Accumulation pattern of intranasally installed metal oxide nanoparticles in the mouse olfactory bulb

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Certain nanomaterials of organic or inorganic nature, as well as some viruses, are capable of translocating from the nasal cavity into the brain bypassing blood-brain barrier (BBB). For example, nanoparticles (NP), such as carbon, gold, metal (manganese, iron, titanium) oxides, etc., could reach deep brain structures after administration into the nasal cavity. Upon intranasal administration, NPs accumulate mainly in the structures of olfactory system, especially in olfactory bulbs and anterior olfactory nuclei. On the one hand, NPs are attractive “platform” for the development of non-invasive nose-to-brain drug delivery systems, and magnetic resonance imaging (MRI) or radiocontrast agents for neuroimaging. But, on the other hand, potential neurotoxicity of air dispersed nano-pollutants raises significant concerns as a risk factor for dementia and other neurological disorders. Thus, it is crucial to detail accumulation patterns of nose-to-brain transport of NPs both qualitatively and quantitatively. In this study, we investigated the distribution of NPs through the layers of mouse olfactory bulb after their intranasal administration. To address this, we prepared negatively charged manganese oxide nanoparticles (MnFe₂O₄-NPs) and platinum oxide particles (PtO-Ps), which were efficiently transmitted from the nasal cavity into the brain. Because the intensity of T1-weighted MRI signal of MnFe₂O₄-NPs strongly correlated with Mn concentration in brain structures, this allowed...
high-resolution quantitative tracking of NP’s brain trafficking with 11.7 T MRI. To estimate the extrapolation of the obtained distribution patterns, we intranasally administered PtO-Ps, available for detection in the brain tissues by transmission electron microscopy (TEM) and micro-X-ray fluorescent analysis (micro-RFA).

To compare the values of the MRI signal with the level of MnFe$_2$O$_4$-NPs accumulation in the brain tissues, the Pepric Particle Spectrometer (PPS) based on the Particle Electron Paramagnetic Resonance (pEPR) technique was used (Gobbo et al., 2015). The MnFe$_2$O$_4$-NPs concentration data in the main olfactory bulb (MOB) highly correlated with the T1-weighted MRI signal ($r=0.96$).

The distribution of the MRI contrast in 24 hours after the intranasal administration of MnFe$_2$O$_4$-NPs reflected a greater accumulation of nanoparticles in the glomerular and mitral layers of the olfactory bulbs formed by synaptic endings of olfactory neurons and mitral nerve cells, respectively (Fig. 1A). The plexiform layer formed by dendrites of mitral neurons and tuft cells had a significantly lower contrast level. These particle distribution patterns were reproduced when carrying out the analysis of MOB paraffin slices applying the micro-RFA method, cut out and fixed in 24 hours after intranasal administration of PtO-Ps (Fig. 1A). Moreover, none of 57 detected PtO-Ps was detected in the extracellular space. In such case, nanoparticles were localized in bodies of neurons, axons, mitochondria and in close proximity to synapses (Fig. 1B).

The diameter of the intranasally administered PtO-Ps varied from 50 to 500 nm (133 ± 110 nm, Fig. 1C), but the diameter of the particles detected in the brain structures did not exceed 200 nm (Fig. 1C). To evaluate the effect of particle size on olfactory transport of MnFe$_2$O$_4$ we compared accumulation in MOB of nanosized and submicron particles (MnFe$_2$O$_4$-NPs and MnFe$_2$O$_4$-Ps, consequently). Only manganese particles with a diameter of less than 150 nm effectively moved from the nasal cavity into the deep regions of the brain. Particles with a diameter > 300 nm (352 ± 70 nm) were not at all detected in the brain tissues (Fig. 1D). This results were confirmed using PPS. Similar results were obtained in experiments with intranasal administration of Ir, carbon and Fe$_2$O$_3$ particles (Kreyling et al., 2009, Braakhuis et al., 2014, Wang, Wang et al. 2016). Thus, the obtained results make it possible to assume that there is a possibility of capture from the nasal cavity, transport and transsynaptic transmission of particles whose diameter is <200 nm, but exceeding the dimensions of synaptic vesicles (50-80 nm).

Thus, using MRI, micro-RFA and TEM imaging of MOB distribution of intranasally administered paramagnetic MnFe$_2$O$_4$-NPs and electron-dense PtO-Ps, we obtained experimentally substantiated arguments in favor of transcellular and trans-synaptic transport of metal oxide nanoparticles from the nasal cavity into the brain. The significance of the olfactory path, for the NP penetration into the brain contributes to deciphering pathogenesis mechanisms of a number of infectious and neurodegenerative diseases of the CNS. Considering that not only pathogens but also therapeutic agents can be delivered the CNS through the olfactory pathway, a
description of the spatial patterns of NP nose-to-brain transport creates the basis for targeted delivery of nanoscale drugs to the affected brain structures.

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Fig. 1. Comparison of accumulation patterns of intranasally administered MnFe$_2$O$_4$-NPs and PtO-Ps in MOB structures. (A) Left, Typical profile of the MRI signal distribution along the coronary section of MOB 24 hours after the intranasal MnFe$_2$O$_4$-NP administration (upper part). T1-weighted images reflecting the MRI signal distribution in MOB departments 24 hours after the intranasal MnFe$_2$O$_4$-NP administration (bottom part). The intensity of the signal is reflected in the scale of pseudo-staining. Manganese free areas within the brain are marked in blue. Right, the platinum content profile along the MOB coronary section 24 hours after the intranasal PtO-Ps administration measured with micro-RFA. The result of scanning spaced at intervals of 10 μm, the time of signal set at the point of 300 s, the spot diameter on the sample is ~ 10 μm. The obtained data were processed by the linear smoothing method at 5 points. EPL - external plexiform layer, GL - glomerular layer, GrL - granular cell layer, ML - mitral cell layer. (B) Detection of intranasally instilled PtO-Ps in MOB using TEM. MOB samples were cut out and fixed 24 hours after administration of PtO-Ps (n = 3). All detected PtO-Ps had intracellular localization. (C, D) Size-dependent olfactory transport of NPs. (D) Size distribution of PtO-Ps in colloidal solution (n = 100) and in mouse brain cells (n = 57). (C) T1-weighted MRI images
reflecting changes in the signal level in MOB 24 hours after a single intranasal administration of MnFe$_2$O$_4$-NPs (d$_{\text{aq}}$ ~ 100 nm) and MnFe$_2$O$_4$-Ps (d$_{\text{aq}}$ ~ 350 nm). By means of pseudocoloring, the spatial patterns of manganese accumulation in MOB are indicated on the scans. Manganese free areas within the brain are marked in blue.