The Olfactory Epithelium as a Gateway for Bloodborne Nanoparticles to the Central Nervous System

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MATERIALS AND METHODS

MTT Assay

To estimate the toxicity of Mn_3O_4-NPs and PtO-NPs, two cell lines were used: rat pheochromocytoma PC12 cells (ATCC CRL-1721) and non-tumorigenic human fibroblasts BJ-5ta (ATCC CRL-4001). Cells preserved in liquid nitrogen were thawed according to standard protocol and cultured in DMEM/F12 (1:1; Biolot, Russia) supplemented with 10% fetal bovine serum (FBS; Biolot, Russia) at 37 °C in plastic flasks (Jet Biofil, China) under humidified conditions with 5% CO_2. For passaging, cells were detached using a 0.25% trypsin and 0.02% versene solution (1:1; Biolot, Russia), and enzymatic activity was quenched by adding complete growth medium. Cell concentration was determined using a Countess automated cell counter (Invitrogen, USA). For the MTT assay, cells were seeded into 96-well plates (SPL, Korea) at 20,000 cells per well and incubated for 24 h at 37 °C, 5% CO_2 (Sanyo MCO-5A incubator). Test NPs were added at an initial concentration of 30 μg/mL and serially diluted in twofold steps (six replicates per concentration). Cells were incubated for 96 h under the same conditions. After incubation, the medium was replaced with 90 μL of serum-free medium and 10 μL of MTT solution (5 mg/mL in PBS; Dia-M, Russia). Following a 2-hour incubation, the solution was

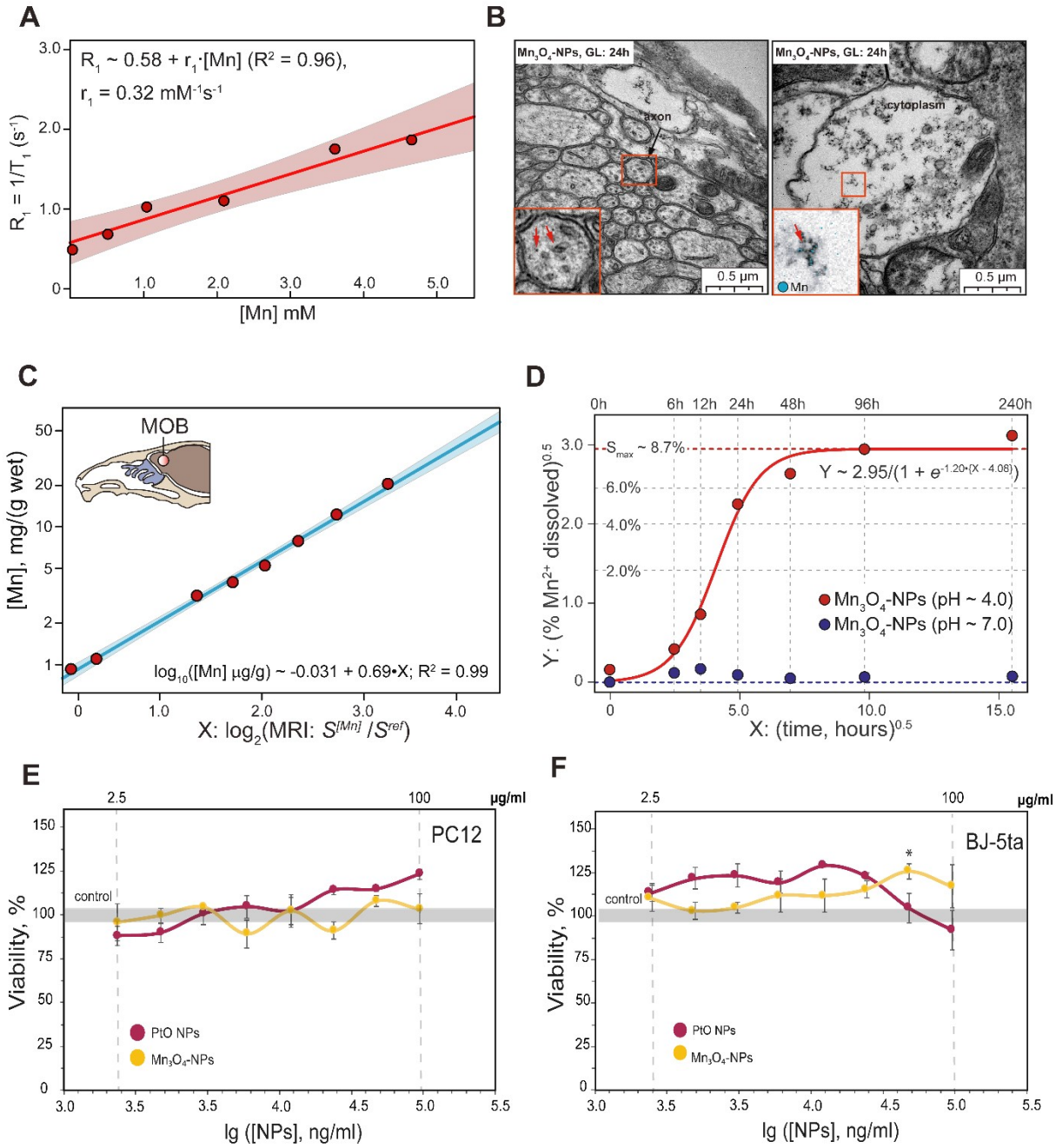
removed, formazan crystals were dissolved in DMSO (Biolot, Russia), and absorbance was measured at 595 nm using a Multiskan SkyHigh plate reader (Thermo Fisher Scientific, USA). Cell viability was calculated relative to the untreated control, which was set at 100%. To confirm that the intrinsic light absorption of the NPs would not interfere with the MTT assay, we characterized their UV-vis spectra. Both Mn_3O_4-NPs and PtO-NPs exhibited a single absorption peak, with maxima at approximately 290 nm and 320 nm, respectively. Since the MTT assay measures formazan absorbance at 595 nm—a wavelength distant from the NP absorption peaks—we concluded that their optical properties did not significantly confound the assay results.

