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Theoretical calculation of NMDA receptor desensitization and intracellular calcium determination through simulation

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Abstract

One of the crucial processes in preventing cellular damage due to excitotoxicity involves the calcium-mediated desensitization of the N-methyl-D-aspartate (NMDA) receptor. The opening of NMDA channels results in the influx of Na^+ and Ca^{2+} ions and efflux of K^+ ions. This condition leads to an increase in intracellular calcium ($[Ca^{2+}]_i$), thereby diminishing the NMDA current and preventing damage induced by constant stimulation of the receptor by glutamate. In this study, a simulator based on a phenomenological mathematical model was developed, employing the Visual Basic 6.0 language for the Windows® environment. Input variables encompass intracellular calcium, peak currents (Ip), and steady-state currents (Iss). Input currents were derived from previously published electrophysiological recordings. The simulator adeptly reproduces the NMDA receptor desensitization phenomenon under varying experimental conditions and accurately calculates internal calcium with a 10% margin of error. It proves valuable in scenarios where simultaneous recordings of NMDA current and $[Ca^{2+}]_i$ via fluorescence labeling techniques are impractical. In the realm of education, it enables students to conduct virtual experiments, providing an immersive introduction to the topic.

Keywords: Desensitization; NMDA Receptor; Simulators; Interactive Education

1. Introduction

The NMDAR (N-methyl-D-aspartate receptor) is a Ca^{2+}, K^+, and Na^+-permeable voltage-gated ligand channel located at the synaptic and extrasynaptic levels. It is made up of four subunits: two NR_1 and two NR_2. The latter are composed of a family of four different subunits (A, B, C, and D) [1]. It is activated by the simultaneous binding of glycine in the NR1 subunits and glutamate in the NR2 subunits. The function of excitatory glutamatergic synapses involves the interaction of the NMDA receptor with the AMPA receptor, both of which are permeable to calcium. At resting potentials, the NMDA receptor is blocked by Mg^{2+}. Activation of the AMPA receptor causes depolarization and unblocks the NMDA receptor. Ca^{2+} permeability is higher at the NMDA receptor (pCa^{2+} AMPA <<< pCa^{2+} NMDA) [2]. Sustained activation of the NMDA receptor produces neuronal damage caused by a substantial increase in the concentration of intracellular Ca^{2+} [3]. Toxic cytoplasmic concentrations of Ca^{2+} trigger changes in the neuron such as an overproduction of free radicals, acidosis, cytoskeletal breakdown, alterations in cell membranes, mitochondrial and reticulum dysfunction, and deoxyribonucleic acid (DNA) fragmentation [4] and leads to pathological apoptosis [5]. Brain trauma has been reported to generate propagated cortical depolarization (SD) that depolarizes neuronal and glial cells in the cerebral cortex. Vascular alterations and an increase in extracellular K^+ and glutamate occur, producing neuronal damage [6]. In hippocampal neurons (CA1 region) following propagated cortical depolarization, intracellular Ca^{2+} concentration increases (~30 µM in the dendrite and soma for several minutes) due to sustained NMDA receptor activation. NMDAR overactivation in various pathologies such as ischemic stroke, and traumatic brain injury, among others, can cause excitotoxicity [7,8].

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The neuron has an intrinsic mechanism that reduces the influx of Ca^{2+} through the NMDA receptor called desensitization. Desensitization could help maintain the integrity of glutaminoceptive neurons.

1.1. NMDA receptor desensitization

NMDA receptor desensitization is a decreased receptor response that occurs when exposed to an agonist for a long period. Electrophysiologically, it is a gradual decrease in the recorded macroscopic peak NMDA current [9]. Two types of desensitization have been characterized: (1) attributed to decreased glycine affinity and (2) to internal Ca^{2+} dependence ($[Ca^{2+}]_i$) [10]. In this work, we are interested in calcium-dependent desensitization. The degree of NMDA receptor desensitization depends on agonist concentration and the increase in internal calcium [10]. The units that make up the receptor determine the degree of desensitization. The GluN1/GluN2A subunits confer greater calcium-dependent desensitization than GluN1/GluN2B [11]. Modulators such as zinc, pH, and polyamines control channel opening, modulate function, and desensitization [12]. With the opening of the NMDA channel, Ca^{2+} enters the neuron. Molecularly, the desensitization of the NMDA receptor by internal Ca^{2+} presents a slow and fast mechanism. The slow is a process in scale of minutes. Calcium acts indirectly through kinases and phosphatases, phosphorylating the receptor and affecting its permeability [13]. The rapid is done on a scale of milliseconds. It has been described in a sequence of events: with the opening of the NMDA channel, the increase in internal calcium favors the binding of calcium to calmodulin and this changes its conformational state. Calmodulin-Ca^{2+} binds to the c-terminal loop of NR1 and modifies its conformational structure by decreasing the NMDA current. Condition reflected in the macroscopic record of the NMDA current as desensitization [10].

