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CALCIUM IMAGING PROTOCOL

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We use this protocol and it's working

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Abstract

This protocol details Ca2+ imaging to analyze activity in astrocytes of the stratum radiatum of the CA1 region of the mouse hippocampus.

Attachments

PDF

Calcium Imaging Prot...

172KB

1 Locate Ca^{2+} levels in astrocytes in the stratum radiatum of the CA1 region of the hippocampus are monitored using the Ca^{2+} indicator fluo-4.

2 Load Astrocytes with the dye by incubating the slices with fluo-4-AM ([M] 2 micromolar (µM) and 0.01% of pluronic) for 00:45:00 - 01:00:00 at Room temperature (Araque et al. 2002; Kang et al. 1998; Nemani et al. 2010; Nett et al. 2002; Navarrete and Araque 2008). 1h 45m

3 This protocol has been proven to be suitable for measuring the calcium activity of astrocytes (Nanclares et al. 2023).

4 Briefly, fluo-4-positive cells present typical electrophysiological properties for astrocytes, this is, low input resistance, a quasi-linear voltage–current relationship under voltageclamped conditions, and absence of action potentials.

5 On the contrary, recordings from fluo-4-negative cells in the stratum radiatum exhibit typical neuronal characteristics, such as higher input resistances, a non-linear voltage–current curve, and firing of action potentials.

6 Additionally, incubate the slices with fluo-4 and sulforhodamine 101 (SR101), a widely used astrocyte marker known to label astrocytes in the hippocampus (Schnell, Hagos, and Hülsmann 2012), provide good co-localization between SR101 and fluo-4-positive cells (Nanclares et al. 2023).

7 Perform the recordings in the presence of a cocktail of neurotransmitter receptor antagonists to minimize neuronal contribution to astrocyte Ca^{2+} activity (CNQX [M] 20 micromolar (µM) , AP5 [M] 50 micromolar (µM) , MPEP [M] 50 micromolar (µM) , LY367385 [M] 100 micromolar (µM) , picrotoxin [M] 50 micromolar (µM) , CGP54626 [M] 1 micromolar (µM) , atropine [M] 50 micromolar (µM) , CPT [M] 2 micromolar (µM) , suramin [M] 100 micromolar (µM) , flupenthixol [M] 30 micromolar (µM) , AM251 [M] 2 micromolar (µM) and TTX [M] 1 micromolar (µM) to block glutamatergic, GABAergic, cholinergic, purinergic, endocannabinoid and dopaminergic transmission).

8 Image Astrocytes using a custom-made confocal microscope (Thorlabs).

9 Obtain the videos at 512 × 512 resolution with a sampling interval of 00:00:01 . 1s

10 Use ImageJ software (NIH) for quantitative fluorescence measurements.

11 Estimate Ca^{2+} variations as changes in the fluorescence signal over baseline (DF/F0), and cells are considered to show a Ca^{2+} event when DF/F0 increases two times the standard deviation of the baseline.

12 Quantify the astrocyte Ca^{2+} signal as the number of Ca^{2+} events per min within 00:10:00 of recording. 10m

13 Obtain Astrocyte Ca^{2+} events from 5 to 24 astrocytes in the field of view during the recording.

Protocol references

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