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Supplemental Information

Optocapacitive Generation of Action Potentials by Microsecond Laser Pulses of Nanojoule Energy

João L. Carvalho-de-Souza, Bernardo I. Pinto, David R. Pepperberg, and Francisco Bezanilla

Methods

DRG neuron preparation

Dorsal root ganglia were removed from 1-3 days old Sprague-Dawley rats following euthanasia and were immediately placed in ice-cold Dulbecco Modified Eagle Medium (DMEM). The tissue was rinsed multiple times with modified Earle's balanced salt solution (EBSS, see composition below), then digested with 0.25% trypsin in EBSS for 20 minutes at 37° C under mild shaking. The trypsin-treated tissue was then centrifuged, the supernatant was discarded, and the softened tissue was resuspended in EBSS + 10 % FBS for mechanical trituration with Pasteur pipettes of decreasing tip sizes. Following a final centrifugation of the cells and removal of the supernatant, the cells were supplemented with DMEM + 5 % FBS. Cells were seeded into sterilized poly-L-lysine solution-treated glass-bottom culture dishes and allowed to sit for 30 minutes to facilitate DRG neuron adhesion to the glass. A 2.5 ml volume of DMEM + 5 % FBS + 100 U/ml penicillin + 100 µg/ml streptomycin was then added to the dish, and the cells were incubated at 37 °C with 5 % CO_2 until use.

Experimental setup

Dishes containing DRG neurons and bath solution (see composition below) were mounted on a Zeiss IM 35 microscope (Carl Zeiss Microscopy, Thornwood, New York) and visualized through objective lenses ranging from 10x/0.25NA to 40x/0.55NA. Patch pipettes were pulled on a Sutter Instruments P-2000 CO_2 laser micropipette puller (Novata, California) and flame polished to produce approximately 2 MΩ resistances when filled with internal pipette solution (see composition below). An analog waveform from an AD/DA converter board (Innovative Integration SBC-6711-A4D4, Simi Valley, CA) drove the amplifier (Axopatch 200B, Molecular Devices, Sunnyvale, California) to clamp the current through the cell's membrane. The amplifier output, the membrane voltage, was digitized at 16-bit resolution and sampled at 20 kHz by the same AD/DA converter board and stored in a personal

computer for analysis. The AD/DA converter board was controlled by customized software. The laser source used in a given experiment (405 nm diode laser, HangZhou NaKu Technology Co. Ltd, HangZhou, China; 532 nm DPSS laser, UltraLasers, Ontario, Canada; or 785 nm diode laser, Laserglow Technologies, Toronto, Canada) was mounted and aligned to the central axis of the microscope objective. TTL-controlled acousto-optic-modulators were used with 532 nm (NEOS Technologies, Gooch & Housego, PLC., Melbourne, Florida) and 785 nm (Brimrose Corporation of America, Sparks, ND, USA) laser lines to enable presentation of the faster laser pulses. The 405 nm laser line was TTL-modulated directly. In all cases, the power of the laser beam incident on the cell preparation was adjusted with the use of neutral density filters (Thorlabs Inc., Newton, NJ, USA). The focused laser beam, a spot with diameter of about 5 μm (about 1/5 of the cell diameter), was centered on the cell under investigation in the experiments with AuNPs and AuNRs, and on a cell-associated GrP or CNTm particle in experiments with these carbon-based materials.

Materials and solutions

Material suppliers:

Saline components, streptomycin (Cat #: S6501), penicillin (Cat #: 13750) and poly-L-lysine (Cat #: P8920): Sigma-Aldrich, St. Louis, Missouri, USA.

Trypsin (Cat # TRL3): Worthington, Lakewood, New Jersey, USA.

Fetal bovine serum (FBS, Cat # 30-2020): ATCC, Manassas, Virginia, USA.

DMEM (Cat # 21063-029): Life Technologies, Grand Island, New York, USA.

Gold nanorods (AuNRs, Cat # C12-25-780-TS-50) and spherical gold nanoparticles (AuNPs, Cat # C11-20-TS-50): Nanopartz, Inc. Loveland, Colorado, USA.

Graphite particles (GrPs, Cat # SA090315): XG Sciences, Inc. Lansing, Missouri, USA.

Carbon nanotubes meshes (CNTms, Cat # SKU 010402): Cheap Tubes Inc. Cambridgeport, Vermont, USA.

Solutions concentrations are in mM unless otherwise stated:

EBSS: 132 NaCl, 5.3 KCl, 1 NaH_2PO_4, 10 HEPES, 5.5 glucose, pH 7.4.

Patch bath solution: 132 NaCl, 6 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, 5 glucose, pH 7.4.

Patch pipette solution: 150 KF, 10 NaCl, 4.5 $MgCl_2$, 2 ATP, 9 EGTA, 10 HEPES, pH 7.4.

Materials features and their delivery to DRG neurons

The AuNRs used in the present experiments were of diameter 25 nm and length 94 nm (aspect ratio: 3.8), and exhibit strong plasmonic light absorption with a peak at 780 nm. The AuNR preparation contained a solubilizing coating that included streptavidin molecules. However, in the present study, the streptavidin was not intended for use in anchoring the nanoparticles. Rather, the AuNR coating served merely to facilitate AuNR monodispersion in the recording buffer solution. AuNRs were prepared as a 10 nM solution in saline of composition identical to that of the cell bathing solution (12) and delivered to isolated single DRG neurons from a perfusion micropipette. The AuNPs, prepared at a concentration of 50 nM in bathing solution, were similarly superfused onto the DRG neurons.

Because the carbon-based GrP and CNTm particles used here absorb broadly over UV to near-IR wavelengths, we investigated the action of laser pulses at both 405 and 532 nm for GrP-treated cells, and 405 and 785 nm for CNTm-treated cells. The GrPs or CNTms were first suspended in a test tube and sonicated for 5 min in the cell bathing solution at 0.1% and 0.7% w/v concentration, respectively, then allowed to settle. After a 5-min period, a considerable portion of material had settled to the bottom of the test tube, and the remaining suspension consisted largely of GrPs or CNTms of 1-2 μm in size. A 5 μl volume of a suspension of one of these carbon-based particles was then drop-casted onto the DRG neurons in the dish. After 5 minutes, most of the carbon particles were at the bottom of the dish, many of them touching a side or on top of a neuron.

Using whole-cell current clamp, we recorded membrane voltage responses to laser pulses. Unless otherwise indicated, procedures for the presentation of laser pulses to the cells were similar to those previously described (12).