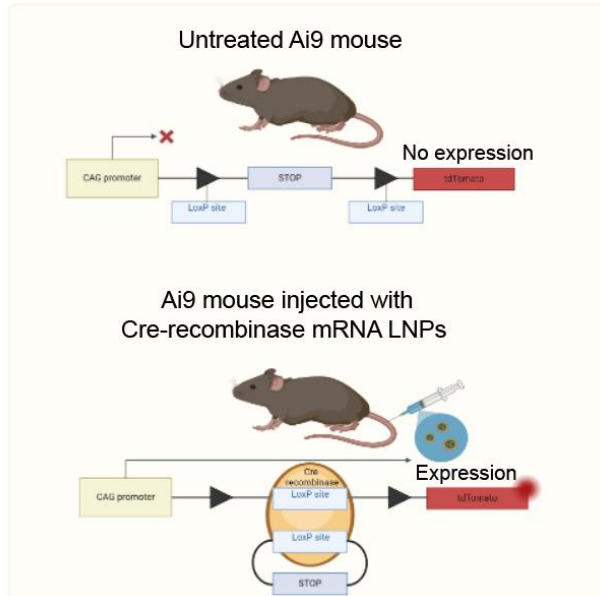
Model developed by Dr. Kira Radinsky and Sally Turotov



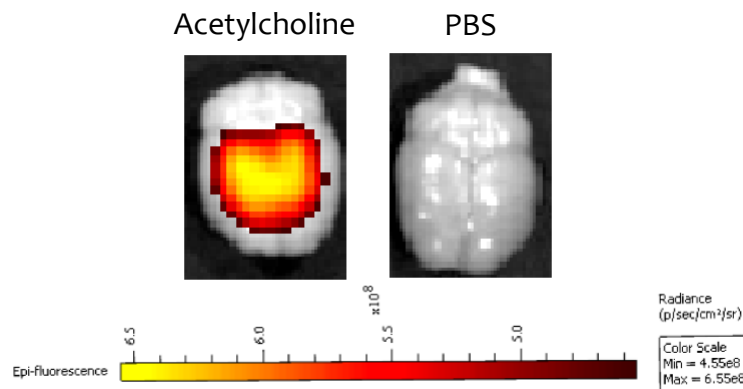
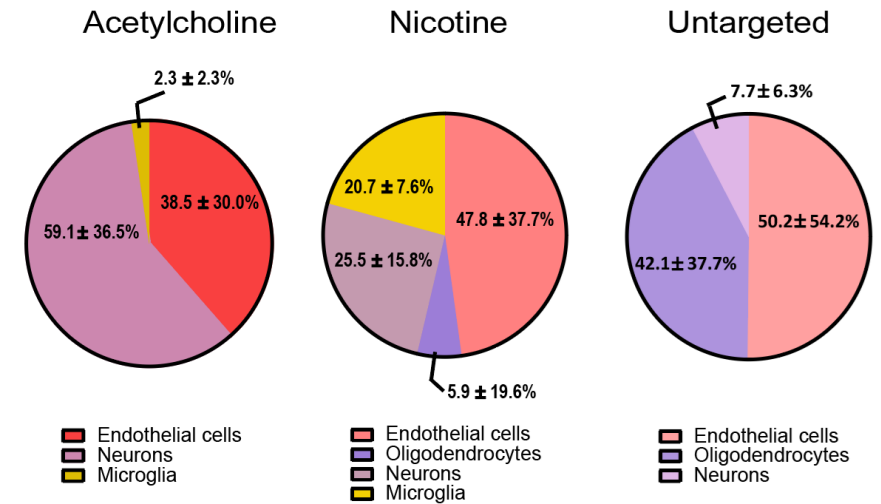
Normalized luminescence results in the brain