The objective of this study is to computationally simulate the desensitization of the NMDA receptor by internal calcium, and estimate the internal calcium reached by the receptor stimulation by glutamate (or its NMDA (N-methyl-D-aspartate) agonist) and glycine for educational purposes.

2. Material and methods

The simulators were developed using Visual Basic® ver. 6.0 for the Windows® environment. Reproduction of the macroscopic NMDA current and its desensitization during an NMDA pulse was achieved using Equation 1. Voltage-dependent Mg^{2+} block is governed by Equation 2.

$$I_{NMDA} = \left[I_p [Ca^{2+}]_i \left(1 - exp\left(\frac{-t\,[Ca^{2+}]_i}{\tau} \right) \right) + I_p \right] B(V, Mg^{2+})\ (V - E_{NMDA}) \text{.....(1)}$$

I_{NMDA} represents the NMDA current, Ip stands for peak current, $[Ca^{2+}]_i$ represents the internal calcium and corresponds to the intracellular calcium concentration at rest plus the concentration derived from the NMDA current, tau (τ) represents a time constant, and E_{NMDA} signifies the reversal potential of the NMDA current.

$$B(V, Mg^{2+}) = \left(1 + \frac{[Mg^{2+}]}{3.57} \exp\left(\frac{-V}{16.13}\right) \right) \text{..... (2)}$$

$B(V, Mg^{2+})$ is the activation function, $[Mg^{2+}]$ is the external magnesium concentration, V is the voltage, and 3.57 mM and 16.13 mV are data fitting parameters derived from the experimental cures [14,15].

3. Results and Discussion

The process of calcium-induced desensitization manifests during the macroscopic recording of lNMDA in neurons. It is postulated to function as a protective mechanism, mitigating damage caused by excessive internal calcium concentrations. The implication of the NMDA receptor in various diseases [8] has spurred comprehensive investigations. Experiments have been conducted on neurons or transfected cells. To replicate these experiments, a mathematical model estimating the calcium concentration required to achieve NMDA receptor desensitization under different experimental conditions was formulated. This mathematical model was implemented in a simulator.

The simulator encompasses two distinct data input modules and an integrated oscilloscope (Figure 1A). Input data is sourced from two primary channels: (1) experimental records generated directly by the user and (2) data reported in peer-reviewed scientific publications. Essential parameters for simulating the electrophysiological trace involve the specification of peak current (Ip) and steady-state current (Iss). A tabulated representation of exemplary data sourced from diverse scientific publications is illustrated in Figure 1B. Access to this table is facilitated through the selection of <EXAMPLES> from the simulator´s top-tier menu.

The compilation of current data within the table emanates from experiments conducted under rigorously controlled conditions, with manipulations involving varying concentrations of NMDA, Glycine, or Glutamate. The identification and retrieval of pertinent scientific articles were achieved through systematic online searches, employing author names or Digital Object Identifiers (DOIs) as search parameters. Measurement of Ip and Iss values was executed meticulously, utilizing a ruler on the NMDA current trace.

The initial subset of examples within the table corresponds to experiments conducted under the influence of Mg^{2+}, whereas the subsequent three sets were conducted in the absence of Mg^{2+}. Noteworthy is the observable amplitude disparity in peak currents across different experimental conditions. The amplitude of peak current is intricately influenced by factors such as the concentration of NMDA (or glutamate) and glycine, the presence or absence of Mg^{2+}, and the specific neuronal subtype (Figure 1B).

