Table S3. Activity Readouts for Functional Neural Circuit Analysis

Selected techniques currently available for achieving brain activity readouts and covering a broad range of capabilities are summarized. Three main categories are listed: electrophysiological, optical, and immediate early gene (IEG)-based. We also list fMRI as an important method for achieving whole-brain activity readouts, especially given compatibility with small mammals and optogenetics. For recent discussion of other activity readouts available for use in humans, beyond the scope of this review, see Poldrack and Farah (2015).

<table>
<thead>
<tr>
<th>Method</th>
<th>Species Compatibility</th>
<th>Compatibility with Awake Behavior</th>
<th>Major Applications/ Advantages</th>
<th>Major Caveats</th>
<th>References</th>
</tr>
</thead>
</table>
| Whole-cell in slice              | primarily mice, rats  | not compatible                     | • Experimenter control over ion concentrations  
• Easily controlled pharmacological manipulation  
• Intracellular access  
• Single cell resolution | • No behavioral context  
• Full circuits and circuit dynamics may not be preserved in slice | Walz et al., 2002 (Neuromethods textbook) |
| Whole-cell in vivo               | widely compatible     | compatible                          | • Intracellular access in an intact circuit  
• Intracellular access during behavior  
• Single cell resolution | • Low throughput, technically demanding approach  
• Not currently compatible with behavior over days | Lee et al., 2006  
Kitamura et al., 2008 ("shadow patching" of unlabeled cells)  
Kodandaramaiah et al., 2012 (automation)  
Munoz et al., 2014 (channelrhodopsin-assisted cell targeting) |
| Extracellular in vivo            | widely compatible     | compatible                          | • Well-established method for monitoring neuronal activity during free behavior  
• Excellent temporal resolution  
• Multi- or single-unit recordings  
• Action potential collision tests can be used to establish projection targets | • Cell type identification (e.g. using juxtascellular labeling) is low throughput  
• Biased towards isolating active cells | Chorev et al., 2009 (review)  
Lipski et al., 1981 (collision testing) |
| Extracellular in vivo with optotagging | mice              | compatible                          | • Combines a well-established method for monitoring neuronal activity with a potentially higher throughput method of cell type identification | • Although cell type identification is higher throughput than juxtascellular labeling, it can be difficult to definitively ID cells. Arbitrary cutoffs are often employed. | Lima et al., 2009  
Cardin et al., 2010 |
| Voltage imaging                  | flies, mice          | not yet tested                      | • An optical readout of neuronal activity that permits single cell resolution from many, even densely packed cells  
• Good temporal resolution  
• Access to subthreshold membrane voltage dynamics  
• Compatible with in vivo or slice preparations | • Sensors are still largely under development | Gong et al., 2015 (recent indicator improvement)  
St.-Pierre et al., 2014 (recent indicator improvement)  
Knopfel, 2012 (indicator review)  
Hamel et al., 2015 (recent brain imaging review) |
| Calcium imaging                  | widely compatible     | compatible                          | • An optical readout of neuronal activity that permits single cell resolution from many, even densely packed cells  
• Good temporal resolution  
• Access to subthreshold membrane voltage dynamics  
• High signal-to-noise sensors available in green and red | • No access to subthreshold membrane voltage dynamics  
• Relatively slow kinetics compared to electrophysiology | Hamel et al., 2015 (recent brain imaging review) |
| Fiber photometry                 | mice, rats           | compatible                          | • An optical readout of neuronal activity from a genetically defined population of neurons  
• An easy-to-implement technique that is highly comptable with freely moving behavior  
• Compatible with any optical indicator | • Lack of single cell resolution | Lutcke et al., 2010  
Schulz et al., 2012  
Cui et al., 2013  
Gusain et al., 2014 (deep brain axonal signals relevant to ODEs)  
Lerner et al., 2015 (isosbestic control excitation wavelength)  
Guo et al., 2015  
Kim et al., 2016  
Zalocusky et al., 2016 (rat) |
| IEG histology                    | widely compatible     | compatible                          | • Allows a broad readout of recently activated neurons  
• Access to subthreshold membrane voltage dynamics  
• In vivo electrophysiology  
• Cell type identification (e.g. using juxtascellular labeling) is low throughput  
• Biased towards isolating active cells | • Poor temporal resolution (hours)  
• Post-mortem fixed tissue readout | Guzowski et al., 2005 (review) |
| IEG transgenic reporters (Fos-GFP, Arc-GFP) | mice          | compatible                          | • Allows a broad readout of recently activated neurons  
• Access to subthreshold membrane voltage dynamics  
• In vivo electrophysiology  
• Cell type identification (e.g. using juxtascellular labeling) is low throughput  
• Biased towards isolating active cells | • Poor temporal resolution (hours) | Barth et al., 2007 (review) |
| TRAP (FosTRAP, ArcTRAP)           | mice                 | compatible                          | • Allows a broad readout of recently activated neurons  
• Access to subthreshold membrane voltage dynamics  
• In vivo electrophysiology  
• Readout occurs during a chemically-defined window  
• Whole brain readout visible in a live subject | • Poor temporal resolution (hours) | Guenthner et al., 2013 |
| fMRI                             | Human, non-human primate, rodent | compatible with awake, but still, subjects | • Non-invasive, compatible with human studies  
• In non-human studies, can be combined with optogenetic manipulation (ofMRI) | • Poor temporal resolution (seconds)  
• Lack of single cell resolution | Poldrack and Farah, 2015 (review)  
Lee et al., 2010 (ofMRI) |
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