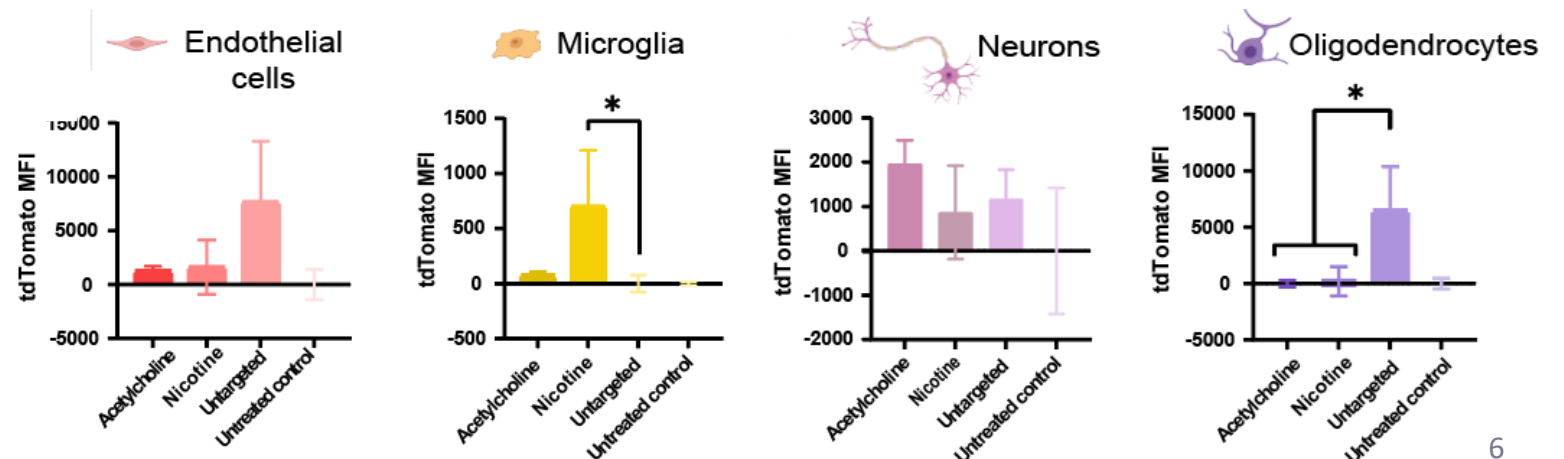
Targeting Moiety Alters LNP Brain-Cell Transfection Selectivity



Analysis was performed on +tdTomato live cell population, indicating successful mRNA transfection and translation