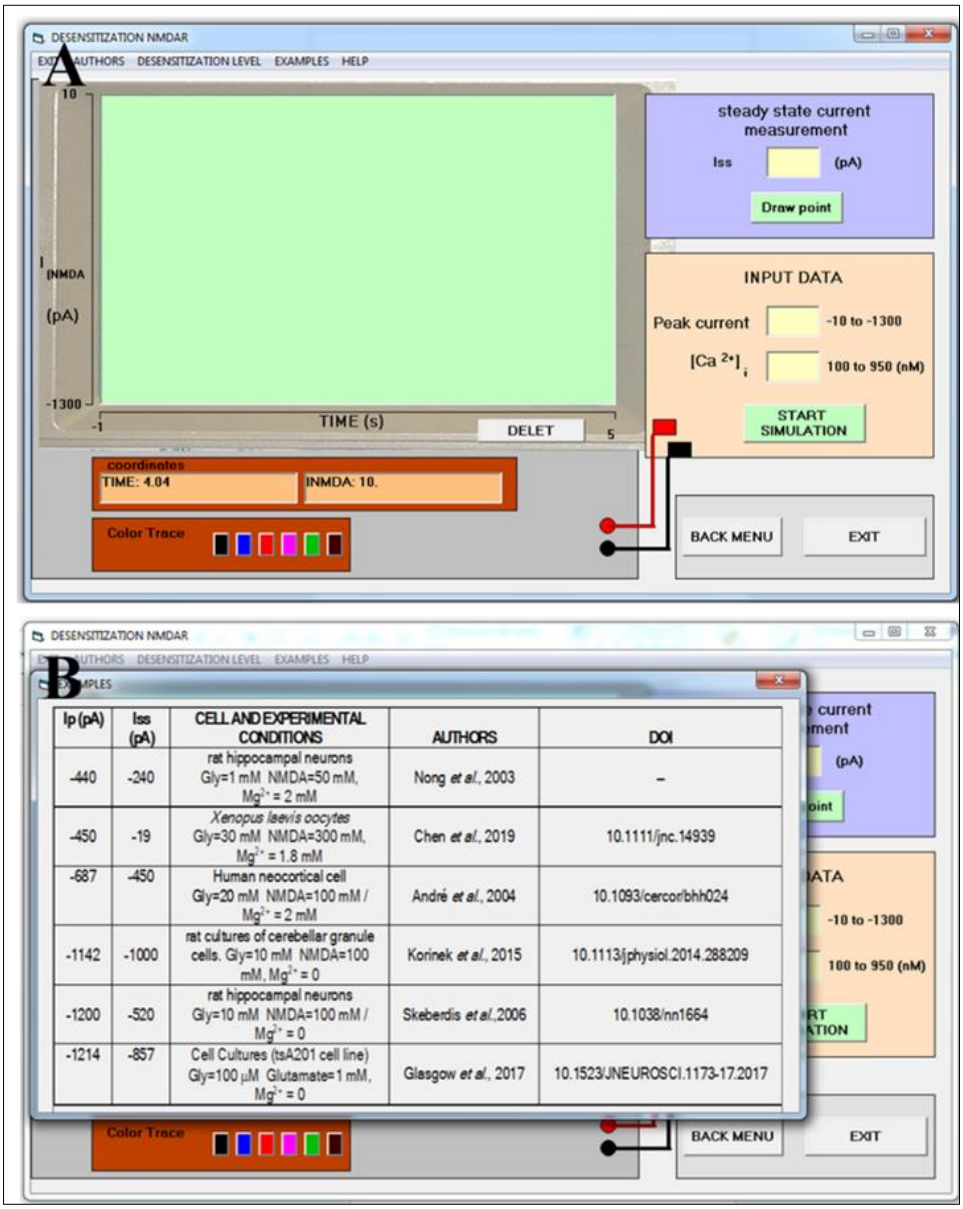

Figure 1 (A) Simulator Interface. On the right side, boxes are displayed for entering Iss (upper box), peak current, and $[Ca^{2+}]_i$ in the corresponding boxes below. (B) Table of examples from experiments reported by different authors. With this data, the user can conduct corresponding simulations and compare them with actual experiments. Users can input their own data or use data from any other reported experiment

The calcium concentration entered into the simulator corresponds to the intracellular calcium concentration at rest plus the concentration derived from the NMDA current. Desensitization of the NMDA receptor by Ca^{2+} is greater with

higher concentrations of internal Ca^{2+}. To achieve the desired desensitization, these values must be varied, as illustrated in the desensitization example.

