Supplementary Material for:

BDNF and Huntingtin protein modifications by Manganese: Implications for striatal medium spiny neuron pathology in manganese neurotoxicity

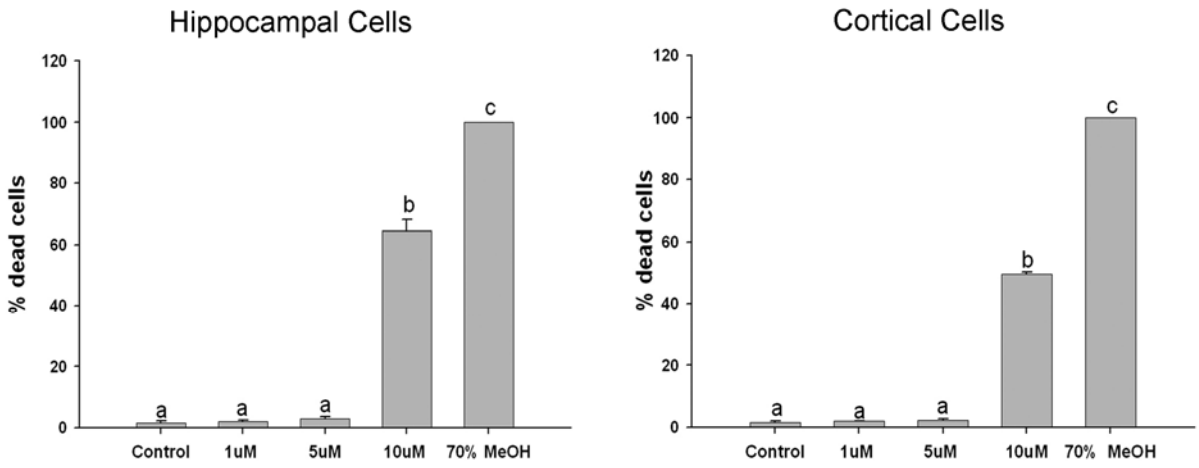
Kirstie H. Stansfield[1], Terry Jo Bichell[2], Aaron B. Bowman[2],

and Tomás R. Guilarte[1*]

[1]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY.

[2]Department of Neurology, Center for Molecular Toxicology and Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN.

(*) Correspondence to:
Tomás R. Guilarte, PhD
Department of Environmental Health Sciences
Columbia University
Mailman School of Public Health
722 West 168th Street, Room 1105E
New York, NY 10032
Phone: 212-305-3959
Fax: 212-305-3857
E-mail: trguilarte@columbia.edu



Supplementary Figure 1.

Cytotoxicity of Mn and methanol (MeOH): Both 1 and 5 μM of Mn were determined to be noncytotoxic in both hippocampal and cortical neurons based on the Live/Dead Viability/ Cytotoxicity assay. After exposure to 0, 1, 5 and 10 μM Mn for 5 days *in vitro*, hippocampal cell viability (% dead cells) was 1.5 ± 0.6%, 2.11 ± 0.3%, 3.07 ± 0.4% and 64.5 ± 3.5%, respectively and for cortical cell viability was 1.6 ± 0.3%, 2.0 ± 0.1%, 2.3 ± 0.4% and 49.4 ± 0.6%, respectively. Cell viability after exposure to excitotoxic levels of methanol (70%) for 20 minutes, used as a positive control (*n* = 3 independent assays).

Methods for Supplementary Figure 1: LIVE/DEAD® Cell Viability Assay: LIVE/DEAD® Cell Viability Assays were performed as per the manufacturer's instructions (Molecular Probes, Invitrogen). Briefly, hippocampal and cortical neurons were plated on glass coverslips at 14,000 cells/cm^2. Cells were exposed to vehicle, 1 μM, 5 μM or 10 μM Mn for 5 days or to 70% methanol for 20 minutes as a positive cytotoxic control. After the exposure period, 100 μl of the LIVE/DEAD reagents (2 μM calcein AM and 4 μM ethidium homodimer-1) were added to the coverslips for 30 min at RT. Coverslips were viewed under a fluorescent scope and number of live and dead cells were counted.