May 21, 2024

Whole-cell patch-clamp in vitro

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.n2bvjn84pgk5/v1

Nicola Biagio Mercuri[1]

[1]Fondazione Santa Lucia IRCSS

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Nicola Biagio Mercuri
Fondazione Santa Lucia IRCSS

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.n2bvjn84pgk5/v1

Protocol Citation: Nicola Biagio Mercuri 2024. Whole-cell patch-clamp in vitro. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvjn84pgk5/v1

Protocol status: Working

Created: May 20, 2024

Last Modified: May 21, 2024

Protocol Integer ID: 100139

Abstract

Evoked firing of single neurons

Patch-clamp recordings

1 A single slice is transferred to a submerged recording chamber (2.5–3 ml/min, 33.5°C), on the stage
of an upright microscope equipped for infrared video microscopy (Hamamatsu, Tokyo), allowing direct visualization of the recorded neurons.

1.1 ACSF composition is (in mM): 126 NaCl, 2.5 KCl,1.2 $MgCl_2$, 1.2 NaH2PO4, 2.4 CaCl2, 10 glucose and 25 NaHCO3. The solution is saturated with a mixture of O2/CO2

2 Neurons were recorded in whole cell patch-clamp configuration using 1.5-mm borosilicate glass electrodes (3–4 MOhm), pulled with a vertical puller (PP-83 Narishige) and filled with (in mM): K-gluconate (135), KCl (10), HEPES (10), $MgCl_2$(2), EGTA (0.1), $CaCl_2$ (0.05), Mg_2-ATP (4), Na_3-GTP (0.3), Phosphocreatine-Na_2 (10), adjusted to pH 7.3 with KOH. Membrane currents were
recorded in voltage-clamp mode at a holding potential of -60 mV, using a differential amplifier (Multiclamp 700B, Molecular Devices, LLC., 3860 N First Street San Jose, CA 95134 USA). Signals were filtered at 3 kHz, and digitized at 10 kHz.