SypHy imaging and dextran uptake assays from Yao CK et al. (2017)

Chi-Kuang Yao, Yu-Tzu Liu, I-Chi Lee, You-Tung Wang, Ping-Yen Wu

Abstract

This protocol is from 'Flower Ca^{2+} channel in CME and ADBE' of Yao CK et al.

Please see the manuscript for the full method details.

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Guidelines

Experiments were performed at either room or physiological temperatures controlled by Warner temperature controller (TC-344B).

Before start

You'll need:

chamber (Warner instruments RC-25F) with two parallel platinum wires separated by 5 mm

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imaging buffer:

- 136 mM NaCl
- 2.5 mM KCl
- 2 mM $CaCl_2$
- 1.3 mM $MgCl_2$
- 10 mM glucose
- 10 mM HEPES [pH 7.4]
- 10 mM CNQX
- 50 mM AP-5

acidic buffer:

- 136 mM NaCl
- 2.5 mM KCl
- 2 mM $CaCl_2$
- 1.3 mM $MgCl_2$
- 10 mM glucose
- 10 mM 2-[N-morpholino] ethane sulphonic acid [pH 5.5]
- 10 mM CNQX
- 50 mM AP-5

Acidic buffer was prepared by replacing HEPES in the imaging buffer with 2-[N-morpholino] ethane sulphonic acid [47,48].

imaging solution:

- 144 mM NaCl
- 2.5 mM KCl
- 2.5 mM $CaCl_2$
- 2.5 mM $MgCl_2$
- 10 mM HEPES (pH 7.5)
- 10 mM CNQX
- 50 mM AP-5)

Materials

- imaging buffer by Contributed by users
- acidic buffer by Contributed by users
- imaging solution by Contributed by users
- pSpCas9(BB) by Contributed by users
- pCMV-SyphyA4 #24478 by addgene
- 50 µM 40 kDa TMR-dextran (Invitrogen) by Contributed by users

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Protocol

SypHy imaging

Step 1.

Transfect DIV7 cultured rat hippocampal neurons cultured on 12-well plate with pSpCas9(BB) and pCMV-SyphyA4 (addgene#24478) plasmids [5] by Ca^{2+} phosphate method.

SypHy imaging

Step 2.

Bath DIV13-15 neurons in the imaging buffer in a chamber (Warner instruments RC-25F) with two parallel platinum wires separated by 5 mm.

SypHy imaging

Step 3.

Elicit SV exocytosis with a train of 200 action potentials delivered with 20-Hz electric field stimulation (50 mA, 1-ms pulse width).

NOTES

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See "before start" for the imaging buffer.

SypHy imaging

Step 4.

Capture single images every 1 s using MetaMorph software and ANDOR iXon 897 camera.

SV re-acidification

Step 5.

For SV re-acidification experiments, perfuse the imaging chamber with the imaging buffer.

SV re-acidification

Step 6.

Perfuse with an acidic buffer (see 'Before start' for exact composition).

SV re-acidification

Step 7.

Perfuse the imaging buffer to allow surface SypHy to be fluorescent.

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Maintain experimental temperatures at physiological temperatures.

Counting

Step 8.

Count the final SypHy fluorescence intensity in the presynaptic terminals by subtracting the background fluorescence intensity on the surrounding coverslip from the SypHy fluorescence intensity within presynaptic terminals.

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Each data value was obtained from a single terminal.

40 kDa TMR-dextran uptake assays

Step 9.

For 40 kDa TMR-dextran uptake assays, stimulate DIV13-15 neurons transfected with pSpCas9(BB) plasmids by a train of 1600 action potentials delivered with a 80 Hz electric field stimulation (50 mA, 1-ms pulse width) in the imaging solution in the presence of 50 µM 40 kDa TMR-dextran (Invitrogen).

40 kDa TMR-dextran uptake assays

Step 10.

Perfuse neurons with the same buffer for 5 min to remove excess dextran dye.

DURATION

00:05:00

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Experiments were performed at room temperature. Imaging was achieved through MetaMorph software and ANDOR iXon 897 camera. Image processing was achieved using Image J and LSM Zen.

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