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Highly conductive carbon nanotube matrix accelerates developmental chloride extrusion in central nervous system neurons by increased expression of chloride transporter, KCC2**

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Supplementary File

Supplementary Figure Legends

Figure 1. X-ray photoelectron spectroscopy (XPS) of fwCNT preparation

XPS analysis demonstrates high level of purity of fwCNT. XPS of the fwCNT preparation reveals the presence of four elements, C, O, N and Ca. Note the absence of trace element contamination. The element contents are listed on the upper left of the spectrum. A small amount of calcium with 0.26 atm% (atomic percent), which likely represents a contaminant of the deionized water used during purification steps, is the only metallic element detectable in the spectrum, indicating a very high level of purity of our fwCNT preparation.

Figure 2. Control experiments for intraneuronal chloride

a Lack of influence on intraneuronal chloride by SiO_x-nanowires. Left-hand panels show a scanning electron micrograph of SiO_x-nanowires (i), spray-coating of SiO_x-nanowires to cell culture substrate (ii), a clomeleon-transfected cortical neuron on SiO_x-nanowires (iii), and the control neuron on pDL matrix (iv). The right-hand bar graph shows clomeleon-based intraneuronal chloride concentrations (DIV2), there was no difference between groups ($n \geq 25$ neurons/group). Note that SiO_x-nanowires were dispersed in GA, identical to treatment of fwCNT.

b Lack of influence on intraneuronal chloride by gum arabic (GA) and filtrate from fwCNT GA-coating. The bar diagram depicts rather a moderate (GA-coating) or more solid (coating with filtrate) increase of clomeleon-based intraneuronal chloride concentration (DIV2; $n \geq 200$ neurons per group).

Figure 3. Dependence of accelerated chloride shift on VGCC and CaMKII

a Confocal micrograph of pDL control-cultured primary cortical neurons, indicating bodipy-DHP binding. In contrast to main ms. Fig. 4a, this image was acquired using the green fluorescent channel of the confocal microscope. Note that the green laser leads to prohibitive levels of background activity with fwCNT matrix (which is not the case with regular fluorescent microscopy, as shown in main ms. Fig. 4b), which prompted use of the red laser to demonstrate DHP binding to fwCNT-cultured neurons with confocal microscopy. Also note that this image was acquired at a vastly increased level of sensitivity of acquisition as compared to main ms. Fig. 4a.

b Higher magnification of confocal micrograph of fwCNT-cultured primary cortical neurons (main ms. Fig. 4a) showing DHP binding, and detail of the polar expression of L-type voltage-gated calcium channels (VGCC).

c Detection of Cav1.1, 1.3 and 1.4 by immunocytochemistry. Cultures were probed by immuno-labeling for the VGCC isoforms Cav1.1, 1.3 and 1.4. Transcripts of *Cav1.2* channels could not be detected. Cav1.1 had a very low signal for pDL-controls. Note increased level of expression of the three channel isoforms for neurons on fwCNT matrix, in keeping with increased fluorescent DHP-binding, see Supplementary Figs. 3a-b and main ms. Fig. 4a.

d Dependence of acceleration of the chloride shift and upregulation of KCC2 in fwCNT-cultured primary neurons on CaMKII. Shown here are our data obtained when using a second specific CaMKII inhibitor, KN62 (1µM).

e Confocal micrograph panel, double-labeling for KCC2 and ß3-tubulin. Our findings illustrate the strict dependence of upregulation of KCC2 in fwCNT-cultured primary neurons on CaMKII,

by use of specific inhibitor KN93 (1µM) (for its effects, see main ms. Fig. 4d), in contrast to the relative lack of effect of KN93 in Au-cultured neurons.

