Interfacing Neurons with Carbon Nanotubes: Electrical Signal Transfer and Synaptic Stimulation in Cultured Brain Circuits

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Supplemental Methods

Electrophysiological recordings

To deliver electrical stimulations through the SWNT substrate to neuronal cultured circuits, an unchlorinated Ag electrode-wire was driven by a voltage-controlled stimulus isolator (Digitimer, DS2, UK). The wire contacted the SWNT-layer through silver conducting grease (CW7100, ITW Chemtronics, USA). The area of SWNT-deposited glass in contact with the Ag wire was dried and left unexposed to the extracellular solution by an ad-hoc fluidic mechanical septum, placed within the recording chamber. Preventing any flooding in the dry area avoided direct electrical contact between the wire and the cells or the bulk solution. Under such conditions, neurons could only be extracellularly stimulated through the electrolyte-SWNT interface (Fig. 2B, sketch). The stimulator-reference was connected to the bath-reference electrode as in a monopolar configuration for extracellular stimulation.

Scanning electron microscopy (SEM) and immunocytochemistry

For SEM analysis, cultures (control and SWNT, n=10, each condition; from 5 different culture series) after 8-10 days in vitro, were fixed as described (Lovat *et al.*, 2005). Cultures were then dehydrated and uncoated specimens were used for standard SEM analysis.

For immunocytochemistry, cultures (control, SWNT and poly-d-lysine coated; see Supplemental Fig.1) were fixed as described (Lovat *et al.*, 2005) and subsequently incubated with mouse monoclonal anti-microtubule associated protein 2 (MAP-2; Sigma), at 1:400 dilution. The incubation was for 16 hr, at 4° C in a humid chamber.

Cultures were then processed with the corresponding biotinylated secondary as previously described (Lovat *et al*., 2005). Slides were viewed with a Zeiss Axioskop (Germany) microscope equipped with a CCD camera (Optronics, Italy). The morphological data were obtained using the Image pro express program (Media Cybernetics, Des Moines, IA). We observed 8 different coverslips in every growth condition and not less than 30 different fields per coverslips were analyzed. Between 200 and 250 cells per condition were selected at random for analysis and digital images of the MAP-2 positive cells were captured. We quantified the area of the positive-cell body and the number of processes exiting each cell. For these measurements only clearly distinguishable isolated neurons were chosen. A neuronal process was defined as a tapering, MAP-2-stained process that was greater than 30 μm in length.

Data acquisition and analysis

Signals, filtered (10 kHz), were sampled (20 kHz), digitized (Digidata 1200) and analyzed with a Clampex 8.1 software (Axon Instruments). Membrane capacitance and R_{IN} were measured under voltage-clamp (Lovat *et al.*, 2005). Spontaneous action potential (APs) frequency was monitored across epochs of at least 400 s (pClamp 9; Axon Instruments). Numerical values in the text are means ± s.e.m., with n=number of neurons, unless stated otherwise.

Modeling of neuronal excitability and of the neuron-nanotubes junction

Neuronal modeling and numerical computer simulations were performed using the NEURON simulation environment (Carnevale and Hines, 2006). Single-compartmental conductance-based model, incorporating leakage and voltage-dependent Na^+ and K^+ conductances, was based on the hippocampal model neuron introduced by Traub and Miles (1991). Resistive and capacitive electrical circuits, were also simulated in NEURON and coupled to charge-balance membrane equations, as equivalent models of pipette-neuron and SWNT-electrolyte-neuron-pipette interactions, following (Schoen and Fromherz, 2007; Bockris *et al.*, 2000; Grattarola and Martinoia, 1993; Geddes, 1972) and basic concepts from electrochemical impedance spectroscopy (Girault, 2004).

Supplemental References

Bockris JO'M, Reddy AKN, Gamboa-Aldeco M, (2000) Modern Electrochemistry 2B, 2nd edition, Kluwer Academic/Plenum Publishers, New York.

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Geddes, LA (1972) Electrodes the Measurement of Bioelectric Events. New York: Wiley-Interscience.

Schoen I, Fromherz P. (2007) The mechanism of extracellular stimulation of nerve cells on an electrolyte-oxide-semiconductor capacitor. Biophys J 92(3):1096-111.