MRI signal dynamics modelling

MRI scans were performed before administration and at 3, 6, 9, 12, 24, 48, 96, and 168 h post-administration. The MRI contrast was quantified as the $\log_2$ ratio of the signal intensity after ($S^{[Mn]}$) to before (S_0) Mn_3O_4-NP administration: $\log_2(S(t)) = \log_2(S^{[Mn]}/ S_0)$. This model assumed that the MRI signal logarithm log(S) increases linearly over time T with rate R_{acc} until it reaches a peak at time T_{max}. Then log(S) starts decreasing with the rate Rdec, so the model equation becomes:

$\log(S(T)) = \log(S(0)) + R_{acc} * T + H(T - T_{max}) * R_{dec} * T + N(0, \sigma)$, where H is Heaviside step function (if $x <= 0$ then $H(x) = 0$ else $H(x) = 1$), $N(0, \sigma)$ is a normally distributed residual. Fig S2A shows some model predictions with 95% prediction band.

RESULTS



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media	d_{aq}, nm	ζ, mV	*r1*, $mM^{-1}*s^{-1}$	*r2*, $mM^{-1}*s^{-1}$
mQ	142±23	-28±2,1	0,322±0,02	7,6±1,4
saline	227±57	-18±8,1	0,22±0,05	6,8±1,1
saline+BSA	89±45	-28±8,5	1,28±0,07	6,5±5,1
blood	N/A	N/A	0,89±0,04	7,1±1,0
blood serum	N/A	N/A	1,36±0,05	6,8±1,1
nasal mucus	113±36	-23±2,5	1,282±0,04	7,1±1,8
cytoplasme	89±45	-28±8,5	1,96±0,07	6,5±5,1

Figure S1. Characterization of metal oxide NPs. (A) Estimation of longitudinal relaxivity r1 of the Mn_3O_4-NPs in water. (B) TEM analysis of Mn_3O_4-NP localization in mouse main olfactory bulb (MOB). (C) The relation between the concentrations of [Mn] in the MOB (μg per g wet tissue, as determined by ICP-AES) and normalized T1-weighted MRI signals, assessed 24 h after the intranasal administration of 7 μL of Mn_3O_4 -NPs at various concentrations (0, 2, 4, or 6 mg/mL). (D) *In vitro* solubility of the Mn_3O_4-NPs. To estimate the % of dissolved [Mn], the NPs were incubated at neutral and acidic pH for indicated time points, dialyzed, and the remaining insoluble [Mn] content was measured by the ICP-AES. At neutral pH, the Mn_3O_4-NPs were insoluble (blue line). (E, F) Influence of Mn_3O_4 NPs and PtO NPs concentration on rat pheochromocytoma PC12 (E) and non-tumorigenic human fibroblasts BJ-5ta (F) cell viability in the MTT assay. (G) Hydrodynamic diameter (d_h, nm), zeta potential (ζ, mV), longitudinal relaxivity r1 ($mM^{-1} \cdot s^{-1}$) and transverse r2 ($mM^{-1} \cdot s^{-1}$) of the Mn_3O_4-NPs in various biological media. The biological media were prepared in accordance with previously described methods [1-3]. Cytoplasmic extracts were prepared from mouse olfactory bulb tissue.