Supplementary Figures

Figure 1

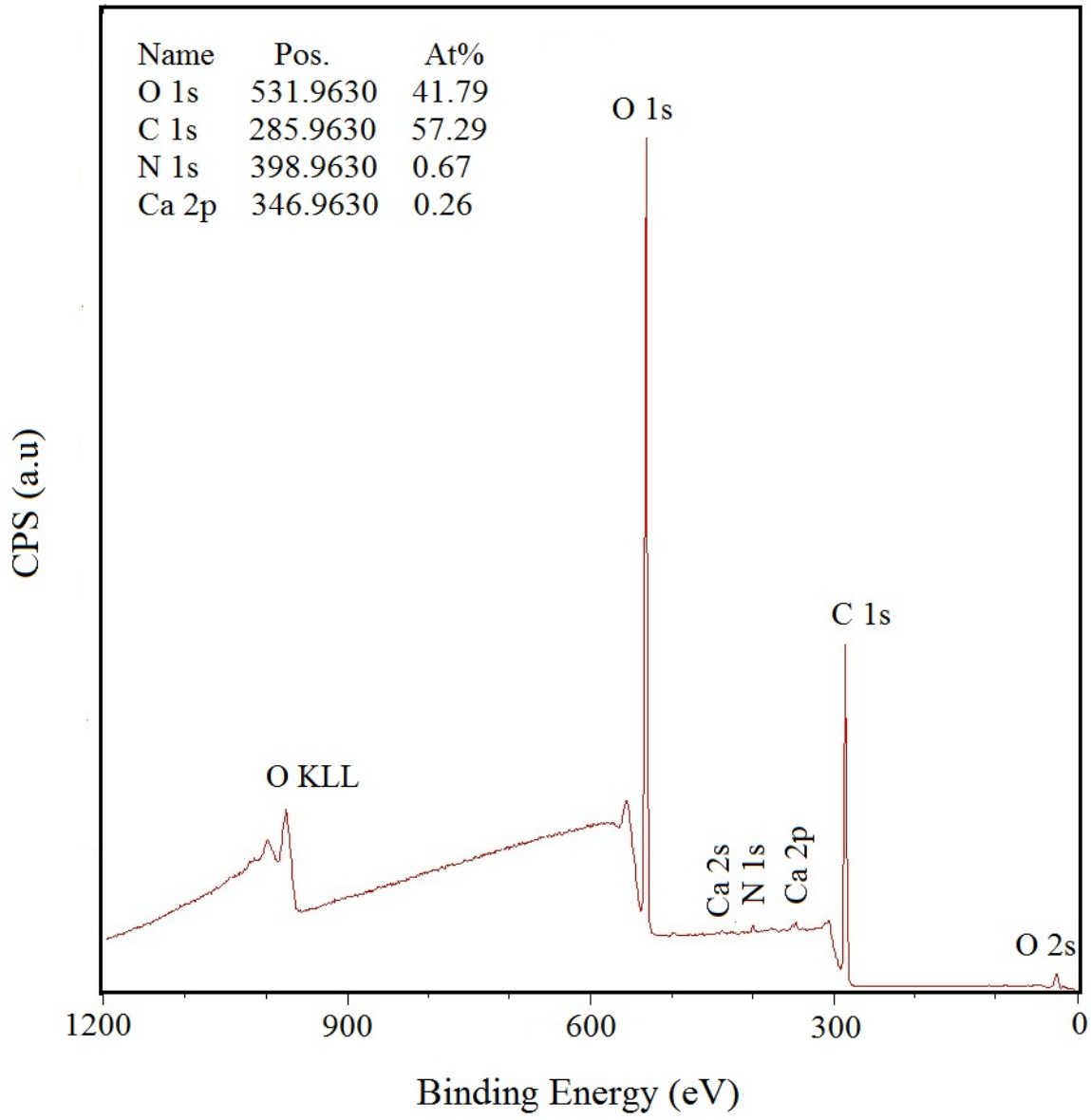

Figure 2

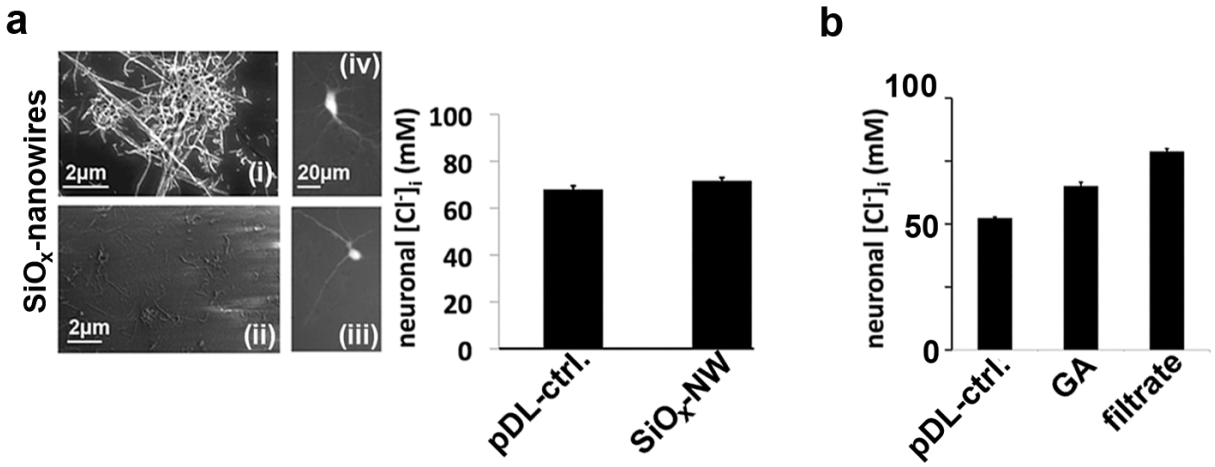

Figure 3