

## Table S1. Tools for Neural Circuit Mapping

Selected techniques currently available for neural circuit mapping and covering a broad range of capabilities are summarized, with attention given both to major applications/advantages (particularly in terms of characterizing IODEs) and to major caveats. The terms IDE and ODE are defined only relative to the cell population of interest; hence transsynaptic markers are particularly useful in this regard for identifying the inputs to a genetically and anatomically specified starter cell population.

Method	Connectivity Information/ Tracing Directionality	Mechanism/ Marker	Properties Checklist						Species Compatibility	Major Applications/ Advantages	Major Caveats	References	
			Long-range Connections	Cell-type specificity	Trans-synaptic	Mono-synaptic Restricted	Single Cell Resolution	Synapse Visualization					
<b>Golgi staining</b>	Detailed local cell morphology	Silver precipitate						✓	widely compatible	<ul style="list-style-type: none"> <li>Complete neuronal morphology and fine structure (e.g. spines) are visible</li> <li>Sparse labeling allows single neurons to be distinguished</li> </ul>	<ul style="list-style-type: none"> <li>Only sparse labeling is useful</li> <li>Staining can only be applied to post-fixed samples</li> <li>Difficult to establish connectivity patterns (esp. long-range)</li> </ul>	Ranjan and Mallick, 2010 (modern updates)	
<b>Dyes</b>	<b>DIX Lipophilic Tracers</b>	Non-specific membrane tracing								<ul style="list-style-type: none"> <li>Compatible with tracing in post-fixed brains as well as with live tissue imaging</li> <li>Efficient transport via membrane diffusion</li> </ul>	<ul style="list-style-type: none"> <li>No cell type specificity</li> <li>No directional specificity for tracing</li> </ul>	Honig and Hume, 1989	
	<b>Dextran Amines</b>	Some anterograde vs retrograde specificity using different mW dextrans leading to preferential uptake by cell bodies vs axons	Fluorescent dye (variety of wavelengths available) incorporated into cell membranes							<ul style="list-style-type: none"> <li>Wide variety of marker conjugates</li> <li>Variety of MWs available to help achieve directional specificity</li> <li>Can be used to identify ODEs</li> </ul>	<ul style="list-style-type: none"> <li>No cell type specificity</li> </ul>	Reiner et al., 2000	
	<b>FluoroGold</b>	Retrograde (axonal uptake)	UV excitation gives gold or blue emission depending on pH	✓					✓ (sparse labeling can allow single cell tracing)	widely compatible	<ul style="list-style-type: none"> <li>Efficient uptake by axons</li> <li>Can be used to identify ODEs</li> <li>Visualization can be enhanced by immunostaining</li> <li>Compatible with EM for ultrastructural studies</li> </ul>	<ul style="list-style-type: none"> <li>No cell type specificity</li> <li>One color option</li> <li>High diffusibility can make local injections difficult</li> </ul>	Naumann et al., 2000
	<b>Retrobeads</b>	Retrograde (axonal uptake)	Latex microbeads used to deliver red or green fluorescent dyes								<ul style="list-style-type: none"> <li>Limited local spread of beads allows local connectivity mapping or very precise ODE tracing</li> <li>Beads are trafficked quickly, yet are non-toxic, allowing a very wide range of survival times post-injection</li> </ul>	<ul style="list-style-type: none"> <li>No cell type specificity</li> <li>Punctate appearance can make cell ID difficult</li> <li>No labeling of cell morphology</li> <li>Less efficient axonal uptake than other options (e.g. FluoroGold)</li> </ul>	Katz et al., 1984 Katz and Iarocci, 1990

