Tracking the pathway of diesel exhaust particles from nose to brain by micro-X-ray florescence analysis

The work presented here is the result of tracking diesel exhaust particles (DEPs) in the olfactory nerve by detecting the metal elements included in those particles using micro-X-ray fluorescent analysis based synchrotron radiation (SR-XRF). Nanomaterials are already being widely used in medical products, such as dressings for injuries and wounds, dental-bonding agents, sunscreens, and cosmetics. It is also common in industrial products such as fuel cells, tires, tonal cartridges, clothing, and electronics. Nanosized particles (NPs) are more dangerous than microsized particles [1]. DEPs are well known for containing many NPs. Most of the particles emitted by engines are NPs with a diameter less than 50 nm in the nuclei mode. Most of the mass is from 50 nm to 1 μm in the accumulation mode. DEPs consist mainly of a highly agglomerated solid carbonaceous material and ash, as well as volatile organic and sulfur compounds. Metal compounds in the fuel and lube oil result in a small amount of inorganic ash. Recent studies have shown that after inhalation of metal NPs, an increase in the quantity of metal is evident in both the olfactory bulb and olfactory cortex [2]. These facts suggest that NPs can invade the olfactory nerve from the olfactory mucosa and are consequently transported to the central nervous system (CNS) via olfactory nerves (Fig. 1). However, recent studies have not shown that the NPs actually exist in olfactory nerves, which leads to the possibility that metal NPs could have been ionized in the nasal mucosa first, and then transported to the CNS as metal ions afterwards. It is proposed in further studies that NPs reach the olfactory bulb through cerebrospinal fluid enveloped by olfactory ensheathing cells [3]. The purpose of this study was reveal whether metal elements in DEPs are detectable in the olfactory epithelium and olfactory bulb after whole-body exposure to DEPs. By employing the method of micro-XRF, we were able to identify the metal elements included in DEPs and generate gradation maps showing the levels of metal elements within the minute tissue layers of the olfactory organ.

The 3D image in Fig. 1 (left: sagittal plate, right: coronal plate) is a micro-CT image measured using the table top system (inspeXio SMX-90CT, Shimadzu). The coronal section in the 3D image clearly shows the otherwise complex anatomy of the nasal cavity. The main area of the olfactory epithelium consists of three major cell types: (1) the olfactory cell, which forms the most superficial layer with support cells (olfactory cell layer: OCL), (2) one type of basal cell, which is a stem cell, and (3) another type of basal cell, which makes up the basal membrane (BM). The lamina propria (LP) consists of Bowman's glands, bundles of olfactory axons, and blood vessels of both small arteries and veins. The main olfactory bulb consists of six types of tissue layer: olfactory neuron layer (ONL), glomerular cell layer (GL), external plexiform layer, mitral cell layer (ML), internal plexiform layer, and granular cell layer. The first olfactory neurons at the olfactory epithelium form a bundle at the lamina propria, projecting to the glomerular cell layer at the olfactory bulb. Many synapses are in the glomerular cell layer and connect to higher neurons. Their cell bodies line up at the mitral cell layer and project to the nucleus of the amygdala and the piriform cortex in the brain. Given the invasion of the NPs into the olfactory nerves from their dendrites, the NPs can be transported to the olfactory bulb by axonal flow. Furthermore, in case the NPs pass the synapses in the glomerular cell layer according to the neurotransmission, the NPs could be transported to the amygdala and the piriform cortex.

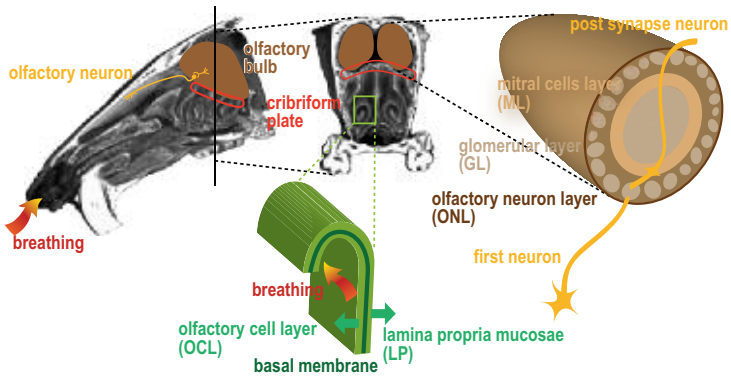

Fig. 1. Anatomy of the olfactory organ. CT image shows sagittal plate (left) and dose coronal plate (right) in lab mouse.