3.1. Example of NMDA receptor desensitization

Elevated internal Ca^{2+} concentration induces a reduction in macroscopic NMDA current, a phenomenon commonly denoted as Ca^{2+}-induced desensitization. Activation of the NMDA receptor by NMDA (or glutamate) and glycine initiates channel opening, facilitating the influx of Na^{+} and Ca^{2+} concomitant with K^{+} efflux. The basal internal Ca^{2+} concentration typically oscillates within the range of 50 nM to 100 nM. Upon NMDA receptor activation, there is a notable surge in this concentration. The simulator emulates this intricate process, necessitating user input of an initial calcium concentration. The degree of NMDA receptor desensitization, induced by calcium, is directly dependent on the internal calcium concentration [10]. Under normal conditions, a resting internal calcium concentration does not elicit desensitization. However, the introduction of a higher concentration, such as $[Ca^{2+}]_i$ = 850 nM, elicits robust desensitization.

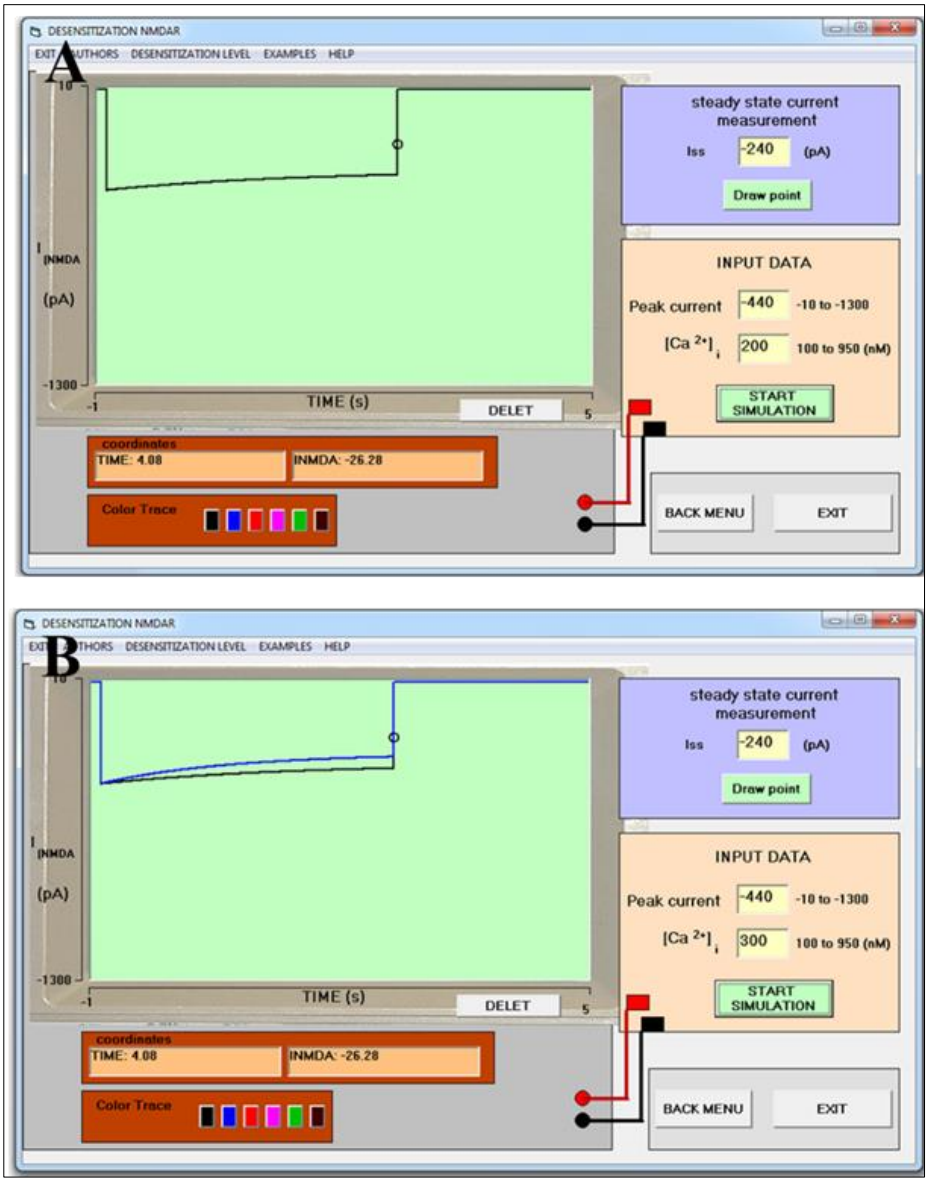

Figure 2 Simulation of the experiment as documented by Nong *et al.* [16]. (A) Recording with intracellular calcium concentration $[Ca^{2+}]_i$ set at 200 nM. The simulated trace is depicted beneath the circular annotation denoting the experimental Iss. (B) Response at $[Ca^{2+}]_i$ = 300 nM. The trace converges towards the circular marker (blue trace) while maintaining the preceding simulation (black trace)