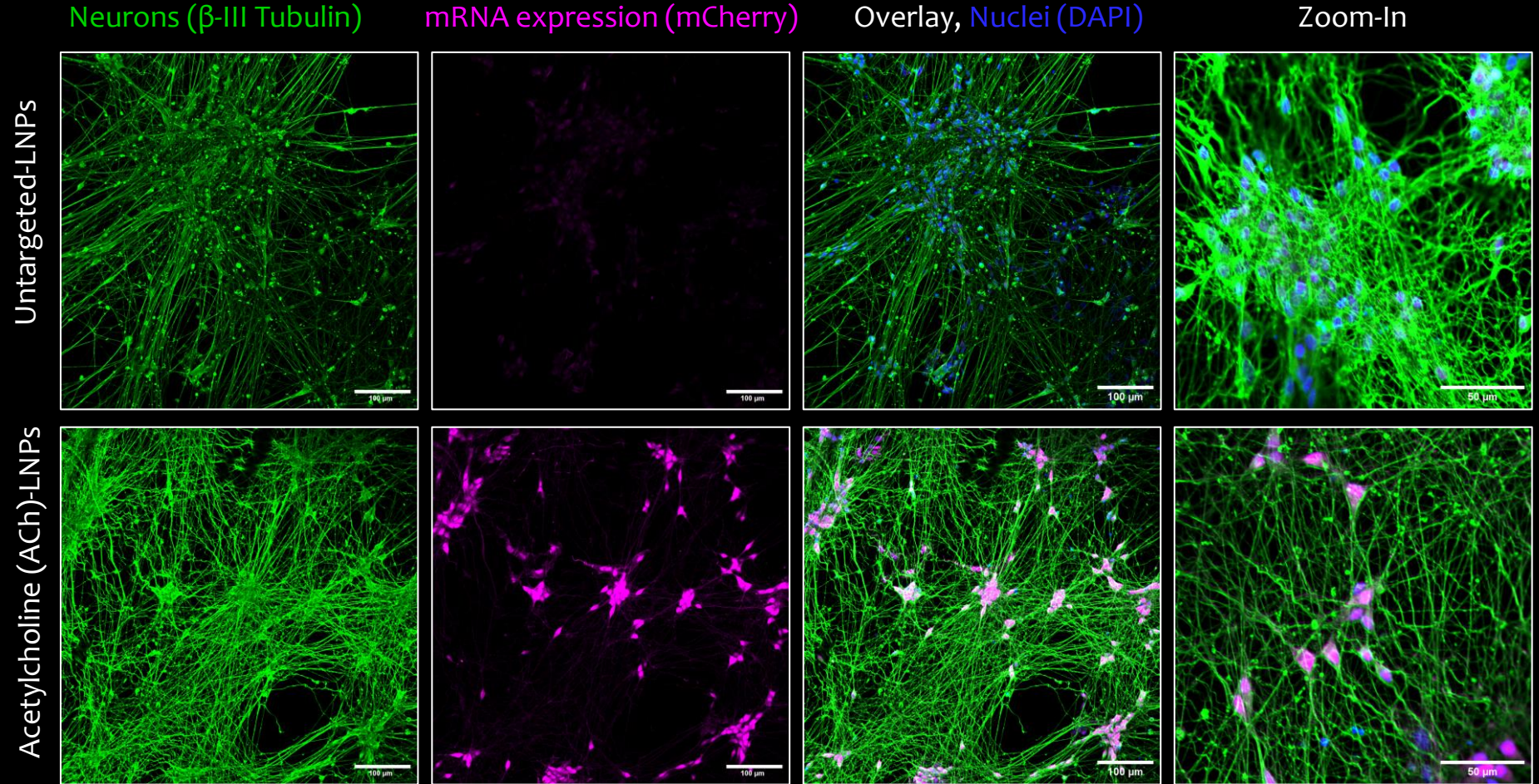
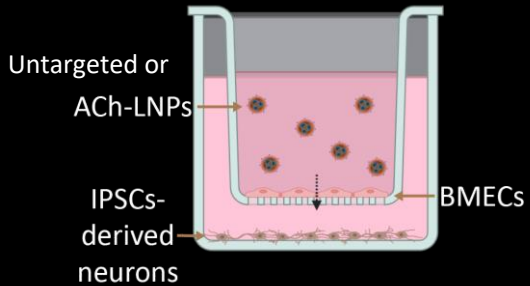


IVIS Imaging of tdTomato signal in the brain



Functional Neuronal Transfection Across a Human iPSC-Derived BBB

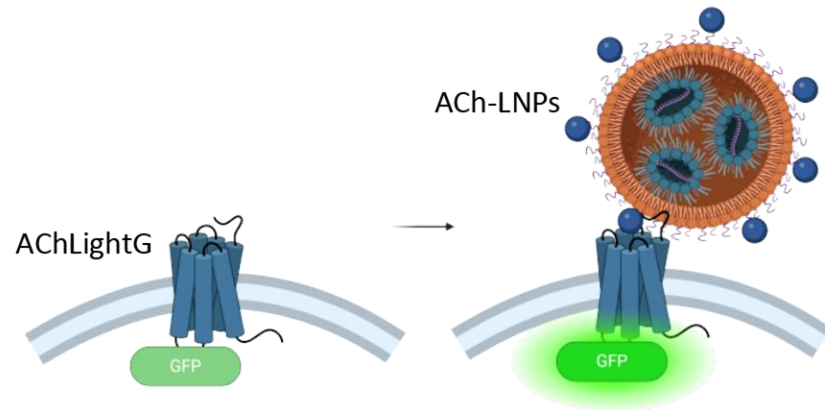
mCherry-loaded LNPs were apically applied and incubated for 24 hours to test *in vitro* BBB passage



