Single cell electrophysiologic recordings in hippocampal slices

Alexey Semyanov (semyanov@brain.riken.jp)
Semyanov Lab, RIKEN Brain Science Institute
Inseon Song
Semyanov Lab, RIKEN Brain Science Institute

Method Article

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Abstract

Electrode-based techniques are the workhorse of modern cellular neurophysiology. Although these techniques are gradually being replaced by optical imaging, which allows for subcellular resolution or simultaneous recording of thousands of cells, electrode-based methods are still useful for revealing novel phenomena. Here we describe three classic techniques that we used to demonstrate biphasic, excitatory, and inhibitory, actions of tonic $GABA_A$ conductance in CA1 hippocampal interneurons. One of these techniques is whole-cell voltage/current clamp recording, which has the advantage of relatively good electrical control of the cell membrane as well as clamping of the concentrations of intracellular ions. The second technique is gramicidin perforated-patch recording, which allows for some electric control of the cellwithout disturbing the intracellular chloride concentration. The third technique, cell-attached recording, is the least invasive, but can only be used to record large signals, such as action potentials. Which of these techniques is optimal depends on the particular research question.

Reagents

• CGP 52342, NBQX disodium salt, QX314 bromide, D-AP5, Tetrotodoxin citrate, Picrotoxin, and NNC 711 from Tocris • Gramicidin from Sigma

Equipment

• Olympus BX51WI microscopy • DAGE-MTI IR-1000 infrared video camera • Sharp Aquos monitor • Axon MultiClamp 700A amplifier Molecular Devices • WPI PV830 Pneumatic picopump • Digitimer Ltd, constant current isolated stimulator • Luigs & Neumann manipulator SM-5 • National Instruments BNC-2110 A/D-D/A board • Borosilicate glass capillaries \(GC120F-7.5) Harvard apparatus • Sutter P-97 micropipette puller Sutter Instrument • Microm HM650V sectioning system

Procedure

Brain slice preparation 1. Decapitate 4-6 weeks-old C57BL/6J mice. 2. Remove brains and cut transversal \(350 μm thick) hippocampal slices in sucrose-based solution\(in mM):87 NaCl, 2.5 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 26.2 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, and 50 sucrose; saturated with 95% CO_2/5% O_2, ~300mOsm using Microm HM650V sectioning system. 3. Collect the slices and maintain in oxygenated artificial cerebrospinal fluid \(ACSF)\(in mM):119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1 $CaCl_2$, 26.2 $NaHCO_3$, 1 NaH_2PO_4, and 11 glucose: for 30 min at 34°C and then at least 1 hr at 25°C before recording. **Whole-cell recording for synaptic and tonic $GABA_A$R signaling** 4. Transfer individual slices to a submersion recording chamber that is continuously perfused with the oxygenated ACSF at 34°C throughout the recording. 5. All recordings were made from CA1 pyramidal cells and str. radiatum interneurons visually identified with an infrared differential interference contrast microscope. 6. Whole-cell pipettes used in voltage-clamp recordings of tonic $GABA_A$ current and IPSCs contained \(in mM): 130

CsCl, 8 NaCl, 10 Cs-HEPES, 2 EGTA, 0.2 $MaCl_2$, 2 MgATP, 0.3 Na_3GTP, and 5 QX314Br \(pH 7.2, osmolarity 295 mOsm) in the presence of NBQX\(25 μM), CGP52342\(5 μM), and D-AP5\(50 μM). 7.Whole-cell current-clamp recordings were performed to obtain input-output characteristics of the neurons with pipettes containing \(in mM): 132.3 K-gluconate, 7.7 KCl, 4 NaCl, 0.5 $CaCl_2$, 10 HEPES, 5 EGTA, 2 MgATP, and 0.5 Na_3GTP. 8. Amplify electrical signals with a MultiClamp 700B amplifier \(Molecular Devices), digitalized at 5 KHz by National Instruments converter board \(BNC-2110, Molecular Devices), acquired and analyzed by WinWCP software \(Univ of Strathclyde). 9. Analyze Series resistance \(R_s), input resistance \(R_i), and membrane capacitance \(c_m) were monitored throughout the recordings. These parameters were obtained in voltage-clamp mode from the current in response to hyperpolarizing voltage steps \(V_{step}=-5 mV). $R_s=V_{step}/I_{peak}$ $R_m=V_{step}/_{Iss}-R_s$ $\_c\_m=\tau\(1/R_s+1/R_i)$ where, I_{peak} is the peak amplitude of the current transient immediately after the step is applied, τ is the decay time constant of the current, and I_{ss} is the steady-state current. Analyze data only when the change in series resistance or input resistance is less than 20% during recording. **Gramicidin-perforated patch recording** 8.Perforated patch recording were conducted with pipettes with a resistance 4-6 MΩ and back-filled with an solution containing \(in mM) 145 KCl, 10 HEPES, 5 ATP-Mg, 0.2 GTP-Na, 2 QX314, and 2 EGTA, adjusted to pH7.2 with KOH. 20μg/ml gramicidin-D\(Sigma) 9.The pipettes tips were first filled with the KCl-based solution described for whole-cell recordings to prevent leakage of the antibiotic while approaching the cell. The pipettes were then back-filled with the same solution containing 20 μg/ml gramicidin. 10. Fresh gramicidin solution was made every 2 hour. 11. After the pipette tip contacted the cell membrane, the Rs was monitored. Recordings were started when the R_s stabilised at 30 to 45 MΩ \(usually within 15-20 min). 12. To get reversal potential of tonic $GABA_A$ currents, the cells were voltage-clamped at -65 mV. Membrane I-V characteristics were then obtained with 2000-ms voltage steps delivered from 90 mV to 70 mV with 10 mV intervals and from 55 mV to 20 mV with 5-mV intervals. 13. I-V characteristics for $GABA_A$ tonic current were obtained as the difference between membrane I-V characteristics in 5 μM GABA and in 100 μM picrotoxin, added sequentially. **Cell-attached experiments for detecting action currents** 1.Pipettes were filled with superfusion solution and loosely attached to the cell membrane to measure interneuron firing without perturbing intracellular ionic concentrations. 2. Recordings were performed in the voltage-clamp mode. The command potential was set to the potential at which the holding current was 0 pA \(0-current potential) to avoid direct cell stimulation by the electrode. 3.The evoked $GABA_A$ mediated postsynaptic conductance were triggered by 200-μs current pulses at 50 μA delivered by a tungsten monopolar electrode placed in the str. radiatum. 4. $GABA_A$ activation was also produced with a 100-μM GABA puff application \(100 ms, 50 psi) using Pneumatic picopump.

Timing

7-8 hours

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