Whole Cell PatchClamp Electrophysiology in Opsin-Expressing Brain Slice with Visible Lights or UCNPs

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Method Article

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Abstract

It describes the flow of whole cell patch-clamp electrophysiology in mice brain slice.

Introduction

Reagents

1.Artificial cerebrospinal fluid (ACSF) : 119 mM sodium chloride, 2.5 mM potassium chloride, 26 mM sodium bicarbonate, 12.5 mM D-glucose, 5 mM HEPES, 2 mM calcium chloride, 2 mM magnesium chloride and 1.25 mM sodium phosphate monobasic monohydrate (pH 7.3).

2.Internal solution : 105 mM potassium gluconate, 5 mM potassium chloride, 0.5 mM calcium chloride, 2 mM magnesium chloride, 5 mM ethylene glycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid, 2 mM adenosine 5'-triphosphate magnesium salt, 0.5 mM guanosine 5'-triphosphate sodium salt hydrate,7 mM phosphocreatine disodium salt hydrate and10.0 mM HEPES. pH 7.2, osmolarity 280 mOsm.

Equipment

Sutter P97, X-cite Illuminator, Multiclamp 700B amplifier, Digidata 1440 digitizer

Procedure

1. Mouse was anesthetized with isoflurane.

2. Mouse was perfused with 4℃ ACSF.

3. Brain was cut and immersed in uniform ice-ACSF mixtures.

4. Brain was dissected, dehydrated and glued on to a plate as soon as possible.

5. Brain was transferred into uniform ice-ACSF mixtures bubbled with carbogen (95% oxygen and 5% carbon dioxide).

6. 250-um-thick slices were obtained and transferred into 37℃ ACSF.

7. Thick-walled borosilicate glass (Sutter BF150-86-10) electrodes were pulled (Sutter P97) to a resistance of 5-7 MΩ.

8. Fill the electrodes with internal solution.

9. Electrode was moved to approach the neuron.

10. The neuron was patched.

11. For visible light, it was filtered to 470 nm, 546 nm and 665 nm through microscope. Light power was controlled through X-cite Illuminators and light pulse was adjust to 1s.

12. For NIR lasers (808 nm, 980 nm and 1532 nm) , they were delivered through Optical fiber.

13. UCNPs illuminant were fixed in the form of transparent polymethylpropanamide (PMMA) film.

14. Specifically to preparation, 1 mL UCNPs stock solution were re-dispersed to 4 mL chloroform with With 0.40 g dissolved PMMA. The solid UCNP film was then obtained by dripping solution on the cover glass and completely evaporating the chloroform solvent.

15. The brain slice was placed onto the UCNP film.

16. The activity was recorded.