To elucidate the mechanism of Ca^{2+}-induced desensitization, we replicated the experiment documented by Nong *et al.* [16]. Input parameters comprised Iss = -240 pA, Ip = -440 pA, and an initial $[Ca^{2+}]_i$ of 200 nM (Figure 2A). The <Draw

point> button generates a circle on the oscilloscope, representing the Iss value (Figure 2A). This circle serves as a benchmark to iteratively increase $[Ca^{2+}]_i$ until the NMDA current trace aligns with the circle. At $[Ca^{2+}]_i$ = 200 nM, the simulated desensitization is less pronounced than the actual experiment, evidenced by the trace falling below the reference circle towards the conclusion of the NMDA and glycine stimulus. The user is required to increment $[Ca^{2+}]_i$, as a higher concentration corresponds to a more substantial NMDA receptor desensitization.

Figure 2B depicts the simulation after elevating $[Ca^{2+}]_i$ to 300 nM, demonstrating augmented desensitization (blue trace). This exemplifies the dependency of NMDA receptor desensitization on the internal Ca^{2+} concentration.

Continuing with the previous simulation, Figure 3C illustrates that the red trace precisely intersects with the circular marker designating the steady-state current (Iss) observed in the authentic experiment. The $[Ca^{2+}]_i$ concentration necessary for desensitization was determined to be 450 nM. Leveraging the Iss and Ip data, the percentage of desensitization was computed, resulting in a value of 45% (Figure 3D). The interface for computing the desensitization percentage is accessible through <DESENSITIZATION LEVEL>, visible in the simulator's top menu.

