ATLAS- A Method For Anterograde Transsynaptic Tracing From Genetically Determined Neurons

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Abstract

The study of neural circuits has been revolutionized by the advent of retrograde monosynaptic tracing, where neurons that synapse onto a select population can be labeled by infecting that population with a deletion-mutant neurotropic virus rabies virus, which then travels backward across synapses. However, mapping circuits in the anterograde direction, i.e, from presynaptic to postsynaptic neuron, has proven challenging. Current anterograde tracing methods all have weaknesses, such as the inability to trace from genetically determined neurons, labeling of neurons presynaptic as well as postsynaptic to infected neurons, multi-synaptic labeling, and toxicity. Here we introduce ATLAS (Anterograde Transsynaptic Labeling using Anti-body-like Sensors), a new method for anterograde tracing. ATLAS uses a rationally designed anterograde tracer that efficiently and specifically maps connections from genetically determined starter cells, monosynaptically, and without retrograde labeling, or toxicity. It is based on the AMPA FingR, a recombinant antibody-like protein that binds specifically to the N-terminal of the AMPA receptor, GluA1, which is the part of the protein that protrudes from the cell. The AMPA.FingR is released from the presynaptic termini, diffuses across the synapse, and then binds to a subset of AMPA receptors undergoing endocytosis (internalization by the cell). ATLAS works by a well-defined mechanism and is made of independent components that can be optimized and exchanged to have a specific functionality. These properties give ATLAS advantages over traditional viral tracers.

Methods

Schema 1: The mechanism for transsynaptic tracing using ATLAS. AMPA.FingeR-Cre is expressed in the presynaptic neuron 1 and transported through the secretory pathway to axon terminals via VAMP2. Inside the vesicle, β-Secretase APP-cleaving enzyme (BACE) cutting site is cleaved, and AMPA.FingR fused with recombinase is released into the synaptic cleft. AMPA.FingR then binds to AMPA receptors on the postsynaptic membrane. The entire complex, including AMPA-FingR Cre and AMPAR, is endocytosed into postsynaptic neurons. AMPA.FingR-Cre is released from the endosome and enters the nucleus of the

ATLAS: Anterograde **T**ranssynaptic **L**abeling using **A**ntibody-like **S**ensors

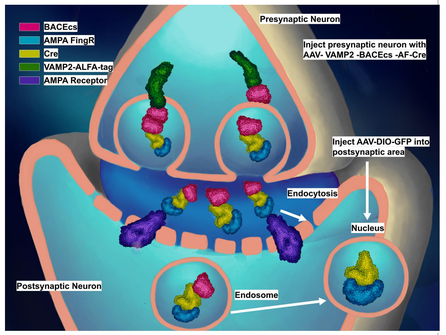


Diagram by Saya Rutgers

Results

AMPA.FingR-Cre is released from presynaptic sites and endocytosed by the postsynaptic neurons via interaction with AMPA receptors

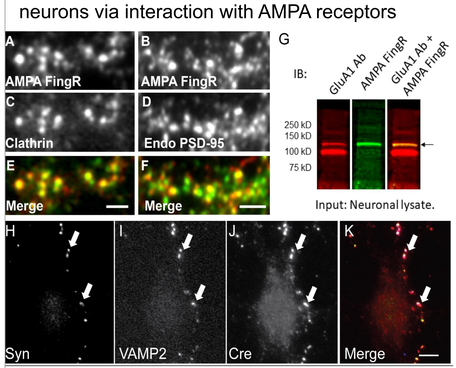
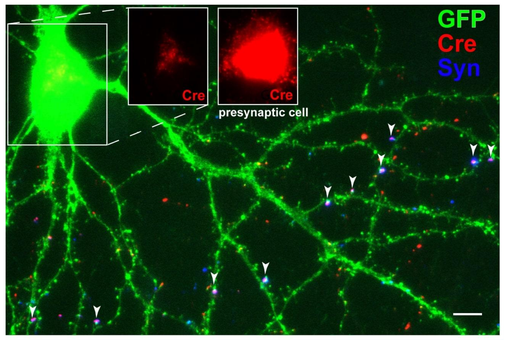


