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Application Report

Primary brain cells on Qube 384

Voltage- and current-clamp recordings in primary neurons and astrocytes

Summary

Native cells represent a promising alternative to cell lines due to more physiological behavior.

- Primary brain neurons and astrocytes were tested on Qube 384
- Neuronal Na^+ and K^+ currents were measured in voltage clamp
- Neuronal action potentials were measured in current clamp
- Astrocyte K^+-currents were elicited with both ramp- and step-protocols

Introduction

Dissociated primary brain cells (such as neurons and astrocytes) are excellent model systems for neurobiological, biophysical, and pharmacological evaluations. The presence of a wide variety of ion channels and receptors ensures a physiological relevant analysis of cell response and signaling. The dissociated cells are devoid of processes, and therefore possess an ideal geometry for rapid and complete control over the membrane potential, which is highly desired in patch-clamp experiments[1].

Furthermore, dissociated cells are entirely isolated from other cells, tissue, and other barriers, making it possible to obtain complete control of the extracellular environment, which, combined with ultra-fast perfusion systems, can facilitate the study of rapidly desensitizing, ligand-gated channels.

While the advantages of isolated brain cells are numerous, it must be considered that enzymatic dissociation can alter membrane characteristic, and removing the cells from their supporting environment may change their properties[1–3]. Nevertheless, patch-clamping of primary brain cells is a powerful tool for pharmacological and biophysiological evaluation of ion channels in both neurons and astrocytes, as well as for monitoring changes in membrane properties and excitability[1,2].

Results and discussion

Primary Neurons

To evaluate the electrical properties of acutely isolated neurons, cells were isolated from P7 male NMRI mouse brains, using a gentleMACS™ Dissociator (Miltenyi Biotec) and a magnetic, MicroBeads based mouse neuron isolation kit (Miltenyi Biotec). For more details, see methods. On the same day of isolation, the neurons were evaluated in whole-cell patch-clamp experiments on Qube 384.

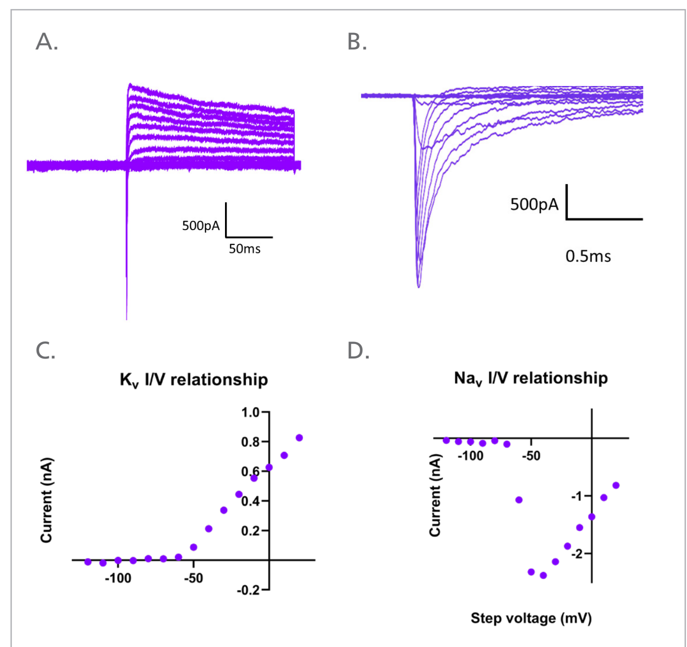

Fig. 1: The electrical properties of acutely isolated neurons. A) Current response to 15 voltage steps from -120 mV to 20 mV. B) Sodium current elicited by 15 same protocol as (A)). C) Potassium current-voltage relationship from (A). D) Sodium current-voltage relationship from (B).

Neuronal ion channel currents

15 depolarization steps from -120 mV to 20 mV elicited sodium and potassium currents in the dissociated mouse neurons. Figure 1A shows the current response to the voltage steps. First, a millisecond long, inward current occurred due to the opening of the sodium channels (see Figure 1B and Figure 1D for current-voltage relationship). This current was followed by a slower outward current, mediated by the opening of the potassium channels (see Figure 1C for current-voltage relationship).

Neuronal action potentials

Current clamp recording is a powerful way of assessing a change in excitability. Monitoring changes in both passive and active properties provide an approach to identify ion channels that may contribute to the changes in excitability[1]. Despite the loss of the dendritic tree and the possible loss of resting potential during the dissociation, the properties of the dissociated cells under current clamp have been found to be remarkably similar to intact neurons in slices or in tissue culture[2,4].

In the acutely isolated mouse neurons, action potential firing was initiated using a current ramp (see Figure 2 for an example).

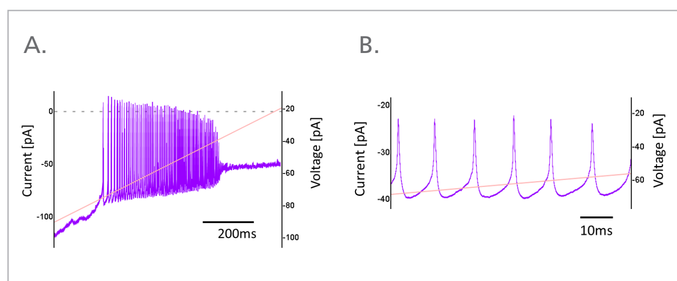

Fig. 2: Example of action potential firing in an acutely isolated mouse neuron. The action potentials were induced using a current ramp. A) Action potential burst firing. This neuron starts firing at a membrane potential of around -75 mV. B) Zoom in of 6 action potentials, displaying the morphology of the action potentials.