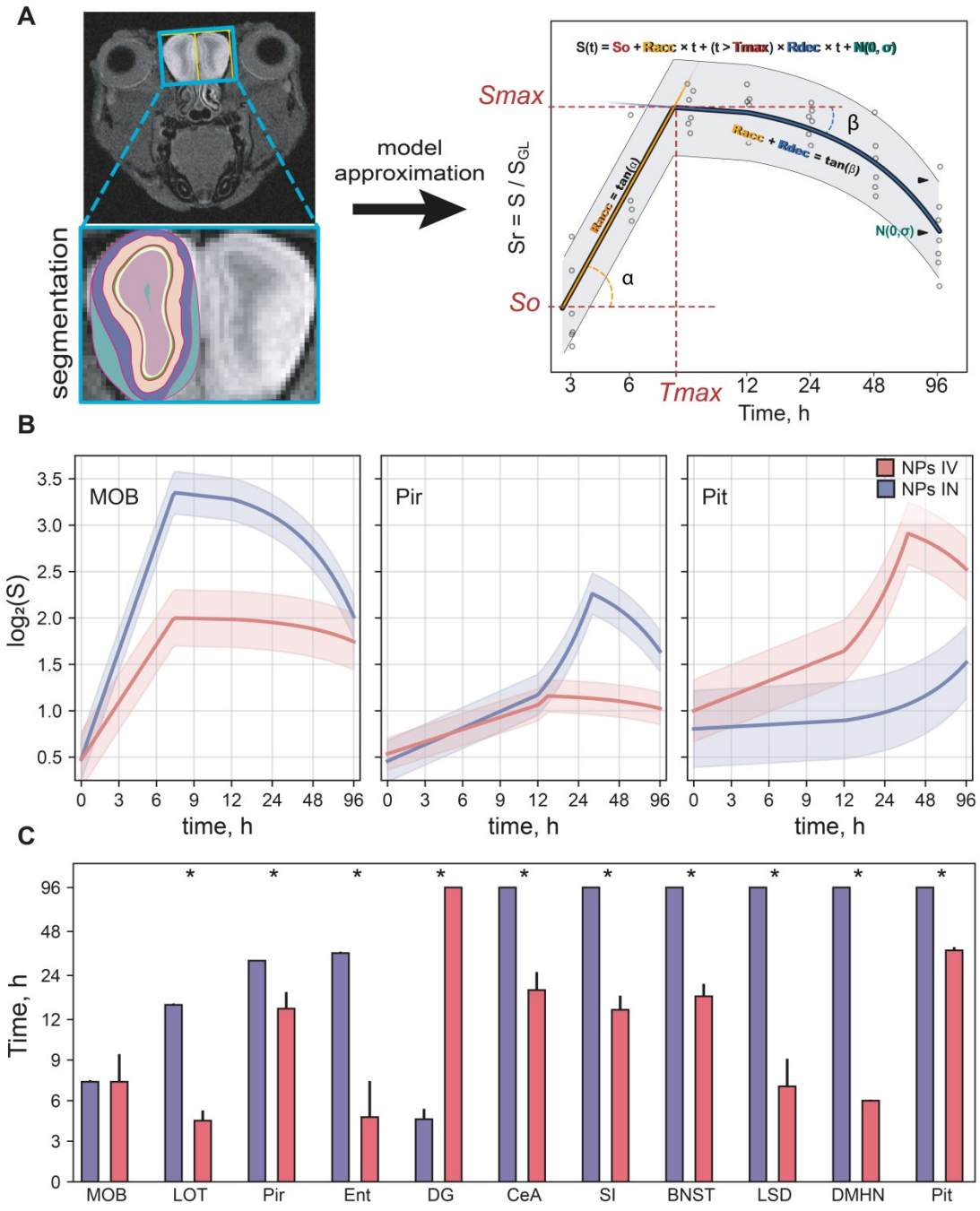

Figure S2. Comparison of Mn_3O_4 NP dynamics in the mouse CNS following IN and IV administration. (A) Mathematical model approximation of the time-dependent change in T1-weighted MRI signal within the mouse CNS after IV and IN administration of Mn_3O_4 NPs. Data points represent measurements taken at 3, 6, 9, 12, 24, 48, 96, and 168 hours post-administration. (B) Normalized T1-weighted MRI signal intensities for key brain structures (dashed lines, n=6 animals per time point) were fitted to a four-parameter model (see Methods for details). (C) Time to maximum T1-weighted MRI signal intensity (T_{max}) across different brain structures following IN and IV administration of Mn_3O_4 NPs. Abbreviations: MOB – main olfactory bulb, Pir – piriform cortex, Ent – entorhinal cortex, DG – dentate gyrus, CeA – Central amygdalar nucleus, VP/SI –