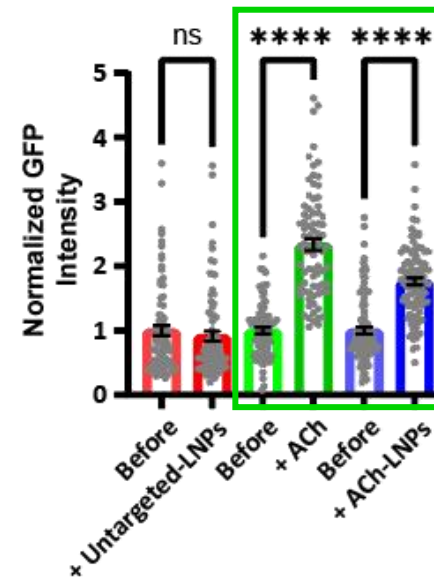
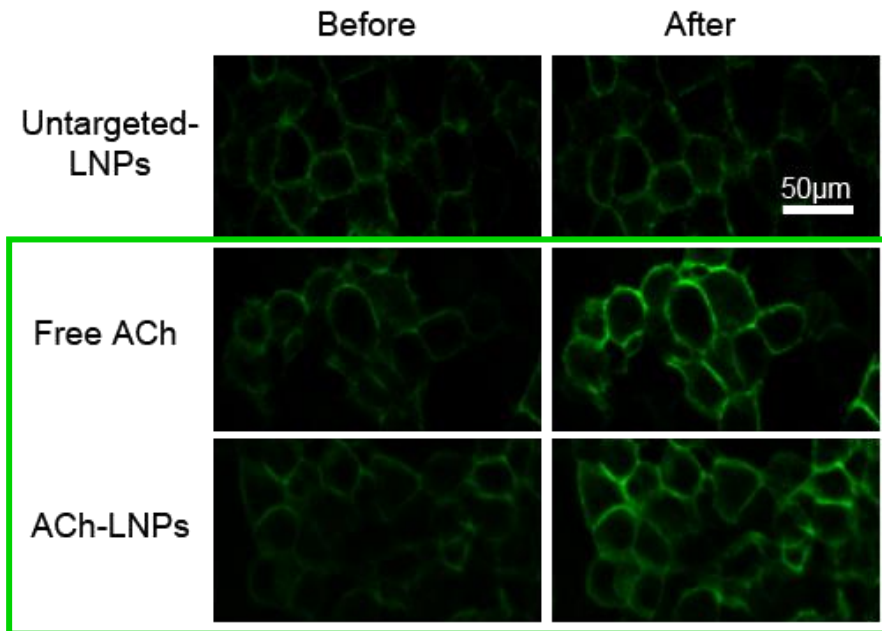
In collaboration with Dr. Raneen Nicola and Prof. Ben M. Maoz

Chen G.*, Sela M.*, Kadosh, H.*, et al. (2025). *Under Revision*.

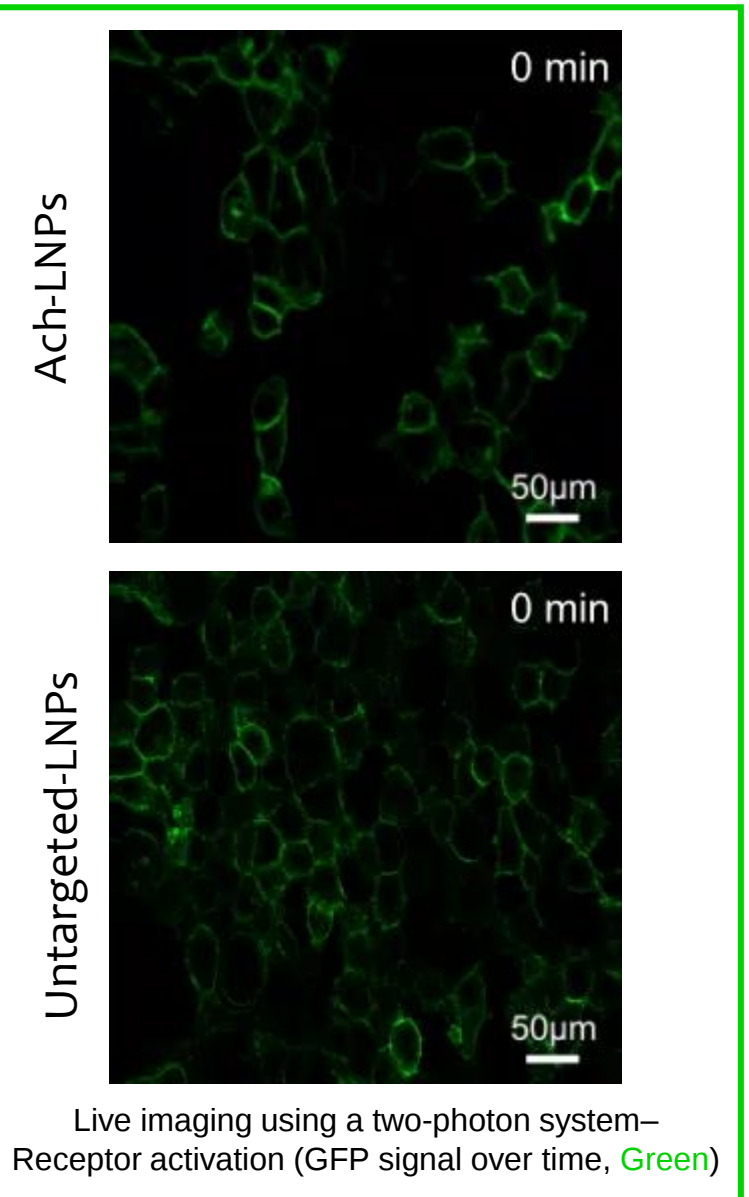
ACh-LNPs Functionally Engage ACh-Receptors



