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Fluorescence recovery after photobleaching (FRAP) in neuro

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We use this protocol and it's working

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Abstract

This protocol describes a fluorescence recovery after photobleaching (FRAP) assay used to measure the mobility of α-synuclein in synaptic boutons of cultured hippocampal neurons.

Materials

Reagents

- **Tyrode's buffer:** 119 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 30 mM glucose, 25 mM HEPES, pH 7.4
- D-AP5 (ab120271, abcam)
- CNQX (ab120044, abcam)

Consumables

- Glass-bottom dishes (P35G-1.5-10-C, MatTek)

Equipment

- Nikon CSU-W1 spinning disk confocal microscope with Andor DU-888 EMCCD camera
- Rapp programmable illumination system
- 60× oil objective (PlanApo λ, NA 1.40)
- Temperature and CO_2 controller (37°C, 5% CO_2)

Neuron preparation and expression of α-synuclein-GFP in hippocampal neurons

1 Culture synuclein triple-knockout hippocampal neurons on MatTek glass-bottom dishes.

2 At DIV7, transduce neurons with lentivirus encoding human α-synuclein-GFP (α-syn-GFP).

3 Maintain the neurons for 14–18 days after transduction.

Photobleaching

4 Calibration using a calibration solution (0.01 mg/ml fluorescein for photobleaching calibration). This step is crucial for obtaining consistent results. Ensure you use the same type of glass-bottom dish that will be used for the actual experiment. Optimize the laser focus to ensure a sharp photobleaching spot, thereby avoiding unintended bleaching of adjacent synaptic boutons.

5 Replace culture medium with Tyrode's buffer with D-AP5 (2-amino-5-phosphonopentanoic acid) and CNQX before imaging.

6 Place the dish on the microscope stage and maintain 37°C with 5% CO_2 throughout imaging.
- Wait for 5-10 minutes until the temperature stabilizes to avoid focal drift during image acquisition.

7 Identify α-syn-GFP–positive synaptic boutons using the spinning disk confocal microscope.

8 Select a single-pixel ROI within a synaptic bouton for bleaching.

9 Photobleach the ROI using a 405 nm laser at 30 mW for 50 ms (no repeat). Immediately begin acquiring fluorescence recovery using 488 nm excitation (5% laser power, 200 ms exposure with an EM gain of 200) for 100 frames (no interval) over 20 seconds using the Andor DU-888 EMCCD camera and Rapp illumination system.

Data analysis

10 Using Fiji (NIH), select the bleached synaptic bouton as an ROI and measure the 488 nm fluorescence intensity using the Time Series Analyzer V3 plugin.

11 Normalize intensity values as follows: Pre-bleach fluorescence = 100%, Post-bleach fluorescence = 0%
Generate fluorescence recovery curves and fit the first 10 s of data with a one-phase exponential model in GraphPad Prism to calculate the recovery time constant (τ).
Determine the extent of fluorescence recovery at 20 s after photobleaching to quantify the recovery rate.

Protocol references

Fortin, D.L.*et al.* Neural activity controls the synaptic accumulation of alpha-synuclein. *J Neurosci* **25**, 10913-10921 (2005).