Investigation_of_mitophagy_in_Hippo_neurons

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ABSTRACT

We developed a method for assessing mitochondrial clearance in primary hippocampal neurons.

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Plating and maintenance of hippocampal neurons 2d

1 Plate primary hippocampal Sprague Dawley rat neurons (embryonic day 18) on 35 mm glass bottomed dishes at 250,00 cells per dish in MEM supplemented with 10% horse serum, 33 mM D-glucose, and 1 mM sodium pyruvate.

1.1 Precoat dishes with 0.5 mg/mL poly-L-lysine.

2 Incubate neurons for at least 2 but no more than 5 hours at 37 degrees C, 5% CO2.

3 Replace media with Neurobasal (Gibco) supplemented with 33 mM D-glucose, 2 mM GlutaMAX (Invitrogen), 100 units/ml penicillin, 100 ug/ml streptomycin, and 2% B-27 (ThermoFisher) (Maintenance Media; MM) and continue to

maintain at 37 degrees C, 5% CO2.

4 Add AraC (5 uM) one day post-plating to prevent glial proliferation

Transfection of primary neurons 4d

5 Transfect neurons 5 days after initial plating. 4d

5.1 Simultaneously transfect 0.8-1.2 ug total plasmid per 35-mm dish and 45 pmol siRNA. Use Lipofectamine 2000 Transfection Reagent (ThermoFisher).

5.2 Incubate transfected neurons 36-48 hours.

Fluorescent labeling in neurons

6 For Halo and/or SNAP tagged expressed proteins, incubate neurons in 100 nM Halo and/or 2 uM SNAP ligand of preferred colors for 30 min. Wash 2X and rest 30 min in conditioned media.

6.1 To measure mitochondrial membrane potential, incubate neurons in 2.5 nM TMRE for 30 min

Mitophagy induction in neurons 2h

7 Replace neuron media with MM containing 3 nM Antimycin A (or, for control conditions, media containing no small molecule inhibitor) and incubate for 2 hours. 2h

Imaging neurons

8 Replace MM with HibernateE (Brain Bits) supplemented with 2% B27 and 33 mM D-glucose and rest at least 15 min in imaging chamber of microscope.

8.1 Add Antimycin A to HibE media for treated conditions. Add TMRE to HibE media for mitochondrial potential measurements.

9 Image neurons, for example, with a Nikon Eclipse Ti Microscope with a 100X objective (Apochromat, 1.49-N.A. oil immersion) and an UltraView Vox spinning disk confocal system (PerkinElmer). Collect Z-stacks at 0.15 nm/step with Volocity acquisition software (PerkinElmer).

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