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ELECTROPHYSIOLOGICAL CHARACTERIZATION OF DIFFERENTIATING NEONATAL RAT HIPPOCAMPAL NEURONS

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ABSTRACT

This study investigates the differentiation and electrophysiological properties of neonatal rat hippocampal CA1 neurons cultured in vitro. Primary hippocampal neurons were extracted from 1-2 day old postnatal rats and cultured for a period of up to 13 days. Electrophysiological recordings were conducted using the patch-clamp technique on days 4 to 11, and 13 in vitro (DIV). Key parameters measured included resting membrane potential, input resistance, and cell capacitance.

Notably, the input resistance started to decrease significantly after 6 DIV, whereas the resting membrane potential and cell capacitance remained relatively constant over time. These findings suggest that these neurons undergo differentiation without a concomitant increase in size and provide valuable insights into the electrophysiological behavior and differentiation patterns of neonatal rat hippocampal neurons in a controlled environment.

This research contributes to the broader understanding of neuronal development and has potential implications for neurological studies.

Key words: patch-clamp, excitability, action potential, input resistance, hippocampal neuron.

Introduction

In vitro studies of hippocampal neurons derived from neonatal rats have been instrumental in advancing our understanding of neuronal development, intrinsic excitability, and synaptic integration. The hippocampus, a central structure in the mammalian brain, plays a critical role in learning, memory formation, and spatial navigation. Within this region, CA1 pyramidal neurons are particularly well-characterized for their distinct electrophysiological profiles and developmental trajectories (Kandel *et al.*, 2013; Spruston and Johnston, 1992).

Culturing hippocampal neurons provides a controlled environment to investigate how intrinsic membrane properties evolve during early differentiation. Parameters such as resting membrane potential, input resistance, and membrane capacitance are key indicators of neuronal maturity and functional readiness. These properties are shaped by the dynamic expression of ion channels, changes in membrane morphology, and the establishment of synaptic networks (Debanne *et al.*, 2019; Bean, 2007).

This study focuses on CA1 neurons cultured from neonatal rats, examining their electrophysiological characteristics between 4 and 13 days in vitro (DIV) – a critical window during which neurons undergo rapid differentiation and begin to exhibit mature firing patterns. By tracking changes in resting membrane potential, input resistance, and capacitance, we aim to elucidate the timeline and mechanisms of neuronal maturation in vitro.

Materials and Methods

Primary cultured hippocampal neurons were extracted from neonatal rats at 1-2 postnatal day. The neurons selected for electrophysiological recordings exhibited prominent pyramidal morphology with well-defined dendritic processes. The cultures were sustained for a period of 13 days. A recovery post-isolation period was obligatory, as no detectable calcium currents were observed prior to 4 days in vitro (DIV). Electrophysiological experiments were conducted on 4 DIV, 5 DIV, 6 DIV, 7 DIV, 8 DIV, 9 DIV, 10 DIV, 11 DIV, and 13 DIV.

In our experimental setup, whole cell currents were measured using an EPC 10 patch clamp amplifier (HEKA Elektronik Dr. Schulze GmbH, Germany). A standard patch clamp configuration was employed, consisting of an Olympus inverted microscope equipped with a Leitz micromanipulator. Experimental control was facilitated by HEKA™ PatchMaster software. Patch pipettes were pulled from borosilicate glass using a DMZ-universal puller (Zeitz-Instruments GmbH, Munich, Germany).

For the recordings, the "whole cell" configuration was employed. The resistance of the seal exceeded 1 GΩ. In voltage clamp experiments, the holding potential (HP) was set to –80 mV, while in current clamp mode, the holding current (HI) was adjusted to ensure the cell membrane potential was approximately –70 mV. The membrane potential of the cell was measured without current injection in the current clamp mode.

For off-line analysis of the experimental data, the software FitMaster and Origin 7.5 were used. Statistical significance of the observed effects was assessed using paired or unpaired Student's t-test, as appropriate.