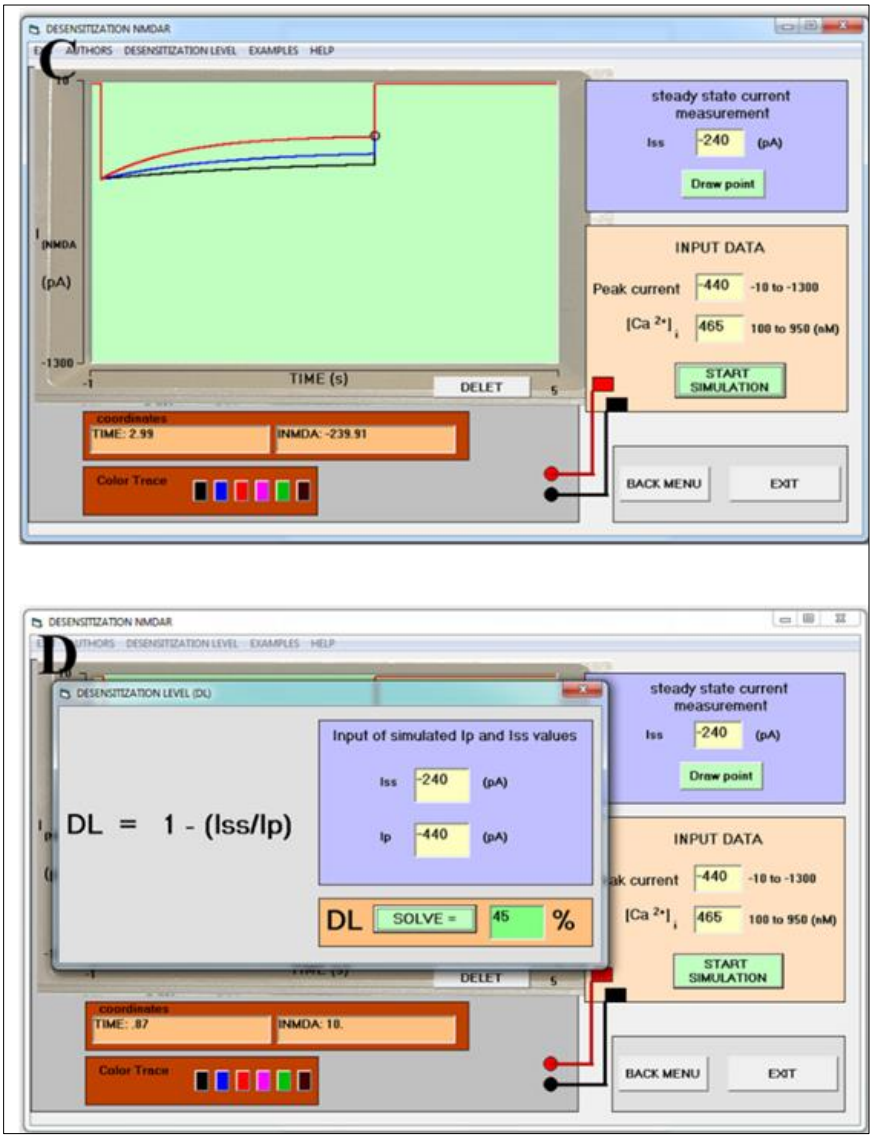

Figure 3 Continuing the simulation of experiments conducted by Nong *et al.* [16]. (C) Simulation of NMDA Current (depicted by the red trace) closely mimics the actual experimental current. The necessary $[Ca^{2+}]_i$ concentration for this simulation was determined to be 450 nM. Experimental parameters included NMDA at 50 mM, glycine at 1 mM, and Mg^{2+} at 2 mM. (D) The popup window is designed for calculating the degree of NMDA receptor desensitization. In this particular case, the calculated desensitization level was 45%

In the antecedent simulation, a systematic presentation illustrated the stepwise replication of macroscopic NMDA current by the simulator, emulating desensitization in rat hippocampal neurons under stipulated experimental conditions.

In genuine experimental scenarios, the precise Ca^{2+} concentration entering through NMDA channels within the macroscopic NMDA current remains undetermined. However, fluorescence techniques have been employed to gauge the internal Ca^{2+} concentration following stimulation with glutamate/glycine or NMDA/glycine, both in neurons and in cells transfected with NMDA receptors and distinct subunits [17,18]. The measured Ca^{2+} concentrations align with those computed by the simulator.

3.2. Simulator Validation

To substantiate the simulator's accuracy, we referenced the work of Medina *et al.* [19], wherein simultaneous recordings of NMDA-induced ionic current and transient Ca^{2+} were performed in cultured hippocampal neurons utilizing Indo-1 labeling. Figure 4A portrays both records. Administration of NMDA elicits an inward ionic current featuring a peak amplitude (Ip) of approximately -370 pA and a steady-state current (Iss) of roughly -252 pA. As per the authors, internal Ca^{2+} concentration increased from 80 nM to 350 nM. The simulator faithfully reproduces the experimental NMDA current (Figure 4A), requiring an internal calcium concentration of approximately 350 nM (Figure 4B).