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			Long-range Connections	Cell-type specificity	Trans-synaptic	Mono-synaptic Restricted	Single Cell Resolution	Synapse Visualization				
Tracer Proteins	HRP	Retrograde (axonal uptake)							widely compatible	<ul style="list-style-type: none"> <li>One component system</li> <li>Can be used to identify ODEs</li> </ul>	<ul style="list-style-type: none"> <li>No cell type specificity</li> <li>One staining option</li> </ul>	LaVail and LaVail, 1972
	WGA	Retrograde (transsynaptic) and anterograde (transsynaptic): specific in some contexts			✓					<ul style="list-style-type: none"> <li>Transsynaptic labeling highly efficient</li> <li>Can be used to identify IDE or ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Transsynaptic labeling is not highly efficient</li> <li>Direction of transsynaptic labeling can be mixed, variable, and circuit-dependent</li> </ul>	Schwab et al., 1978
	PHA-L	Anterograde (transsynaptic)	Fluorophore, biotin, HRP, cre or other marker conjugated to tracer protein	✓	✓ (optionally compatible with viral/genetic techniques for cell specificity)	✓		✓ (sparse expression can allow single cell tracing)		<ul style="list-style-type: none"> <li>Transsynaptic labeling highly efficient</li> <li>Can be used to identify IDEs</li> </ul>	<ul style="list-style-type: none"> <li>Transsynaptic labeling is not highly efficient</li> <li>Not strictly anterograde</li> </ul>	Gerfen and Sawchenko, 1984
	CtB	Retrograde (axonal uptake)								<ul style="list-style-type: none"> <li>Retrograde labeling</li> <li>Cell type specificity possible</li> <li>Can be used to identify ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Not strictly retrograde</li> </ul>	Conte et al., 2009
	TTC	Retrograde (transsynaptic)				✓				<ul style="list-style-type: none"> <li>Transsynaptic labeling highly efficient</li> <li>Can be used to identify IDE or ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Transsynaptic labeling is not highly efficient</li> <li>Not strictly retrograde</li> </ul>	Kissa et al., 2002
AAV	Anterograde (axon tracing) Can also be used to express transsynaptic markers	XFP or cre expressed by virus	✓	✓	✓ (if encoded virus is engineered to express a transsynaptic tracer protein)		✓ (sparse expression can allow single cell tracing)	mammals	<ul style="list-style-type: none"> <li>Versatile, relatively non-toxic package for delivery of numerous tracing components</li> <li>Allows cell type specificity using specific promoters or when combined with recombinase expression strategies</li> <li>Can be used to identify IDEs (e.g. when combined with transsynaptic tracer proteins) and/or ODEs (e.g. via axon tracing)</li> </ul>	<ul style="list-style-type: none"> <li>Packaging size limited to ~5 kB</li> <li>Inconsistent reports of retrograde transport, may require batch-by-batch characterization</li> </ul>	Betley and Sternson, 2011 (review) Wang et al., 2014 (comparison with BDA) Oh et al., 2014 (Allen Mouse Connectivity Atlas)	

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			Long-range Connections	Cell-type specificity	Trans-synaptic	Mono-synaptic Restricted	Single Cell Resolution	Synapse Visualization				
alpha-herpesviruses	HSV-1								mammals, some evidence for fish (see References)	<ul style="list-style-type: none"> <li>Efficient retrograde viral tracer</li> <li>Transsynaptic labeling</li> <li>Can be used to identify IDEs or ODEs (as in Fenno et al., 2014)</li> </ul>	<ul style="list-style-type: none"> <li>Toxicity</li> <li>Careful characterization required to assure that spread is restricted to synaptically connected cells</li> <li>Only particular strains are specifically retrograde</li> </ul>	Ugolini et al., 1987 Zemanick et al., 1991 (strain specificity) LaVail et al., 1997 (strain specificity) Fenno et al., 2014 (cell-type specific approaches) Zou et al., 2014 (fish)
	PRV Bartha	XFP or cre expressed by virus	✓	✓	✓	✓ (Ba2000 variant; see References)			non-primate mammals	<ul style="list-style-type: none"> <li>Efficient retrograde viral tracer</li> <li>Transsynaptic labeling</li> <li>Monosynaptic restriction may be possible</li> <li>Less toxic than other HSV strains</li> <li>Can be used to identify IDEs or ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Toxicity</li> </ul>	Enquist, 2002 Ekstrand et al., 2008 De Falco et al., 2001 (Ba2001 cell-type specific strain) Callaway, 2008 (see comment on Ba2000 for monosynaptic restriction)
	H129 strain	Anterograde (transsynaptic)							mammals	<ul style="list-style-type: none"> <li>Anterograde viral tracer</li> <li>Transsynaptic labeling</li> <li>Can be used to identify IDEs</li> </ul>	<ul style="list-style-type: none"> <li>Toxicity</li> </ul>	Sun et al., 1996 Lo and Anderson, 2011 (cre-dependent cell-type specificity)
VSV	Anterograde (transsynaptic) or retrograde (transsynaptic): Directionality is glycoprotein dependent	XFP label expressed by virus	✓	✓	✓	✓			widely compatible	<ul style="list-style-type: none"> <li>Anterograde viral tracer</li> <li>Transsynaptic labeling</li> <li>Can be used to identify IDEs</li> <li>Can be retracted to monosynaptic labeling using G deletion</li> <li>Cell type specificity using EnvA pseudotyping</li> </ul>	<ul style="list-style-type: none"> <li>Toxicity</li> <li>Poorly understood batch variability, requires careful batch-by-batch characterization (see Correction to Beier et al., 2011)</li> </ul>	Beier et al., 2011 Mundell et al., 2015
CAV	Retrograde (axon transducing)	cre or GFP expressed by virus	✓	✓					mammals	<ul style="list-style-type: none"> <li>Relatively non-toxic retrograde viral tracer. The lack of toxicity makes this virus particularly appealing for examining functional circuit elements in vivo.</li> <li>Can be used to identify ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Not transsynaptic</li> </ul>	Soudais et al., 2001 Junyent and Kremer, 2015 also see TRIO references