Results

Resting potential is determined by the unequal distribution of ions across the cell membrane, varying with cell type, animal age, and experimental conditions. Neurons derived from neonatal animals exhibit a more depolarized resting membrane potential compared to those from adult animals. In our study, the resting membrane potential ranged from –43.2 ± 2.2 mV (n=11) at 7 DIV (the highest value) to –48.8 ± 2.6 mV (n=5) at 9 DIV (the lowest value), with no significant temporal variation observed during the culture period.

Immediate current detection was achieved using a voltage ramp protocol as detailed in Fig. 1. The Na^+ current was detected with amplitudes ranging from 1.5 to 10 nA, followed by Ca^{2+} current, which overlapped with outward K^+ current. The amplitude of the outward K^+ current varied among cells, ranging from 6 to 20 nA at +80 mV.

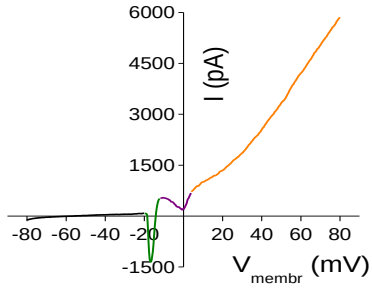

Figure 1: A representative current recording activated by ramp protocol, demonstrated presence of Na^+ current (green line), Ca^{2+} current (violet line) and outward K^+ current (orange line). The current was activated by linear voltage ramp from –80 to +80 mV.

Calcium current density was calculated as the current amplitude divided by cell capacitance. Given that cell capacitance is directly proportional to cell size, where 1 μm² of cell membrane corresponds to approximately 1 pF of capacitance, it serves as a measure of cell surface area. During the initial days post-isolation, neurons underwent differentiation and development, thereby altering the expression of voltage-dependent ion channels. An observed trend indicated an increase in calcium current density, with statistically significant increases compared to 4 DIV noted at 7 DIV, 10 DIV, and 11 DIV (Fig. 2).

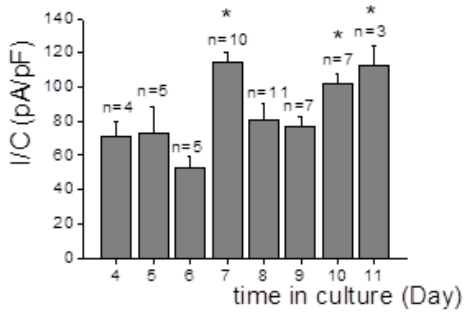

Figure 2: Average calcium current density in hippocampal neurons during increasing time in culture (* - p < 0.05 compared to 4 DIV) – current divided to cell surface (cell capacitance being the "measure" for cell size, as capacitance is directly proportional to cell surface).

The input resistance of each neuron was assessed under current clamp conditions, as delineated in Fig. 4. The resultant voltage traces are illustrated in Fig. 3.

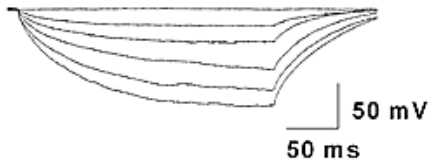

Figure 3: Voltage traces activated by current injections (see Fig. 4). Voltage amplitudes were measured at the pulse's end.

The current-voltage relation for membrane voltages was constructed by measuring the voltage at the end of each pulse, corresponding to a current injection with predefined parameters (Fig. 4), and fitted by a linear function of Ohm's law:

$$I = \frac{1}{R}U$$

where I is the injected current, U is the measured membrane voltage and R is the input resistance of a cell.

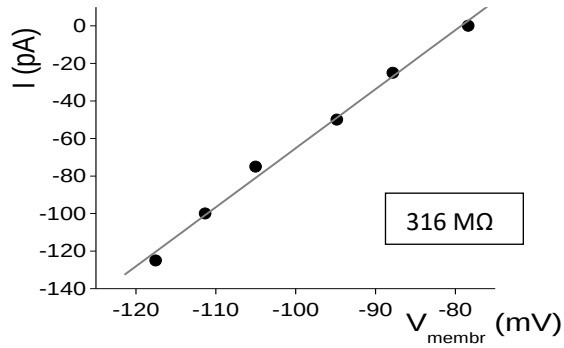

Figure 4: An example of the fitted line of the current-voltage relation (I-V) used to measure the input resistance. Each filled circle represents the voltage amplitude measured at the end of the pulse of traces shown in Fig. 3 and is plotted against the amplitude of the injected current. The line represents the linear fit of experimental data according to Ohm's law, where R = 316 MΩ.

