Ca2+ imaging (GCaMP6f) 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.

Citation: Chi-Kuang Yao, Yu-Tzu Liu, I-Chi Lee, You-Tung Wang, Ping-Yen Wu Ca2+ imaging (GCaMP6f) from Yao CK et al. (2017). **protocols.io**
dx.doi.org/10.17504/protocols.io.hg5b3y6
Published: 04 Apr 2017

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

2 mM Ca^{2+}/5 mM K^+/7 mM glutamate 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
- 2 mM $CaCl_2$
- 7 mM Monosodium glutamate

4 µM Fluo-4 AM (Invitrogen)/100 mM Ca^{2+}/HL-3 solution to be loaded with Fluo-4 AM dye

Materials

✓ 0 mM Ca2 hemolymph-like (HL)-3 solution by Contributed by users

✓ 2 mM Ca2 /5 mM K /7 mM glutamate solution by Contributed by users

✓ 4 µM Fluo-4 AM (Invitrogen)/100 mM Ca2 /HL-3 solution to be loaded with Fluo-4 AM dye by Contributed by users

Protocol

Step 1.

Dissect the third instar larvae in 0 mM Ca^{2+} HL-3 at room temperature.

Step 2.

Incubate in 2 mM Ca^{2+}/5 mM K^+/7 mM glutamate solution for 5 min.

DURATION
00:05:00

NOTES
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Glutamate treatment desensitizes postsynaptic glutamate receptors, thus reducing muscle contraction upon stimulation.

Step 3.

Measure GCaMP6f fluorescence to indicate the resting Ca^{2+} levels.

Step 4.

To image GCaMP6f in high K^+ stimulations, stimulate larval fillets subsequently with 90 mM K^+/2 mM Ca^{2+}/7 mM glutamate solution for 10 minutes.

DURATION
00:10:00

NOTES
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High K^+ and Ca^{2+} lead to bulk Ca^{2+} influxes into the muscles and cause dramatic contractions.

Step 5.

Manually focus the boutons and simultaneously image in the 6^{th} and 10^{th} min every one second.

Step 6.

After 10-min stimulation, rinse larval fillets with 2 mM Ca^{2+}/5 mM K^+/7 mM glutamate solution.

Step 7.

Image again.

Step 8.

Similarly, focus manually the boutons subjected to 1-min 90 mM K^+/0.5 mM Ca^{2+}/7 mM glutamate stimulation and image at the 30^{th} to 60^{th} s every one second.

NOTES
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All images were captured from the muscles 6 and 7 of the abdominal segment 3. Each larva was only used for one recording. The images of clearly focused boutons were further used for data quantifications.

Step 9.

Count the GCaMP6f fluorescence intensity in type Ib boutons and surrounding muscles (served as the fluorescence background).

Step 10.

Calculate final GCaMP6f fluorescence intensity by subtracting background fluorescence intensity in surrounding muscles from GCaMP6f fluorescence intensity in boutons.

NOTES
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The GCaMP6f fluorescence intensity of at least 10 type Ib boutons from the same muscles 6 and 7 at given time period was averaged to obtain each data value.

Electric stimulation

Step 11.

Dissect the third instar larvae in 0 mM Ca^{2+} HL-3 at room temperature.

Electric stimulation

Step 12.

Incubate in 2 mM Ca^{2+}/5 mM K^{+}/7 mM glutamate solution for 5 min.

DURATION
00:05:00

Electric stimulation

Step 13.

Aspirate larval axonal bundle and deliver with 10-40 Hz stimulations via a glass capillary electrode.

NOTES
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The stimulus was fixed at 5 mV and 0.5 ms duration by pClamp 10.6 software (Axon Instruments Inc).

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The images were captured every 2 s using MetaMorph software and ANDOR iXon3 897 camera. All images were captured from the muscles 6 and 7 of abdominal segment 3. Each larva was only used for one recording.

Electric stimulation

Step 14.

Count the GCaMP6f fluorescence intensity in type Ib boutons and surrounding muscles (served as the fluorescence background).

Electric stimulation

Step 15.

Calculate final GCaMP6f fluorescence intensity by subtracting background fluorescence intensity in surrounding muscles from GCaMP6f fluorescence intensity in boutons.

NOTES
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The GCaMP6f fluorescence intensity of at least 5 type Ib boutons from the same muscles 6 and 7 at given time period was averaged to obtain each data value. Images processing was achieved using Image J and LSM Zen.