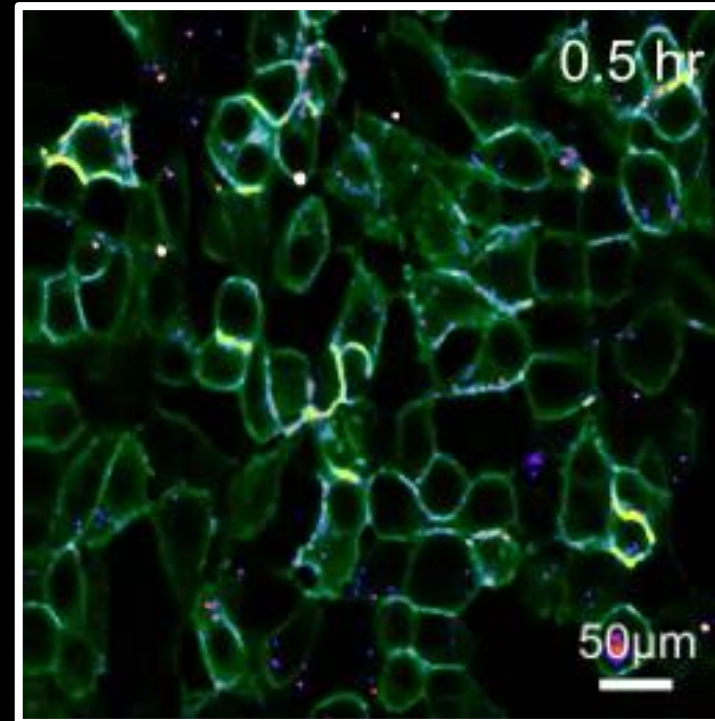
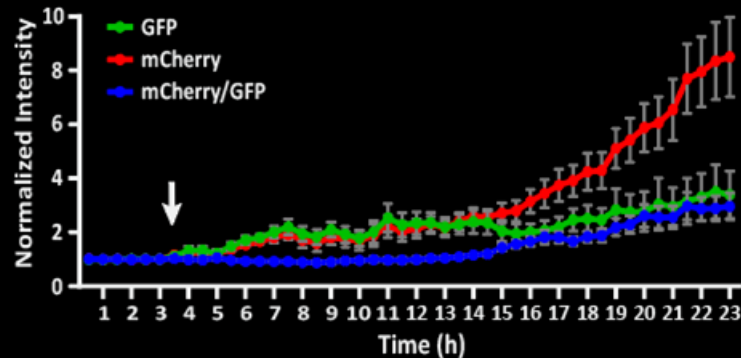
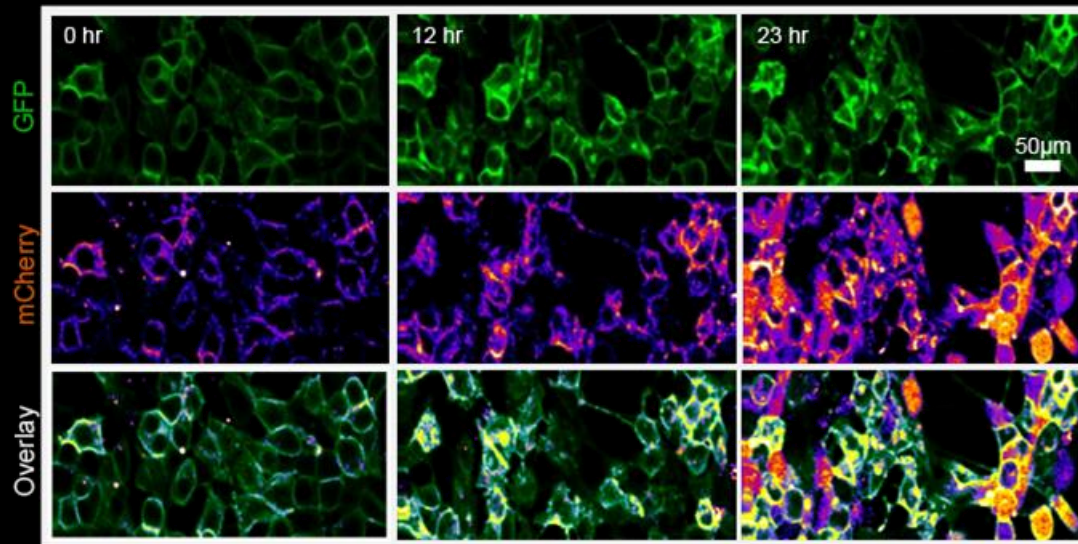
AChLightG is based on an engineered muscarinic receptor coupled to a circularly-permuted GFP, which produces robust green fluorescence upon ACh binding