During prolonged time in culture the input resistance decreased (Fig. 5). This decrease became statistically significant starting at 10 DIV. This decrease is indicative of increased ion channel expression as neuronal differentiation progresses.

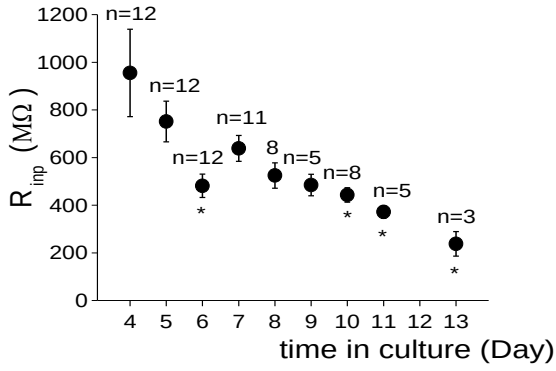

Figure 5: The decrease in input resistance observed in cultured rat hippocampal neurons over time is likely attributable to the upregulation of ion channel expression (* - $p < 0.05$ compared to 4 DIV).

The single action potentials were assessed using the current clamp mode as previously described (Caro, 2025). When the voltage threshold for activation was reached, the neuron generated an action potential (Fig. 6). The following parameters were evaluated: the resting membrane potential of the neuron, the firing threshold, the duration of the action potential, and the peak of the action potential. The activation threshold was identified by a discontinuity in the first derivative of the action potential time course. The duration of the action potential was measured at the voltage corresponding to half the distance between the threshold and the peak. The peak of the action potential represented the maximum membrane voltage, while the amplitude of the action potential was defined as the voltage difference between the threshold and the peak.

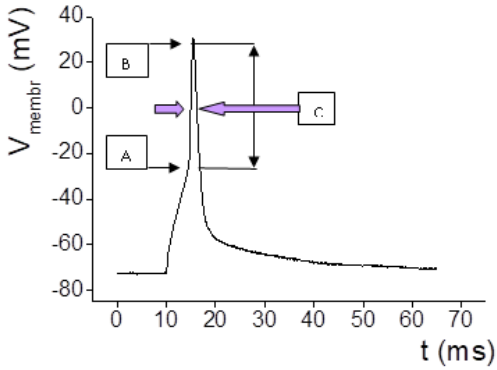

Figure 6: Representation of an action potential recorded from a cultured hippocampal neuron, illustrating the evaluated parameters. The action potential was elicited by the injection of a suprathreshold depolarizing current pulse (typically ranging from 100 to 200 pA) with a duration of 5 ms. A – threshold for firing of the action potential; B – peak (maximum) of the action potential; C – duration of the action potential, also referred to as "half-height width".

The amplitudes of action potentials did not exhibit significant changes during the culture period between 4 DIV and 13 DIV. This interval was determined to be the optimal timeframe for studying the excitability of these cells. Ion channel expression changed markedly during the initial days post-establishment of the cell culture. After two weeks in culture, handling the neurons became challenging.

The resting membrane potential was measured in current clamp mode with no current injection. Its values remained relatively constant throughout the culture period, varying between –37 mV and –55 mV.

The firing threshold did not exhibit a consistent trend of increase or decrease over the culture period. Its values ranged between –25 mV and –40 mV, with a common value around –35 mV. The thresholds form a depolarization baseline, which serves as a modulating factor for neurotransmitter release (Bianchi *et al.*, 2022).

When stimulated with 300 ms long pulses, each hippocampal neuron demonstrated the capability to fire a series of 3 or more action potentials, typically ranging from 5 to 12. These series exhibited the phenomenon of accommodation, characteristic of hippocampal neurons, wherein the time interval between successive action potentials within a series progressively increased (Fig. 7) described by Stuart *et al.* (1997).

