Supplementary Material

Lysosomal iron liberation is responsible for the vulnerability of brain microglial cells to iron oxide nanoparticles: comparison with neurons and astrocytes

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Figure S.1: Physicochemical properties of IONPs. Fluorescent dimercaptosuccinate (DMSA)-coated IONPs were dispersed in water (a,c,e) or glia-conditioned medium (b,d,f) at a concentration of 1 mM (a-f) or 3 mM (e,f) and analyzed by transmission electron microscopy (a,b), energy dispersive X-ray spectroscopy (c,d) and dynamic light scattering (e,f). The scale bar in panel b represents 5 nm and applied to panels a and b.

Figure S.2: Consequences of a 24 h exposure of cultured astrocytes and neurons to IONPs. Neurons (a,b) and astrocytes (c,d) were incubated for 24 h with up to 3 mM iron as IONPs in glia-conditioned medium. Cell viability was assessed by measuring cellular LDH activity (a,c) and MTT reduction capacity (b,d). The data present means $\pm$ SD from values obtained on three independently prepared astrocyte or neuron cultures. Asterisks indicate significant differences compared to control cells that had been incubated in the absence of IONPs (** $p < 0.01$, *** $p < 0.001$).

Figure S.3: Localization of IONPs in astrocytes and neurons after 24 h exposure to IONPs. Cultured astrocytes (a-d) or neurons (e-h) were incubated for 24 h with 0.3 mM BP-DMSA-IONPs in glia-conditioned medium followed by a 90 min incubation with 100 nM lysotracker. Cells were washed with PBS and fixed with 3.5% (w/v) paraformaldehyde prior to microscopy. Co-localization of IONPs and lysotracker is indicated by yellow color (c,g) and by arrows (a-c, e-g). The scale bar in panel h represents 25 μm and applies to all panels.

Figure S.4: Staining for reactive oxygen species (ROS) in cultured astrocytes and neurons after 24 h exposure to IONPs. Cultured astrocytes (a-j) or neurons (k-t) were incubated for 24 h with the indicated concentrations of iron as IONPs in glia-conditioned medium. Subsequently, the cells were incubated for 15 min with dihydrorhodamine 123 to test for ROS generation and with H333342 to stain all nuclei. As positive control for ROS production, cells were incubated for 23 h 45 min with 100 μ M ferric ammonium citrate, followed by an incubation for 15 min with 1 mM H_2O_2 in HEPES-buffered incubation buffer (20 mM HEPES, 5 mM glucose, 1.8 mM CaCl_2 , 1 mM MgCl_2 , 5.4 mM (astrocytes) or 30.4 mM (neurons) KCl, 145 mM NaCl, pH 7.4). The scale bar in t represents 100 μ m and applies to all panels.

Table S.1. Raw data determined for the average hydrodynamic diameter, the polydispersity index and the zeta-potential of BP-DMSA-IONPs dispersed in concentrations of 1 mM or 3 mM in water or glia-conditioned medium.

		[IONP]	n	repetition during measurement							total	
				1	2	3	4	5	6	mean	mean	SD
hydrodynamic diameter (nm)	H ₂ O	1 mM	1	47	50	47	46	44	44	46	44	2
			2	41	44	45	44	40	43	43		
			3	42	43	42	43	42	43	42		
		3 mM	1	49	50	51	48	48	50	49	47	2
			2	44	46	44	45	44	46	45		
			3	48	46	47	47	47	48	47		
	GCM	1 mM	1	81	80	81	81	80	83	81	90	15
			2	112	110	113	114	108	111	111		
			3	77	77	79	80	81	81	79		
		3 mM	4	86	88	86	89	89	91	88	158	28
			1	190	186	187	186	185	190	187		
			2	154	151	143	151	152	147	150		
polydispersity index	H ₂ O	1 mM	3	124	122	123	127	124	127	124	0.190	0.017
			4	172	176	172	169	178	169	172		
			1	0.221	0.084	0.258	0.231	0.234	0.202	0.205		
		3 mM	2	0.198	0.124	0.164	0.159	0.218	0.17	0.172	0.186	0.031
			3	0.189	0.161	0.202	0.197	0.196	0.219	0.194		
			1	0.167	0.139	0.19	0.223	0.227	0.248	0.199		
	GCM	1 mM	2	0.234	0.151	0.225	0.218	0.222	0.195	0.208	0.297	0.029
			3	0.095	0.194	0.162	0.136	0.165	0.151	0.151		
			4	0.318	0.324	0.297	0.307	0.359	0.343	0.325		
		3 mM	1	0.319	0.318	0.298	0.31	0.268	0.281	0.299	0.263	0.025
			2	0.274	0.276	0.279	0.269	0.255	0.25	0.267		
			3	0.271	0.275	0.289	0.269	0.319	0.288	0.285		
zeta-potential (mV)	H ₂ O	1 mM	2	0.238	0.208	0.271	0.208	0.22	0.238	0.231	0.263	0.025
			3	0.24	0.267	0.25	0.231	0.287	0.256	0.255		
			4	0.278	0.278	0.287	0.289	0.28	0.276	0.281		
		3 mM	1	-38.44	-42.79	-26.82	-31.36			-35	-39	8
			2	-24.4	-24.23	-30.67	-52.39			-33		
			3	-36.6	-57.6	-54.65	-55.27	-36.67		-48		
	GCM	1 mM	1	-36.11	-36.91	-57.23	-54.08	-54.18		-48	-50	3
			2	-55.55	-55.95	-55.38	-46.81	-54.1		-54		
			3	-34.06	-56.99	-57.02	-59.9	-36.93		-49		
		3 mM	1	-8.18	-7.76	-8.08	-7.84	-9.14		-8	-10	4
			2	-7.84	-8.9	-8.03	-7.39	-8.21		-8		
			3	-13.62	-14.85	-14.03	-15.68	-14.73		-15		
		3 mM	1	-8.03	-8.38	-8.59	-8.56	-8.27		-8	-10	2
			2	-10.37	-9.98	-10.14	-9.61	-10.29		-10		
			3	-12.29	-12.23	-12.24	-12.06	-11.93		-12		