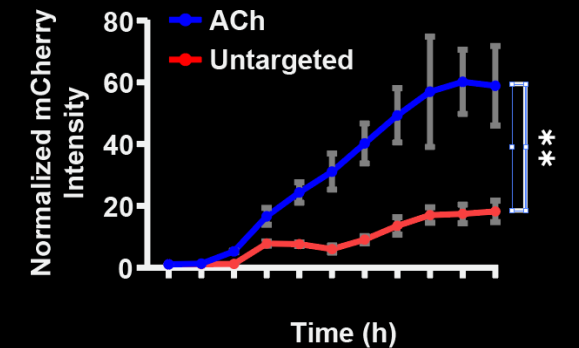
ACh-LNPs and free ACh significantly activate the AChLightG receptor



ACh LNPs Trigger Receptor Binding and Drive Enhanced mRNA Transfection



Two-photon-based live imaging of receptor binding of ACh-LNPs (GFP) and mRNA translation (mCherry)



Significant increase in the expression kinetics of ACh LNPs vs. untargeted LNPs

ACh LNPs Selectively Transfect Neurons and Astrocytes in the Brain

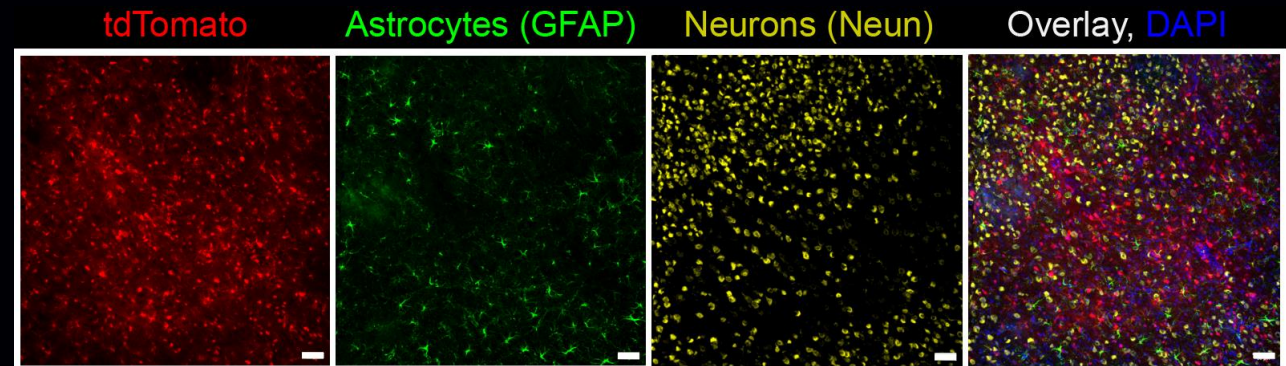
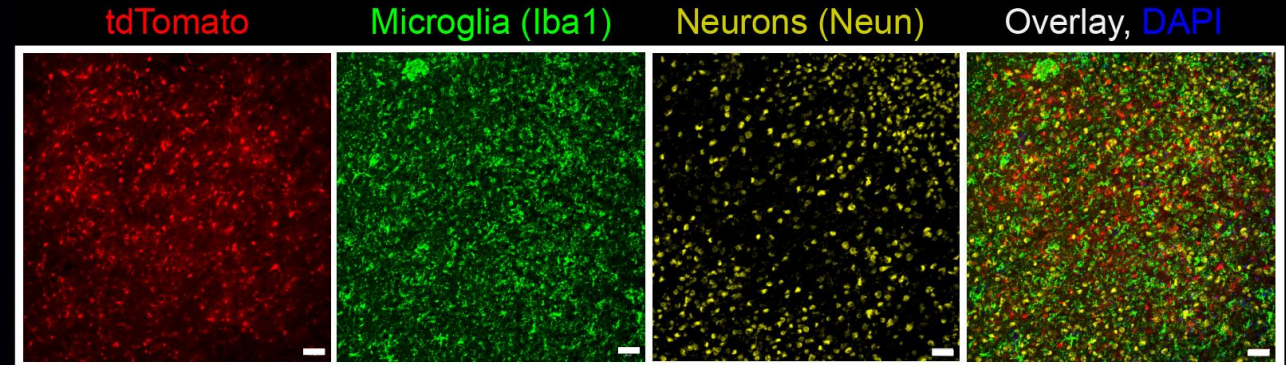
Acetylcholine-Cre mRNA LNPs

Intracerebral
administration

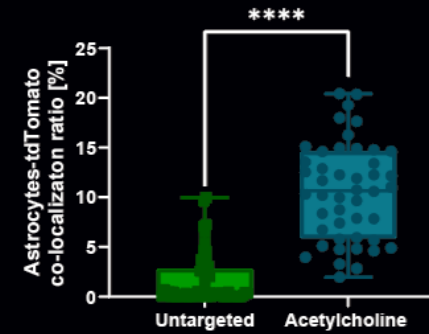
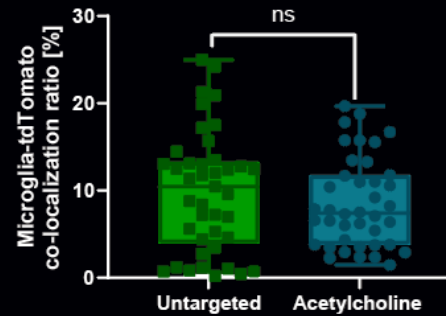
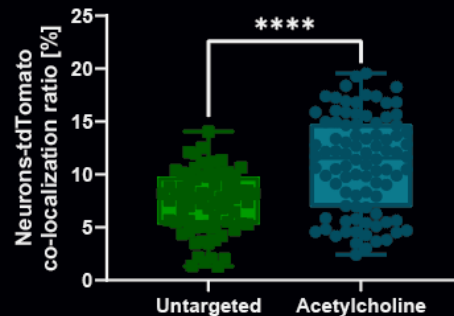
1mm

100μm

tdTomato (Cre-mRNA expression)
DAPI (nuclei)



Scale bar:
50μm



In collaboration with **Prof. Amit Zeisel**
and **Prof. Peleg Hasson Labs**

Chen G.*, Sela M.*, Kadosh, H.,* et al.
(2025). Under Revision.

Summary

- **Small molecule–modified LNPs** enable efficient mRNA delivery across the BBB into the brain.
- **Acetylcholine-LNPs** show strong neuronal and astrocytic selectivity, while **Nicotine-LNPs** were the best to transfect microglia.
- Receptor engagement enhances **mRNA translation**, even without classical internalization.
- This modular, ligand-guided LNP strategy offers a **scalable and tunable platform** for treating neurological diseases at the cellular level.

