Electrophysiology 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.

Citation: Chi-Kuang Yao, Yu-Tzu Liu, I-Chi Lee, You-Tung Wang, Ping-Yen Wu Electrophysiology from Yao CK et al. (2017). **protocols.io**
dx.doi.org/10.17504/protocols.io.hg7b3zn
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

Materials

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

- Axoclamp 900A amplifier (Axon Instruments, Foster City, CA) by Contributed by users
- pClamp 10.6 software (Axon Instruments) by Contributed by users

Protocol

Step 1.

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

Step 2.

Bath in 1 mM Ca^{2+} HL-3 solution for 5-10 min before the recording.

DURATION
00:10:00

NOTES
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The mean value of resistance of recording electrode is ~ 40 MΩ when the electrode is filled with a 3M KCl solution.

All recordings were obtained from muscle 6 of abdominal segment 3. One larva was only used for one recording.

Step 3.

Use recordings from the muscles that hold resting membrane potentials at < - 60 mV for further data quantifications.

Step 4.

Evoke EJPs by stimulating axonal bundle via a glass capillary electrode with an internal diameter of 10-15 mm (Harvard apparatus Glass Capillaries GC120F-15) at 0.2 Hz.

Step 5.

Fix stimulus pulses at 0.5 ms duration (pClamp 10.6 software, Axon Instruments Inc).

Step 6.

Apply 3-5 mV electric stimuli to obtain maximal EJP amplitude.

NOTES
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EJPs were amplified with an Axoclamp 900A amplifier (Axon Instruments, Foster City, CA) under bridge mode, filtered at 10 kHz. EJPs were analyzed by pClamp 10.6 software (Axon Instruments).

For the EJP amplitude at 0.2 Hz, the mean of the EJP amplitude was averaged from the amplitudes of 80 EJPs in one consecutive recording.