After the whole-body DEP exposure for four weeks, the mice were anesthetized by pentobarbital sodium, at which point they were transcardially perfused with phosphate-buffered saline and then fixed with 4% formaldehyde. DEPs were generated by a four-cylinder light-duty diesel engine (2740 cc, Isuzu) removed from a truck in the DEP inhalation facility at the National Institute for Environmental Studies, Japan [4]. The DEP concentration in the chamber was 0.1 mg/m^3 and the count median diameter was 40 nm. The olfactory bulb and epithelium were removed and prepared using a microslicer to a sectional thickness of 100 μm. Micro-XRF imaging was performed at beamline **BL37XU**. A white X-ray from the undulator was monochromatized to 10 keV using a Si (111) double-crystal monochromator and then was adjusted to a fine beam (1.6 H × 2.0V μm^2) using a Kirkpatrick-Baez (K-B) mirror. A silicon drift detector (SDD) was used to measure fluorescence X-ray intensity [5].

The major metal components measured in the Teflon filter that is used for removing NPs or viruses and for collecting the diesel exhaust particles were calcium, copper, iron, nickel and zinc. Figure 2 shows an SR-XRF spectrum of the filter. Figure 3 shows micro-XRF images of the olfactory epithelium and bulb. The gradation maps of iron (a,c) and nickel (b,d) were superimposed on optical microscope images. Fluorescence intensities in high level areas observed in the exposure group were more than 7 times stronger than those observed in the non-DEP exposure group. High-concentration areas marked by arrows appear as red in the LP in the exposure group (arrows, Fig. 3(a)). It is suggested that these high-concentration areas were caused by DEP exposure because all the elements were observed in those areas but not in the non-exposure group. These high concentration areas possibly correspond to the axon bundle of the first olfactory neurons in the LP and perhaps move to the olfactory bulb by axonal transport. In the result of the olfactory bulb, iron shows high-level areas at the ONL and GL, and nickel is observed on the ONL. One nickel high-level area was observed in the GL (arrow, Fig. 3(d)). Something probably existed as organic compounds or remained as DEPs at the terminal of the first neuron in the GL. In addition, the concentrations of iron and zinc were slightly higher in the ML after DEP exposure, showing that these metal elements were transported to the second neurons just beyond the synapse.

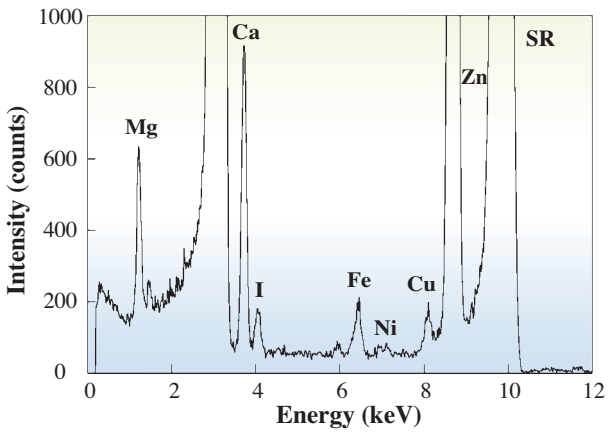

Fig. 2. XRF spectra of diesel exhaust nanosized particles (median diameter: 40 nm).

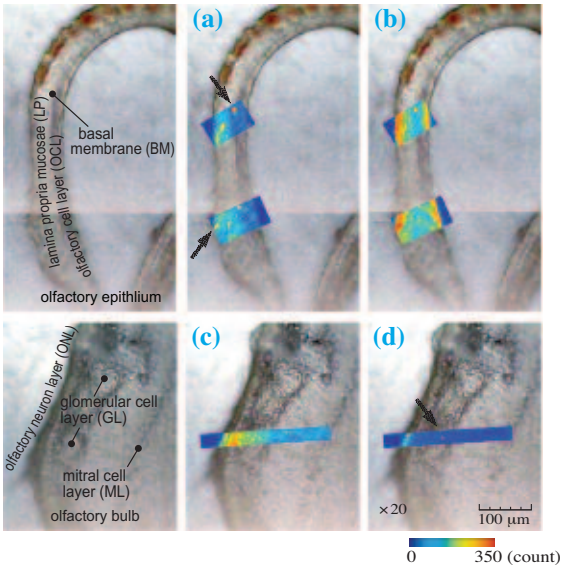

Fig. 3. Elemental mapping images of iron **(a, c)** and nickel **(b, d)** at the olfactory epithelium (upper) and olfactory bulb (lower) after DEP exposure. Exposure air included gaseous components.

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