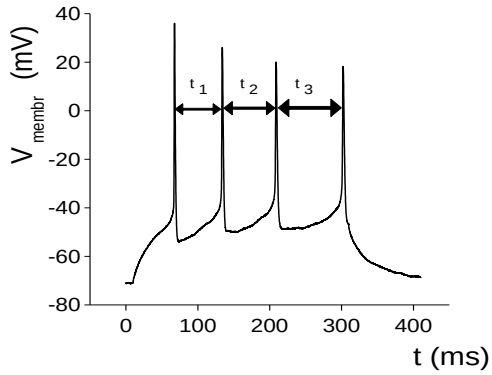

Figure 7: A representative trace of series of action potentials, induced by suprathreshold current stimulation. The time interval t_i between two consecutive action potentials increases after each action potential demonstrating the phenomenon known as spike-frequency adaptation.

Discussion

The observed decrease in input resistance during the early days of hippocampal neuron culture likely reflects a developmental upregulation of ion channel expression as differentiation progresses. Input resistance is a fundamental determinant of neuronal excitability and synaptic integration, influencing both the amplitude of postsynaptic potentials and the threshold for action potential initiation (Kandel *et al.*, 2013; Spruston and Johnston, 1992). Our findings are consistent with previous reports showing that maturing CA1 pyramidal neurons exhibit reduced input resistance due to increased membrane conductance and dendritic arborization (Spruston and Johnston, 1992). Moreover, Debanne *et al.* (2019) and Smith and Walsh (2020) emphasized that activity-dependent modulation of Kv and Na^+ channel densities play a central role in shaping intrinsic excitability during early neuronal development, contributing to homeostatic regulation and circuit formation.

Despite the dynamic changes in membrane properties, the duration of action potentials in our cultures remained relatively stable throughout the 4–13 DIV period, ranging from 0.9 to 1.2 ms. This stability suggests a tightly regulated balance between inward and outward ionic currents during early maturation. Bean (2007) reported that action potential duration in mammalian central neurons typically spans 1–2 ms, with variability arising from differences in channel subtypes and kinetics. Hille and Catterall (2012) and Nanou and Catterall (2018) attributed such diversity to the differential expression of voltage-gated Na^+, K^+, and Ca^{2+} channels, which are not only critical for excitability but also for developmental processes such as synaptogenesis, migration, and differentiation. Supporting this, Lacinova *et al.* (2008) demonstrated that Cav1.2 channels modulate spike frequency adaptation and firing patterns, while Caro *et al.* (2011) found that nimodipine-sensitive currents influence excitability primarily through effects on delayed rectifier K^+ channels.

The robustness of action potential trains observed in our recordings further supports the functional maturation of these neurons. Reliable spike generation is essential for synaptic amplification and input cooperativity, as described by Harnett *et al.* (2012), who showed that dendritic spine compartmentalization enhances local depolarization and NMDA receptor activation. Stuart *et al.* (1997) also demonstrated that backpropagating action potentials into dendrites are actively modulated by A-type K^+ channels, contributing to synaptic plasticity and learning-related signaling.

These mechanisms are tightly linked to the developmental remodeling of ion channel expression, which underlies intrinsic plasticity and network integration (Debanne *et al.*, 2019).

Taken together, our data highlight the 4–13 DIV window as a critical period for studying hippocampal neuron excitability. During this timeframe, neurons undergo substantial changes in ion channel composition and membrane properties, reflecting a coordinated developmental program that prepares them for functional integration into neural circuits. The consistency in action potential duration, alongside the decline in input resistance, aligns with foundational models of excitability (Hille, 2001; Hodgkin & Huxley, 1952) and supports the use of patch-clamp electrophysiology for probing both passive and active properties of developing neurons (Caro, 2025; Santillo, 2024; Kodirov, 2023).

Conclusion

This study demonstrates that hippocampal neurons cultured between 4 and 13 days in vitro (DIV) maintain stable excitability, as evidenced by consistent action potential amplitudes and resting membrane potentials. The observed decline in input resistance over time likely reflects the progressive differentiation of neurons and the upregulation of voltage-gated ion channels, particularly potassium and sodium channels, which are known to shape membrane conductance and excitability during development.