ventral pallidum/substantia innominata, BNST – bed nuclei stria terminalis, DMHN – dorsomedial nucleus of hypothalamus, Pit – pituitary. * - p<0.05 between routes (Student's t-test).

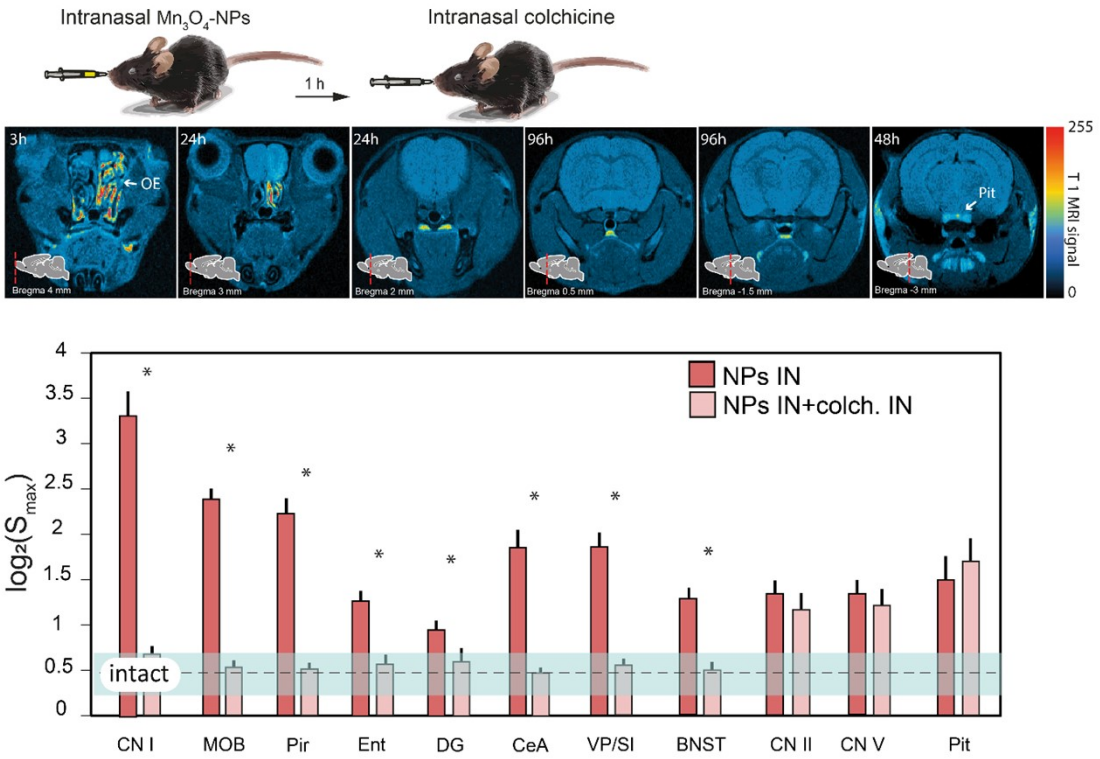

Figure S3. Role of axonal and non-axonal transport mechanisms in Mn_3O_4-NP accumulation in the mouse CNS after IN administration. Representative T1-weighted MR images of mouse brain after Mn_3O_4-NPs IN injection with a follow-up IN colchicine instillation; images above are selected from time points in which T1 MRI signal elevation is near the maximum for the marked structures; images are color-mapped in the identical way, red color marks super-intensive signal, which corresponds to high NPs concentration, blue color marks near-baseline signal. Whiskers mark standard error; pre-administration baseline signal level is marked by dashed line. * - $p < 0.05$ t-test significant differences between colchicine and non-colchicine groups (Student's t-test).

References:

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