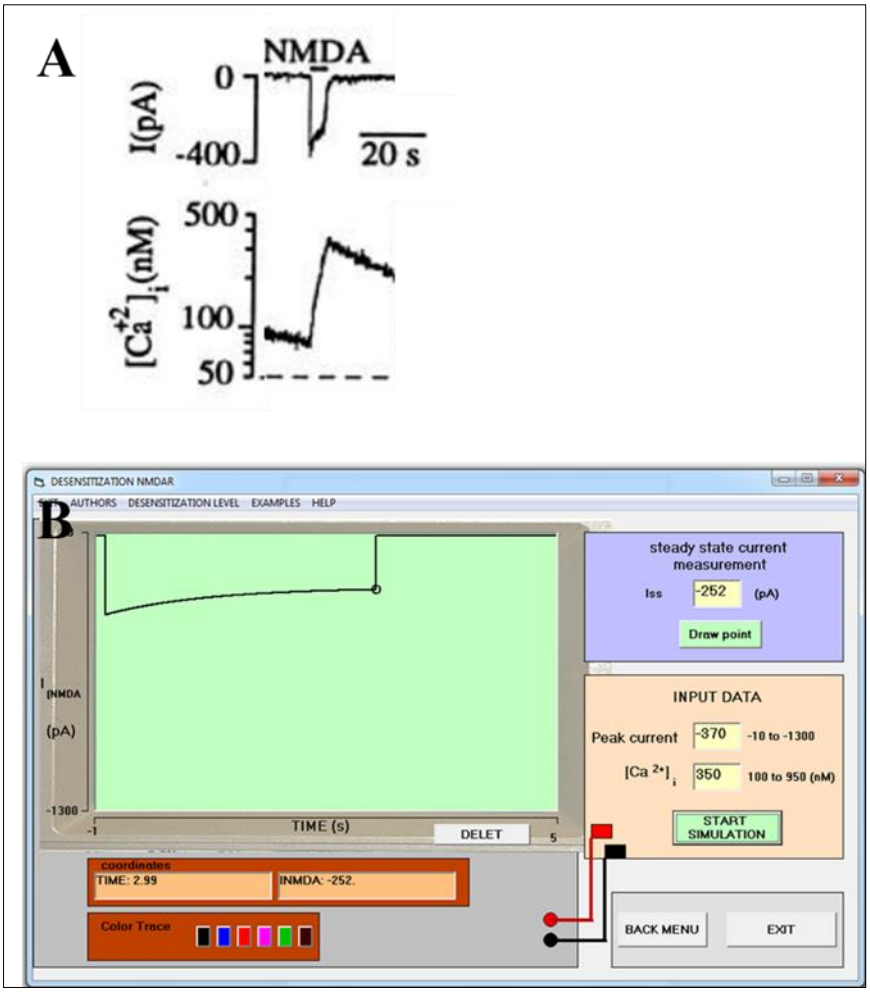

Figure 4 Experimental record and its simulation. (A) Concurrent recording of the NMDA current and the elevation in internal calcium. The peak current measured approximately -350 pA, and the internal calcium concentration, $[Ca^{2+}]_i$, was determined to be 350 nM (Modified figure from Medina *et al.* [19]). (B) Simulation of the experimental NMDA trace. The $[Ca^{2+}]_i$ concentration necessary to replicate desensitization was established at 350 nM

Values for peak currents (Ip) and steady-state currents (Iss) input into the simulator were determined by measuring reported figures using a ruler. In the original figure, authors indicated an Ip amplitude of around -350 pA, while ruler

measurements suggested Ip to be approximately -370 pA, resulting in an 8% discrepancy. The use of a cursor introduces a minor error, leading to a total deviation between 8% and 10%. $[Ca^{2+}]_i$ is assessed as 350 ± 35 nM.

The average $[Ca^{2+}]_i$ across six recorded neurons shifted from 61.7 ± 4.5 nM to 353 ± 37.4 nM. The simulator's computed $[Ca^{2+}]_i$ aligns with the range observed in the studied hippocampal neurons [19]. These findings validate the proposed mathematical model implemented in the simulator.

3.3. Illustration of desensitization in rat hippocampal neuron

The ensuing simulation corresponds to a control experiment conducted in rat hippocampal neurons, as documented by Skeberdis *et al.* [13]. The recorded peak current extracted from the trace in the article was -1200 pA, while the steady-state current was documented as -520 pA. To faithfully reproduce desensitization observed in the authentic experiment, the intracellular calcium concentration ($[Ca^{2+}]_i$) was configured to 570 nM.

Figure 5A showcases the simulated NMDA current, emphasizing the intersection of Iss with the reference circle. The resultant desensitization percentage for the NMDA current was computed to be 57% (Figure 5B).