Primary Astrocytes

Primary rat astrocyte cultures were prepared from P1-P5 rat pups as described by Liu et al., 2003[5], and the cultures were deprived from neurons by repeated subculturing (the culture was reseeded minimum 5 times). The resulting mixed glia cultures were astroglia-enriched through mechanical removal of microglia.

The astrocytes were electrophysiologically characterized, applying two voltage protocols: a voltage protocol consisting of both a step part and a ramp part (Figure 3) and voltage protocol with 20 steps from -110 mV to 80 mV (Figure 4). The currents elicited were characteristic potassium currents with rectification.

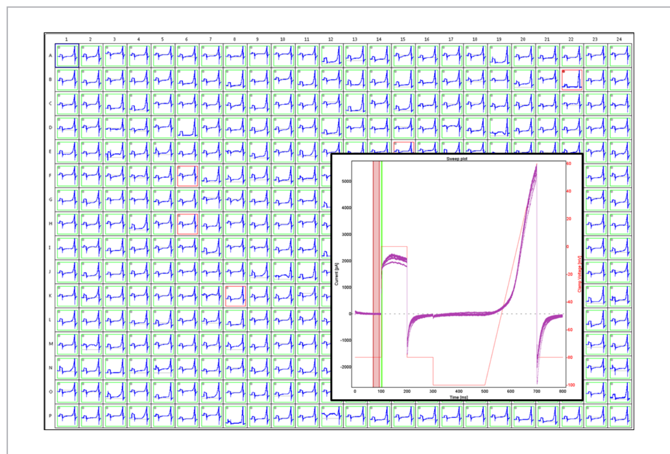

Figure 3: The electrical properties of primary astrocytes. Plate view of a 10-hole QChip: primary rat brain astrocyte potassium currents activated by a voltage step from -80 mV to 0 mV followed by a voltage ramp from -100 mV to +60 mV. Insert: View of one experimental side, displaying the current in relation to the applied voltage protocol.

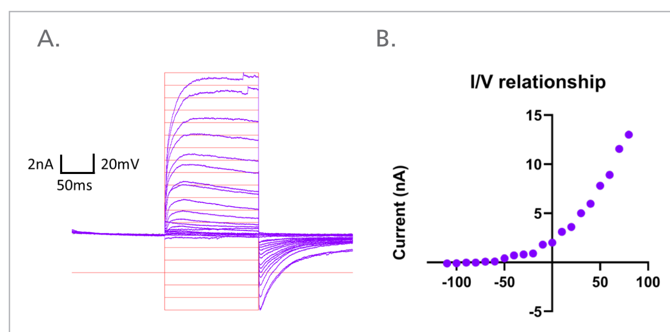

Figure 4: Primary rat astrocyte potassium currents. A) Current response to from -110 mV to 80 mV. B) Potassium current-voltage relationship.

Methods

Primary mouse neurons

Primary neurons were isolated using the 'Neural Tissue Dissociation' guide provided by Miltenyi Biotec[6]. In short, whole brains were collected from P7 male NMRI mice. At this age the cells are expected to already display a sodium current[7] while the cells' surviving rate is still suitable.

The tissue was dissociated using a gentleMACS™ Dissociator (# 130-095-937) and the Neural Tissue Dissociation Kit – Postnatal Neurons (# 130-094-802), both from Miltenyi Biotec, for protocol see Reference 6. The neurons were isolated from the cell suspension via negative selection using a magnetic MicroBeads based isolation kit (# 130-115-389, Miltenyi Biotec). After isolation, the cells were seeded in culture for 1-3 hours. All cells and debris not attaching to the flask were washed away, and

the neurons were harvested using detachin. On the same day of isolation, the neurons were evaluated in whole-cell patch-clamp experiments on a Qube 384 at ambient temperature using single-hole consumables.

Primary rat astrocyte cultures

The brain was isolated from P1-P5 rat pups and astroglia-enriched cultures were grown according to Liu *et al.*, 2003[5]. Patch-clamp: All experiments were carried out at ambient temperature using Qube 384 multi-hole consumables.

Data Analysis

Analysis was performed using the Sophion Analyzer Software and GraphPad Prism 9.00 (GraphPad Software Inc.).

References:

1. Gold MS. Whole-cell recording in isolated primary sensory neurons. Methods Mol Biol. 2012;851:73-97. doi:10.1007/978-1-61779-561-9_5
2. Raman IM, Bean BP. Resurgent sodium current and action potential formation in dissociated cerebellar Purkinje neurons. J Neurosci. 1997;17(12):4517-4526. doi:10.1523/jneurosci.17-12-04517.1997
3. Gao H-M, Hong J-S, Zhang W, Liu B. Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. J Neurosci. 2002;22(3):782-790.
4. Gähwiler BH, Llano I. Sodium and potassium conductances in somatic membranes of rat Purkinje cells from organotypic cerebellar cultures. J Physiol. 1989;417(1):105-122. doi:10.1113/jphysiol.1989.sp017793
5. Liu B, Hong J-S. Primary rat mesencephalic neuron-glia, neuron-enriched, microglia-enriched, and astroglia-enriched cultures. Methods Mol Med. 2003;79:387-395.
6. Miltenyi Biotec. Neural Tissue Dissociation Kit – Postnatal Neurons.; 2018. www.miltenyibiotec.com.
7. Tadros MA, Farrell KE, Graham BA, Brichta AM, Callister RJ. Properties of sodium currents in neonatal and young adult mouse superficial dorsal horn neurons. Mol Pain. 2015;11:17. doi:10.1186/s12990-015-0014-5

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