The firing threshold remained relatively stable throughout the culture period, suggesting that while membrane conductance increased, the balance of inward and outward currents was tightly regulated. The ability of neurons to fire multiple action potentials in response to sustained stimulation, demonstrating accommodation, further supports their functional maturation and readiness for synaptic integration.

Moreover, the electrophysiological stability observed in this timeframe aligns with the developmental window during which intrinsic plasticity mechanisms are actively shaping neuronal identity and responsiveness. The consistent action potential duration and robust firing patterns suggest that key ion channels, including Cav1.2 and delayed rectifier K^+ channels, are functionally expressed and contribute to spike generation and adaptation.

The 4–13 DIV period represents a critical phase for studying the maturation of hippocampal neurons in vitro. The combination of stable excitability parameters and dynamic changes in input resistance provides a valuable framework for investigating ion channel regulation, synaptic development, and the electrophysiological foundations of learning and memory.

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References

1. Bean, B. (2007). *The action potential in mammalian central neurons*. Nature Reviews Neuroscience 8, 451-465.

2. Bianchi, D., R. Migliore, P. Vitale, M. Garad, P. Pousinha, H. Marie, V. Lessmann, M. Migliore. (2022). *Membrane electrical properties of mouse hippocampal CA1 pyramidal neurons during strong input*s. Biophysical Journal, 121(4), 644-657.
3. Caro, A., B. Tarabova, J. Rojo-Ruiz, L. Lacinova. (2011). *Nimodipine inhibits AP firing in cultured hippocampal neurons predominantly due to block of voltage-dependent potassium channels*. Journal of General Physiology and Biophysics 30, S44-S53.
4. Caro, A. (2025). *The patch clamp method and protocols for assessing neuronal excitability*. Tradition and Modernity in Veterinary Medicine, vol.10, No 1(18): 54-61, ISSN 2534-9333, e-ISSN 2534-9341.
5. Debanne, D., Y. Inglebert, M. Russier. (2019). *Plasticity of intrinsic neuronal excitability*. Current opinion in neurobiology, 54, 73-82.
6. Harnett, M., J. Makara, N. Spruston, W. Kath, J. Magee. (2012). *Synaptic amplification by dendritic spines enhances input cooperativity*. Nature, 491(7425), 599-602.
7. Hille, B. (2001). *Ion channels of excitable membrane*s. Sinauer, Sunderland. ISBN 978-0-87893-321-1.
8. Hille, B., W. Catterall. (2012). *Electrical excitability and ion channels*. In Basic neurochemistry (pp. 63-80). Academic Press.
9. Hodgkin, A., A. Huxley. (1952). *A quantitative description of the membrane current and its application to conduction and excitation in nerve*. Journal of Physiology 117, 500-544.
10. Kandel, E., J. Schwartz, T. Jessell, S. Siegelbaum, A. Hudspeth, S. Mack. (2013). *Principles of Neural Science*. New York: McGraw-Hill.
11. Kodirov, S. (2023). *Whole-cell patch-clamp recording and parameters*. Biophysical Reviews 15, 257-288.
12. Lacinova, L., S. Moosmang, N. Langwieser, F. Hofmann, T. Kleppisch. (2008). *Cav1.2 calcium channels modulate the spiking pattern of hippocampal pyramidal cells*. Life Sciences 82, 41-49.
13. Nanou, E., W. Catterall. (2018). *Calcium channels, synaptic plasticity, and neuropsychiatric disease*. Neuron, 98 (3), 466-481.
14. Santillo, S. (2024). *Patch-Clamp: Studying the Passive and Active Properties of Excitable*. Patch Clamp Technique-Current Methods and Future Prospects: Current Methods and Future Prospects, 29.
15. Smith, R., C. Walsh. (2020). *Ion channel functions in early brain development*. Trends in Neurosciences, 43(2), 103-114.
16. Spruston, N., D. Johnston. (1992). *Perforated patch-clamp analysis of the passive membrane properties of three classes of hippocampal neurons*. Journal of Neurophysiology, 67(3), 508-529.
17. Stuart, G., N. Spruston, B. Sakmann, M. Hausser. (1997). *Action potential initiation and backpropagation in neurons of the mammalian CNS*. Trends in Neurosciences, 20(3), 125-131.