Figure 1.A, B: Cultured neurons from the cerebral cortex stained with AMPA.FingR used as an antibody. **C.** AMPA.FingR occurs together with endogenous clathrin, **(E)** but not with endogenous PSD95 **(D,F)**, consistent with AMPA.FingR being present in clathrin coated pits and undergoing endocytosis. Scale bar 5mm. **G.** Western blot of lysate from cultured neurons probed simultaneously with GluA1 antibody (red) and AMPA.Fing-HA (green). Each labels an identical band (arrow). **H-K.** Coexpressed synaptophysin (syn), VAMP2, and Cre in cultured cerebral cortex neurons all confine at presynaptic sites (arrow). Cre is found in the cell body of a postsynaptic neuron. Scale bar 10 µm.

Results

Labeling **postsynaptic** rat cortical neurons in culture using ATLAS

Figure 2. Postsynaptic rat cortical neuron in culture infected with DIO-GFP virus. Presynaptic neurons introduced to the tracer ATLAS and presynaptic marker Synaptophysin. Presynaptic terminals expressing Cre and Synaptophysin overlap with dendritic spines which form the functional contacts with other axons (arrowheads). Diffuse staining of Cre in cell body of post-synaptic cell indicated successful transsynaptic transport of AF-Cre (inset, left). Cre staining in a presynaptic cell body is intense (inset, right). Scale bar 10 µm.



Tracing anterograde connections from the prefrontal cortex (PFC) to striatum using ATLAS

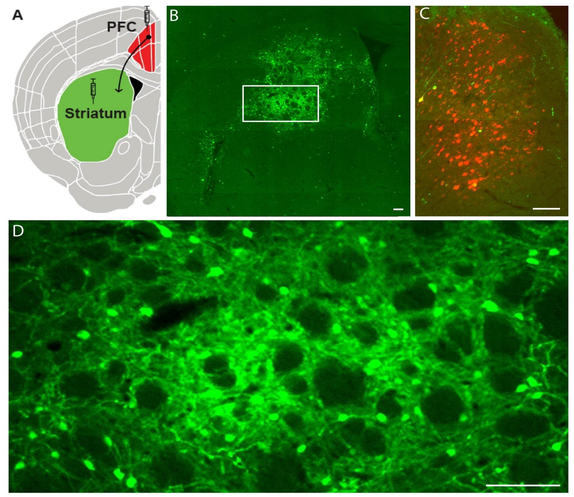


Figure 3. A. AAV8-VAMP2-AMPA.FingR-Cre injected into mouse prefrontal cortex (PFC). AAV8-Dio-GFP injected into striatum 7 days later, followed by perfusion 7 days later. **B.** VAMP2 staining (Alfatag, red) marks presynaptic neurons. Ratio of presynaptic neurons indicates efficient transsynaptic labeling. **C.** Closeup labeling presynaptic (red) and postsynaptic (green) in the PFC. **D.** High magnification image of postsynaptic neurons in the striatum. Scale bar 150 µm.

Tracing anterograde connections from genetically determined VIPR2 neurons from the lateral geniculate nucleus (LGN) to primary visual cortex (V1) using ATLAS

