SUPPLEMENTARY MATERIAL

Exposure to e-cigarette aerosol over two-months induces accumulation of neurotoxic metals and alteration of essential metals in mouse brain

Diane B. Re[1-3‡*], Markus Hilpert[1,2*], Brianna Saglimbeni[1-3], Madeleine Strait[1-3], Vesna Ilievski[1,2], Maxine Coady[1,4], Maria Talayero[1,2], Kai Wilmsen[1,4], Helene Chesnais[1], Olgica Balac[1,2], Ronald A. Glabonjat[1,2], Vesna Slavkovich[1,2], Beizhan Yan[2,5], Joseph Graziano[1,2], Ana Navas-Acien[1,2] and Norman J. Kleiman[1,2‡]

[1]Department of Environmental Health Sciences, Columbia University, New York, NY 10032, USA
[2]NIEHS Center for Environmental Health in Northern Manhattan, Columbia University, New York, NY 10032, USA
[3]Center for Motor Neuron Biology and Disease, Columbia University, New York, NY 10032, USA
[4]Master in Public Health Program, Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, 10032, USA
[5]Lamont-Doherty Earth Observatory, Geochemistry Department, 203 Comer, 61 Route 9W - PO Box 1000
Palisades NY 10964-8000, USA
[*]Co-first authors equally contributed to this work

[‡]Corresponding authors:
Diane B. Re, 630 W 168th St, P&S Bldg. Suite 16-421B, New York, NY 10032, USA. Email: dr2240@cumc.columbia.edu (**Editorial office contact**)
Markus Hilpert, 722 W 168th St, ARB Suite 1107C, New York, NY 10032, USA. Email: mh3632@cumc.columbia.edu
Norman Kleiman, 722 W 168th St, ARB Suite 1113, New York, NY 10032, USA. Email: njk3@cumc.columbia.edu

Abbreviations: As, Arsenic; C, Carbon; Cr, Chromium; Cu, Copper; Ga, Gallium; Ir, Iridium; Pb, Lead; Lu, Lutetium; Hg, Mercury; Mn, Manganese; Ni, Nickel; QC, quality control; Rh, rhodium; Sb, Antimony; Se, Selenium; Si, Silicon; Sn, Tin; Sr, Strontium; Tl, Thallium; U, Uranium; V, Vanadium; W, Tungsten; Zn, Zinc; CNS, Central Nervous System; DRC, dynamic reaction cell; ICP-MS, inductively coupled plasma mass spectrometry; MDL, Method detection limits; MQL, Method quantitation limits.

A. PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) analysis was performed with R version 4.03 using the “ggfortify” package. Metal concentrations were centered and scaled to obtain zero mean and uniform standard deviation. A factor loading, which measures the correlation between an original variable (here metal level) and a principal component (PC), was considered to be highly correlated with the PC if the absolute value of the loading exceeded 0.3 (Field, 2013). Overall, PCA of the distributions of metals in the 15 mice confirmed the dose responses observed in the box plots for certain metals individually. The controls separate well from the low- and high-dose exposure groups in several CNS areas including the striatum (ST) and the ventral midbrain (VM) (**Figure S14**).

For the ST, Cr, Cu, Ni, Pb and Se were highly correlated with PC1 (**Table S2**). Since the median score of the high-dose group was positive and the scores of the control and low-dose groups were negative, mice exposed to the high aerosol dose therefore tended to exhibit the highest levels of these metals in the ST. Moreover, PCA suggests a common source of these five metals and common transport of the metals via the same pathway from the nose to the ST. The common source is likely the inhaled e-cig aerosol. Indeed, in this and a previous study (Zhao et al., 2019) that used the same e-cig device also operated with a Kanthal™ coil, Cr, Cu, Ni and Pb were found in the e-cig aerosol. Among these four metals, Cr is the second most abundant element in the Kanthal™ heating coil of the e-cig, and Cu, Ni and Pb are also common components of e-cigarette hardware.

For the VM, the controls also separate from the exposed groups. Cr, Fe, Ni, Pb and Sr were highly correlated with PC1. Since the median scores of the exposed groups were positive and the median score of the control was negative, exposure to this group of five metals was associated with higher metal levels in the VM. Among these five metals, Fe and Cr are the two most abundant components of the Kanthal^{TM} e-cig heating coil. However, also Ni and Pb are common components in e-cigarette hardware.

It is striking that both in the ST and VM, Cr, Ni and Pb, which are neurotoxic or carcinogenic, are part of PC1. This similarity perhaps arises, because the two tissues are anatomically and functionally connected via the striatonigral pathway (Bartholomew et al., 2016).

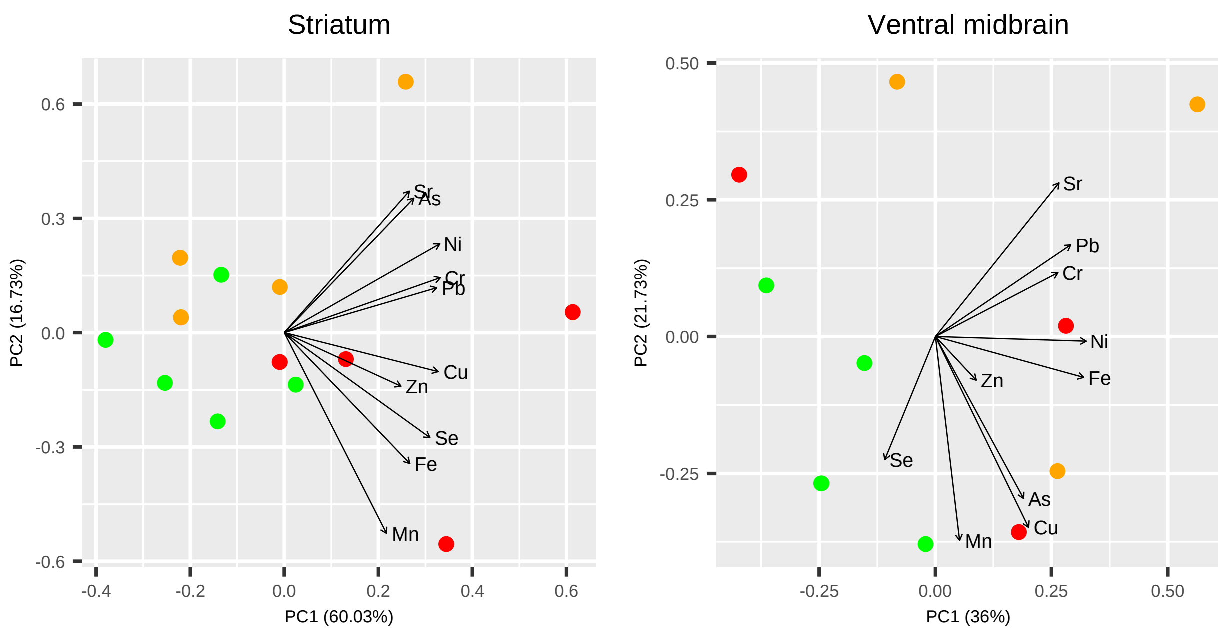

Figure S14: Principal component analysis of the distribution of metals in the 15 mice for two selected brain tissues. Green, orange, and red circles indicate the three exposure groups: control, low dose, and high dose, respectively. Arrows indicate factor loadings of the 10 tissue metal concentrations which are for the most part above their MDLs. Some data points are missing because they were identified as outliers.