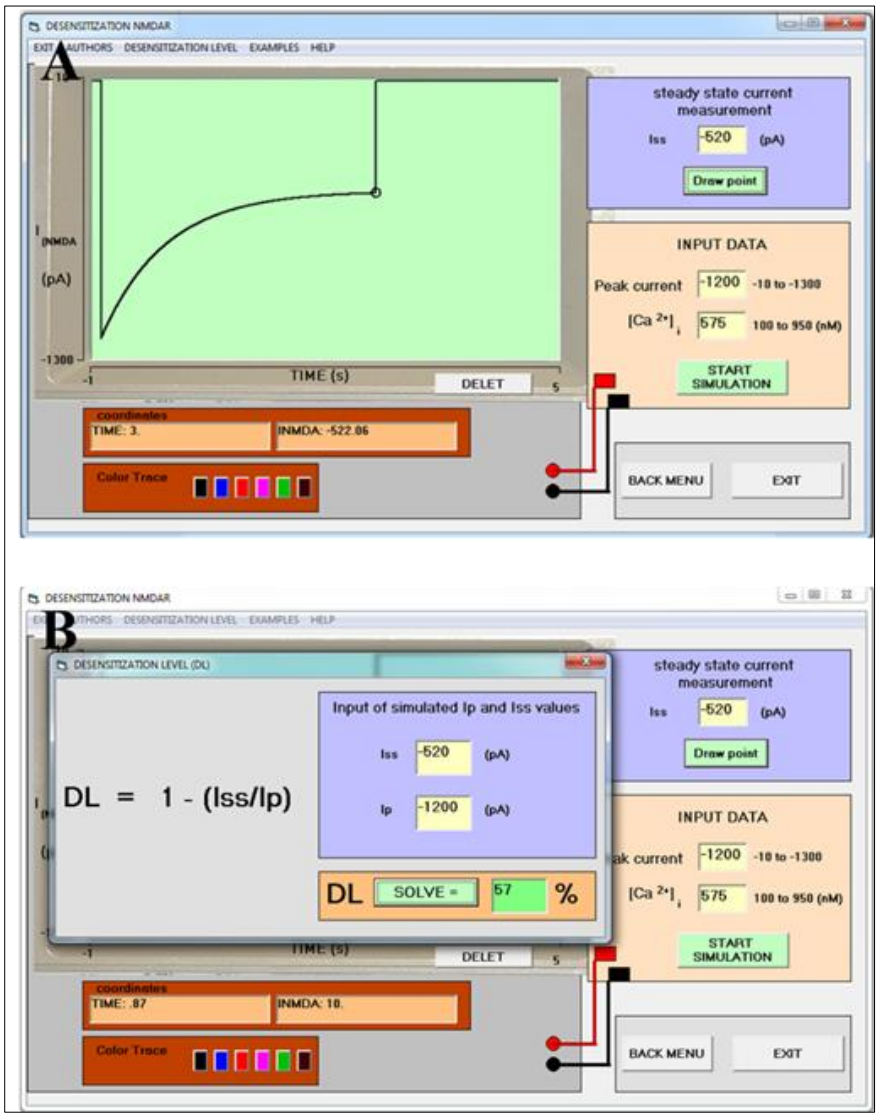

Figure 5 Simulation of an experiment in rat hippocampal neurons [13]. (A) Concluding trace after to the elevation of intracellular calcium concentration ($[Ca^{2+}]_i$) to 570 nM, replicating desensitization observed in the authentic experiment. (B) Computation of the desensitization level. The resultant desensitization level reached 57%

3.4. Simulator configuration and remarks

In this simulator, the NMDA receptor response is decoupled from the input of NMDA and glycine concentrations for the replication of electrophysiological traces. Adopting such an approach would necessitate the development of a simulator tailored to each specific neuron, rendering it contingent on the specific experiment to be recreated. To enable the simulation of any neuron under diverse experimental conditions, we opted to associate the values of peak current (Ip) and steady-state current (Iss) derived from electrophysiological records. This condition facilitates the simulation of any neuron or transfected cell across varying concentrations of NMDA, glycine, glutamate, temperatures, and NMDA receptor configurations.

4. Conclusion

A simulator, executable on the Windows® platform supporting from Windows 7 to Windows 11, has been successfully developed. Its execution necessitates no additional programs or libraries. The simulator adeptly replicates the desensitization process of the NMDA current induced by its agonists and glycine in authentic experiments. Grounded in a phenomenological mathematical model, the simulator estimates the requisite internal Ca^{2+} concentration for the reproduction of calcium-dependent NMDA receptor desensitization, exhibiting an error margin of approximately 10%. This represents a substantial theoretical advancement, enabling the simultaneous acquisition of electrophysiological records and internal Ca^{2+} concentrations. Its utility is particularly noteworthy when simultaneous electrophysiological and calcium fluorescence labeling recordings are impractical. Leveraging the Ip and Iss data, the electrophysiological record of the NMDA current from any neuron or transfected cell can be accurately replicated. The simulator is available for non-commercial use at all times.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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