Protocol: Live-imaging of axonal cargoes in human iPSC-derived neurons or mouse primary neurons

Dan Dou[1,2], C. Alexander Boecker[3], Erika L.F. Holzbaur[1,2]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; [2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA; [3]Department of Neurology, University Medical Center Goettingen, 37077 Goettingen, Germany

Key words

iPSC, iNeuron, live-imaging, axon, confocal

Abstract

Here, we describe procedure and equipment used for live-imaging of axonal cargoes. This was performed both using primary mouse cortical neurons and human iPSC-derived excitatory glutamatergic neurons. Equipment and software used varied based on laboratory site and scheduled upgrades to microscopy equipment during the course of this study.

Reagents

- Hibernate E low fluorescence media (CATALOG)
- GlutaMAX (Thermo Fisher, Cat# 35050061)
- B-27 supplement (Thermo Fisher, Cat# 17504044)
- Hibernate A low fluorescence media (BrainBits, Cat# HALF)
- NT-3 (Pepro Tech, Cat# 450-03)
- BDNF (Pepro Tech, Cat# 450-02)

Equipment

- Heated environmental imaging chamber (37°C)
- Spinning disk confocal microscope (see Materials and Methods for specific systems and cameras used)
- 60x 1.40 NA oil immersion objective
- VisiView software

Safety warnings

Investigators should be trained and familiar with the confocal microscope to avoid eye damage from lasers.

Steps

1. See “Protocol: Primary neuron culture for live-imaging of axonal cargoes” and “Protocol: Culture and transfection of iPSC-derived neurons for live-imaging of

axonal cargoes" for plating and transfection instructions. Primary mouse cortical neurons were imaged on DIV7. Human iNeurons were imaged on DIV21.

2. Replace culture media with low fluorescence imaging media. For primary mouse neurons, Hibernate E medium supplemented with 2% B-27 and 2 mM GlutaMAX was used. For iNeurons, Hibernate A medium supplemented with 10 ng/mL BDNF, 10 ng/mL NT-3, and 2% B-27 was used.
3. Image using spinning disk confocal microscope under 60x magnification (oil immersion objective). See "Materials and Methods" for specific microscopes and cameras used.
4. Identify axons of transfected neurons based on morphological parameters. (Boecker et al., 2020; Kaech and Banker, 2006). For example, axons can most reliably be identified by their length and should span over at least 500 μm.
5. Acquire time lapse recordings at a frame rate of 1 frame per second for 5 minutes. Time lapses were taken in the mid-axon, defined as >300 μm from the soma and > 100 μm from the distal axon terminal. Knowledge of the pixel/micron ratio for the specific objective and camera being used is necessary for accurately measuring these distances.

Citations

Boecker, C.A., Olenick, M.A., Gallagher, E.R., Ward, M.E., and Holzbaur, E.L.F. (2020). ToolBox: Live Imaging of intracellular organelle transport in induced pluripotent stem cell-derived neurons. Traffic *21*, 138–155.

Kaech, S., and Banker, G. (2006). Culturing hippocampal neurons. Nat. Protoc. *1*, 2406–2415.