Thank You!



Prof. Avi Schroeder
Dr. Mor Sela* and Haim Kadosh*
Schroeder's lab members

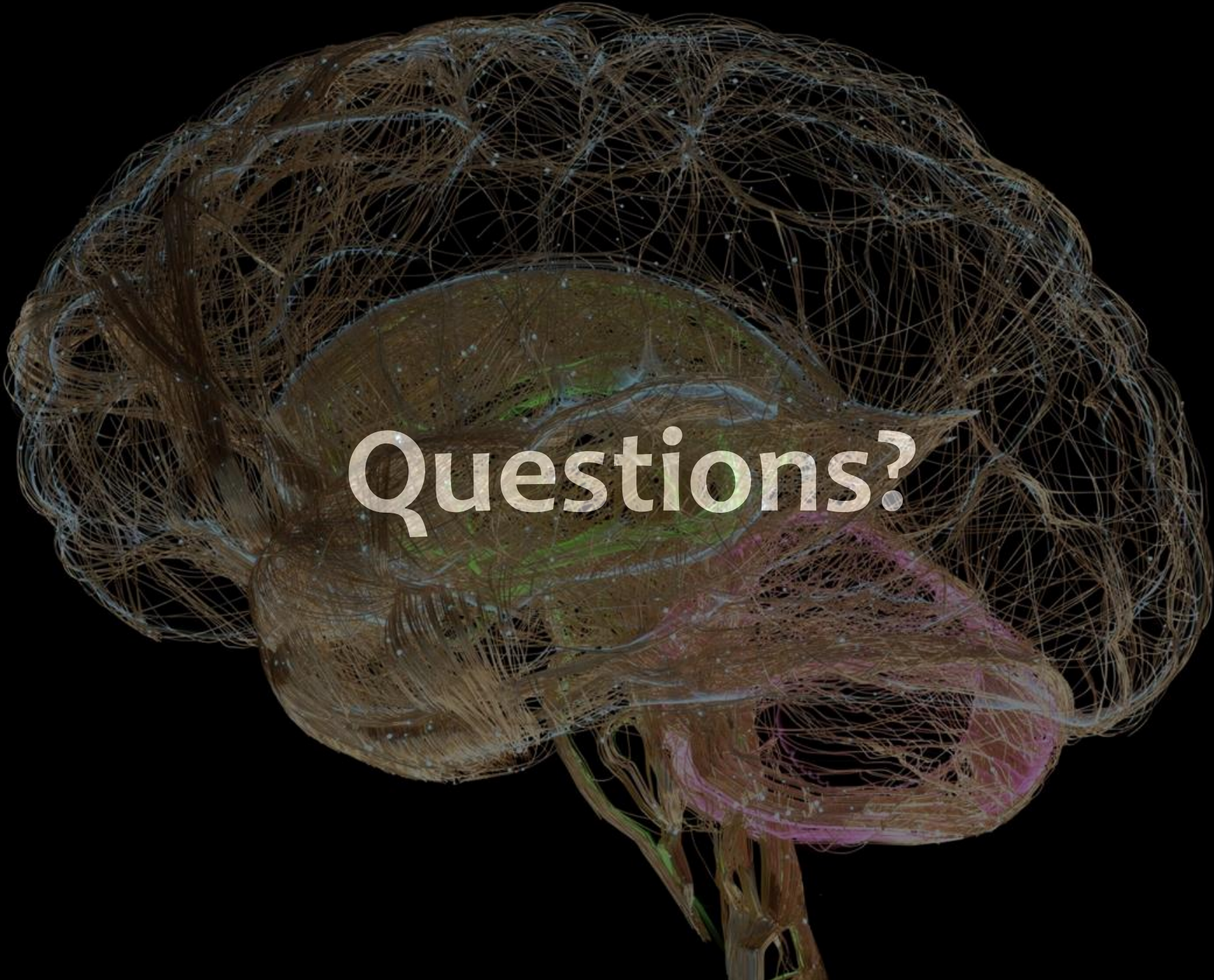
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Figures created using **Biorender.com**

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Questions?