TEM from Yao CK et al. (2017)

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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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Before start

You'll need:

0 mM Ca^{2+} hemolymph-like (HL)-3 solution:

- 70 mM NaCl
- 5 mM KCl
- 10 mM $MgCl_2$
- 10 mM $NaHCO_3$
- 5 mM trehalose
- 5 mM HEPES (pH 7.2)
- 115 mM sucrose

90 mM K^+/0.5 mM Ca^{2+} stimulation:

(or alternative 60mM K^+/1mM Ca^{2+})

- 25 mM NaCl
- 90 mM KCl
- 10 mM $MgCl_2$
- 10 mM $NaHCO_3$
- 5 mM trehalose
- 5 mM HEPES (pH 7.2)

- 30 mM sucrose
- 0.5 mM $CaCl_2$

Materials

- propylene by Contributed by users
- resin by Contributed by users
- ethanol by Contributed by users
- 0 mM Ca2 hemolymph-like (HL)-3 solution by Contributed by users
- 90 mM K /0.5 mM Ca2 stimulation by Contributed by users
- 4% paraformaldehyde/1% glutaraldehyde/0.1 M cacodylic acid (pH 7.2) solution by Contributed by users
- 0.1 M cacodylic acid (pH 7.2) solution by Contributed by users
- 1% OsO4/0.1 M cacodylic acid solution by Contributed by users

Protocol

Step 1.

Dissect larval fillets in 0 mM Ca^{2+} HL-3 solution at room temperature.

Step 2.

To trigger CME, stimulate samples with 90 mM K^+/0.5 mM Ca^{2+} HL-3 solution for 1 min or 60 mM K^+/1 mM Ca^{2+} HL-3 solution for 10 min.

DURATION
00:10:00

Step 3.

To induce ADBE, subject larval fillets to stimulation of a 90 mM K^+/2 mM or 5 mM Ca^{2+} HL-3 solution in the presence or lack of 10 mM La^{3+} for 10 min.

DURATION
00:10:00

Step 4.

Rinse the samples with 0 mM Ca^{2+} HL-3 solution. (1/3)

Step 5.

Rinse the samples with 0 mM Ca^{2+} HL-3 solution. (2/3)

Step 6.

Rinse the samples with 0 mM Ca^{2+} HL-3 solution. (3/3)

Step 7.

Fix further at least for 12 h in 4% paraformaldehyde/1% glutaraldehyde/0.1 M cacodylic acid (pH 7.2) solution

DURATION
12:00:00

Step 8.

Rinse the samples with 0.1 M cacodylic acid (pH 7.2) solution.

Step 9.

Fix the samples in 1% OsO4/0.1 M cacodylic acid solution at room temperature for 3 h.

DURATION
03:00:00

Step 10.

Subject the samples to a series of dehydration from 30 to 100% ethanol.

Step 11.

After 100% ethanol dehydration, incubate the samples in propylene, mixture of propylene and resin and pure resin.

Step 12.

Lastly, embed them in 100% resin.

Step 13.

Capture the images of type Ib boutons using Tecnai G2 Spirit TWIN (FEI Company) and Gatan CCD Camera (794.10.BP2MultiScanTM) at ³ 4,400 x magnifications.

Step 14.

Count the size of SVs and bulk cisternae and the area of type Ib boutons using Image J.

Step 15.

Identify type Ib boutons by multiple layers of subsynaptic reticulum.

NOTES

Chi-Kuang Yao 29 Mar 2017

The radius of bulk cisternae is calculated from A(area)=πr^2. Isolated membranous structures larger than 80 nm in diameter were defined as bulk cisternae.