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Determination of Effective Synaptic Conductances Using Somatic Voltage Clamp

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ABSTRACT

By using dynamic clamp to generate synaptic inputs, we demonstrate the deficiency of the traditional slope-and-intercept method for the determination of effective synaptic conductances using somatic voltage clamp, and and we test the effectiveness of the intercept method for the measurement of effective conductance.

EXTERNAL LINK

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MATERIALS TEXT

The preparation of acute hippocampal slices (350 μm thick) from Sprague Dawley rats of postnatal days 15-20 followed a method described in our previous study (Hao etal, PNAS, 2009). The animal experimental protocol was approved by the Animal Use and Care Committee of State Key Laboratory of Cognitive Neuroscience & Learning at Beijing Normal University. In brief, rats were deeply anesthetized by i.p. injection of pentobarbital (30 mg/kg), and the brain was quickly dissected and then incubated in the ice-cold artificial cerebrospinal fluid (aCSF), which was oxygenated with 95%O_2 / 5%CO_2. Coronal hippocampal slices were sectioned with vibratome (VT1200, Leica) and incubated in oxygenated aCSF at 34 degree for 30 min, followed by an incubation at 20-22 degree till the use for the electrophysiological recording. The aCSF contained (in mM) 125 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 1.3 sodium ascorbate, 0.6 sodium pyruvate, 26 $NaHCO_3$, and 11 D-glucose (pH 7.4 bubbled with 95%O_2 / 5% CO_2).

Whole-cell recording was made on the hippocampal CA1 pyramidal cell (PC) in slices in a chamber perfused with the aCSF solution (2 ml/min; 30-32 degree), under an Olympus upright microscope (BX51WI) that was equipped with the differential interference contrast (DIC) and fluorescence optics as well as an infrared camera (IR-1000E, DAGE-MTI). The borosilicate-glass micropipettes were pulled by a Sutter puller (P-1000) and filled by an internal solution containing (in mM) 145 K-gluconate, 5 KCl, 10 HEPES, 10 disodium phosphocreatine, 4 Mg-ATP, 0.3 Na-GTP and 0.2 EGTA (pH 7.3, 295 mOsm). Simultaneous recordings from the cell body and dendrite of a PC followed a procedure reported previously (Davie etal, Nature Protocols, 2006), in which whole cell recording on the soma was first made using a micropipette (3-5 MΩ; with 20 μM Alexa Fluor 488, InvitroGene), followed by another recording on Alexa Flour 488 (green)-labeled apical dendritic arbor at position ~100 μm away from the soma with a micropipette (10-15 MΩ, filled with the internal solution without Alexa Fluor 488). The serial resistance was compensated by >90% using the built-in function of the amplifier MultiClamp 700B (Molecular Devices). Holding potentials of recorded cells were corrected for a calculated liquid junction potential (Barry etal, Journal of Neuroscience Methods, 1994) of ~15 mV. In the dynamic clamp recording experiments, either AMPA type glutamate receptor-mediated excitatory conductance or $GABA_A$ receptor-mediated inhibitory conductance was intracellularly injected to the recorded PCs through the whole-cell recording pipette, using the built-in dynamic-clamp function of a 1401 Power3 digitizer (CED) and the Spike2 software (v5.08; CED). Kinetics of AMPA or $GABA_A$ receptor conductance were in the form of two exponential functions with different rise/decay time constants: 5/7.8 ms for AMPA conductance; 6/18 ms for $GABA_A$ conductance. Their respective reversal potentials, E_{AMPA} and E_{GABAA}, were set as 0 mV and -70 mV. Membrane voltage or current signals were amplified with a MultiClamp 700B amplifier (Molecular Devices), filtered at10 KHz (low-pass), digitalized by an analog-digital converter (1401 Power3, CED) at 50 KHz, and then acquired by the Spike2 software into a computer for further analysis.

1 Clamp the somatic voltage at various levels using voltage clamp, generate simultaneous E and I synaptic input currents using dynamic

clamp on the dendrite, and measure the corresponding synaptic currents arriving at the soma. In this step, one shall exert a well control of the experimental condition such that the E and I synaptic inputs received by the neuron under different holding voltage are approximately the same.

2 Fit a linear relation between the holding voltage and the synaptic current and read out the intercept value from the I-V relation at each time point. Holding potentials of recorded cells shall be corrected for a calculated liquid junction potential (Barry etal, Journal of Neuroscience Methods, 1994).

3 Vary the reversal potential to a different value through dynamic clamp and repeat the first two steps to obtain its corresponding intercept value at each time point.

4 Recover the effective E and I condcutances through the intercepts of the two I-V relations.