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			Long-range Connections	Cell-type specificity	Trans-synaptic	Mono-synaptic Restricted	Single Cell Resolution	Synapse Visualization				
<b>Rabies</b>	Retrograde (transsynaptic and axon transducing)	XFP label expressed by virus	✓	✓ (EnvA variants; see References)	✓	✓ (G-deleted variants; see References)			mammals	<ul style="list-style-type: none"> <li>Specific, efficient retrograde viral tracer</li> <li>Transsynaptic labeling</li> <li>Can be used to identify IDEs or ODEs</li> <li>Can be retracted to monosynaptic labeling using G deletion</li> <li>Cell type specificity using EnvA pseudotyping</li> </ul>	<ul style="list-style-type: none"> <li>Toxicity</li> <li>Down-regulation of host gene expression</li> </ul>	Wickersham et al., 2007 Callaway and Luo, 2015 (review)
<b>TRIO/cTRIO</b>	Retrograde (axon transducing; CAV) and Retrograde (transsynaptic; rabies): Allows three steps of a circuit to be examined	XFP label expressed by virus	✓	✓ (cTRIO variant; see References)	✓	✓			demonstrated in mice, likely compatible with other mammalian systems	<ul style="list-style-type: none"> <li>Same advantages of rabies (above)</li> <li>Specification of inputs based on output target, allowing visualization of the relationship between IDEs and ODEs (IODEs)</li> </ul>	<ul style="list-style-type: none"> <li>Toxicity</li> <li>Down-regulation of host gene expression</li> </ul>	Schwarz et al., 2015 Beier et al., 2015 Lerner et al., 2015
<b>GRASP/mGRASP</b>	Synaptic partners	Split GFP reconstituted at synapses	✓	✓				✓	currently optimized for worms (GRASP) and mammals (mGRASP)	<ul style="list-style-type: none"> <li>Synapse visualization from defined partners</li> <li>Can be used to further characterize the fine structure of IDEs or ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Possible bias for false positives in synapse detection</li> </ul>	Feinberg et al., 2008 Kim et al., 2011
<b>SynView</b>	Synaptic partners	Split GFP reconstituted at synapses	✓	✓				✓	currently optimized for mammals	<ul style="list-style-type: none"> <li>Synapse visualization from defined partners</li> <li>Can be used to further characterize the fine structure of IDEs or ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Currently limited to examining synaptic contacts initiated by specific adhesion molecules</li> </ul>	Tsetsenis et al., 2014
<b>Brainbow</b>	Anterograde (axon tracing)	Stochastic expression of 3 XFPs	✓	✓				✓	widely compatible - currently adapted for worms, flies, fish, mice	<ul style="list-style-type: none"> <li>Combination of single cell resolution and dense labeling is possible (up to 100s of colors)</li> <li>Can be used to identify ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Imaging is a major challenge (chromatic aberrations, bleaching, etc can make analysis difficult)</li> </ul>	Livet et al., 2007 Pan et al., 2011 (fish) Hampel et al., 2011 (flies) Hadjieconomou et al., 2011 (flies) Cai et al., 2013
<b>Electron Microscopy</b>	Ultrastructural cell morphology	HRP/Diaminobenzidine (DAB) reaction, electron-dense membrane contrast agents, and/or heavy metal-conjugated antibody labeling	✓ (can be combined with long-range techniques e.g. FluoroGold, GESEM)	✓ (limited multifeature immunostaining)				✓ (synapses can be identified by morphology and/or limited immunostaining)	widely compatible	<ul style="list-style-type: none"> <li>The most complete picture of neuronal morphology and circuit structure is obtained</li> <li>Can be used to identify or further characterize the fine structure of IDEs or ODEs</li> </ul>	<ul style="list-style-type: none"> <li>Extensive time and cost, even for imaging very small tissue volumes</li> </ul>	Jurrus et al., 2009 Kleinfeld et al., 2011 Ward et al., 1975 Bock et al., 2011 Briggman et al., 2011 Atasoy et al., 2014 (GESEM)

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<b>Functional Magnetic Resonance Imaging (fMRI)</b>	Functional connectivity	BOLD signal (correlation)	(inference by correlation, need not be direct)						Human, non-human primate, rodent	<ul style="list-style-type: none"> <li>• Whole brain functional connectivity visible in a live subject</li> <li>• Non-invasive, compatible with human studies</li> </ul>	<ul style="list-style-type: none"> <li>• Indirect (non-anatomical) measure of connectivity precludes IODE identification</li> <li>• No cell type specificity</li> </ul>	Friston, 2011 (functional and effective connectivity review) Poldrack and Farah, 2015 (recent review of human imaging methods, with a focus on fMRI)
<b>Diffusion Weighted Imaging (DWI)</b>	White matter tract structure	Visualization of water diffusion preferentially along white matter tracts	(inference by diffusion, need not be direct)						Human, non-human primate, rodent	<ul style="list-style-type: none"> <li>• Whole brain structural pathways visible in a live subject</li> <li>• Non-invasive, compatible with human studies</li> </ul>	<ul style="list-style-type: none"> <li>• Resolution limited to large white matter tracts</li> <li>• No functional information</li> <li>• No cell type specificity</li> </ul>	Le Bihan and Johansen-Berg, 2012

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