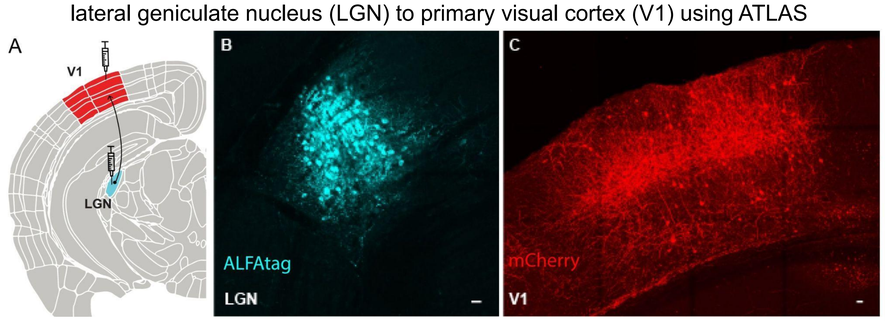


Figure 4. A. AAV8-DIO-VAMP2-AMPA.FingR-FLPo was injected into the LGN of a VIRP2-Cre mouse, which expresses Cre in projection neurons, who have distantly reaching axons, of the LGN. A second injection of AAV-fDIO-mCherry was made in the V1 cortex at the same time. After 14 days, the mouse was perfused, and sections were cut and stained. LGN is known to make anterograde connections with V1 cortex. **B.** LGN neurons expressing AAV8-DIO-VAMP2-ALFAtag-AMPA.FingR-FLP. **C.** V1 cortical neurons expressing mCherry indicating postsynaptic labeling. Scale bar 50 µm.

No observable retrograde labeling from LGN to retina using ATLAS

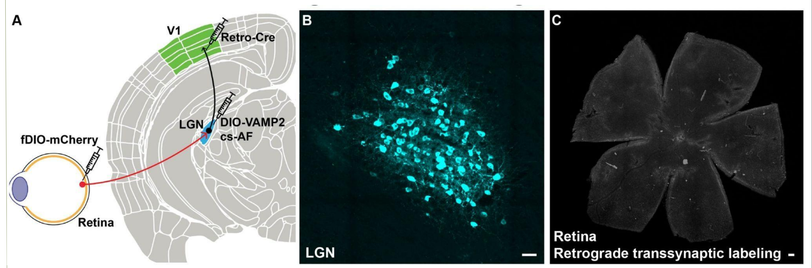


Figure 5. A. Schema for detecting retrograde labeling from the LGN to the retina. Retina projects to LGN, and LGN projects to V1. **B.** Cells in the LGN labeled retrogradely from V1. Scale bar 50 um. **C.** No labeled cells in the retina indicating no retrograde transsynaptic labeling. **D.** Schema for control experiment to confirm LGN cells receive input from retina. **E.** Abundant labeled in the retina indicate that the retina projects to the cells in LGN that are converted retrogradely from V1. Scale bar 250 µm.

Conclusion and Future Studies

We have developed an efficient and customizable method for transsynaptic tracing based on the AMPA.FingR, a recombinant antibody like protein that binds to the N-terminal extracellular domain of the GluA1 AMPA receptor. The method can be used to efficiently trace anterograde circuits monosynaptically, without detectable toxicity or retrograde labeling, from genetically determined excitatory neurons. Currently, no other method can trace anterograde circuits in this manner. The use of recombinases allows the expression of proteins such as GCAMP and channelrhodopsin in synaptic neurons, which allows for functional examination of brain circuits. This method is particularly useful as each individual component can be exchanged or upgraded as needed. This study tested this method on cultured neurons from the cerebral cortex and then in live mouse brains. Within the mouse brains it is clearly seen, when stained, that the circuits are being traced, defining how the starter cell project to other parts of the brain. A control experiment was also conducted to check for retrograde labeling using the retinal projection to the LGN. In the future, this template can be used to generate a modified tracer with improved performance and tracers that can perform specialized tasks such as tracing inhibitory circuits, tracing circuits from individual starter neurons, or mapping how circuits change over time. This will be exceeding helpful in learning to map the brain and understanding the circuits underlying specific behaviors.

References

1. Wickersham IR, et al. (2007) Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. Neuron 53(5):639-647
2. Zingg B, et al. (2017) AAV-Mediated ANterograde Transsynaptic Tagging: Mapping Corticocollicular Input-Defined Neural Pathways for Defense Behaviors. Neuron 93(1):33-47
3. Li E, et al. (2021) ANterograde transneuronal tracing and genetic control with engineered yellow fever vaccine YFV-17D. Nat Methods 18(12):1542-1551.
4. Lo L & Anderson DJ (2011) A Cre-dependent, anterograde transsynaptic viral tracer for mapping output pathways of genetically marked neurons. Neurons 72(6): 938-950.

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