

Increased alpha-synuclein and neuroinflammation in the substantia nigra triggered by systemic inflammation are reversed by targeted inhibition of the receptor for advanced glycation end products (RAGE)

Daniel Oppermann Peixoto^{1,2}, Reykla Ramon Bittencourt¹, Juciano Gasparotto³, Flávio Gabriel Carazza Kessler¹, Pedro Ozorio Brum⁴, Nauana Somensi¹, Carolina Saibro Girardi¹, Lucas dos Santos da Silva¹, Tiago Fleming Outeiro^{5,6,7,8}, José Cláudio Fonseca Moreira¹, Daniel Pens Gelain^{1*}

¹ Centro de Estudos em Estresse Oxidativo, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde – Universidade Federal do Rio Grande do Sul (ICBS-UFRGS), 90035-003, Porto Alegre, RS, Brazil.

² Instituto de Neurociencias, Universidad Miguel Hernández – Consejo Superior de Investigaciones Científicas (UMH-CSIC), 03550, Sant Joan d'Alacant, Alicante, Spain.

³ Instituto de Ciências Biomédicas – Universidade Federal de Alfenas (ICB-UNIFAL), 37130-001, Alfenas, MG, Brazil.

⁴ Max F. Perutz Labs, University of Vienna, 1030, Vienna, Austria.

⁵ Department of Experimental Neurodegeneration, Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany.

⁶ Max Planck Institute for Natural Sciences, 37075 Göttingen, Germany.

⁷ Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Framlington Place, Newcastle Upon Tyne, NE2 4HH, UK.

⁸ Scientific employee with an honorary contract at Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Göttingen, Germany.

Correspondence

Daniel Pens Gelain, Centro de Estudos em Estresse Oxidativo, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde – Universidade Federal do Rio Grande do Sul (ICBS-UFRGS), 90035-003, Porto Alegre, RS, Brazil. Email: daniel.gelain@ufrgs.br, ORCID: <https://orcid.org/0000-0001-5254-0509>

	r^2	p-value
Total Distance Traveled (session 1)	0.0171	0.3809
Total Distance Traveled (session 2)	0.0001	0.9418
Time in the Corners (session 1)	0.0271	0.2688
Time in the Corners (session 2)	0.0021	0.7588
Time in the Center (session 1)	0.0271	0.2686
Time in the Center (session 2)	0.0021	0.7583
Freezing Episodes (session 1)	0.0607	0.0968
Freezing Episodes (session 2)	0.0006	0.8686
Freezing Time (session 1)	0.0034	0.6954
Freezing Time (session 2)	0.0032	0.7025

Table S1. Linear regression analysis. Correlation between weights and all parameters measured on the open field test.

Staining - Brain Region	ANOVA		Tukey's <i>post hoc</i> test (p-values)					
	F (3,32)	p-value	Control vs FPS	Control vs LPS	Control vs LPS+FPS	FPS vs LPS	FPS vs LPS+FPS	LPS vs LPS+FPS
RAGE - SNpc	27.680	<0.0001	0.9918	<0.0001	0.8446	<0.0001	0.9498	<0.0001
RAGE - CA1sp	9.211	0.0002	0.9856	0.0011	0.9998	0.0004	0.9747	0.0014
RAGE - CA3sp	6.132	0.002	0.9641	0.014	0.9951	0.0041	0.9952	0.0076
RAGE - LIIEC	4.720	0.0077	0.9998	0.0228	>0.9999	0.019	0.9999	0.0225
RAGE - DG/GCL	2.953	0.0473	0.9955	0.1019	0.9992	0.0624	0.9835	0.1323
RAGE - DG/Hilus	1.579	0.2136	0.7668	0.6388	0.9992	0.1568	0.8345	0.5588
RAGE - DG/ML	2.722	0.0606	0.9662	0.0936	>0.9999	0.2263	0.9519	0.0822
GFAP - SNpc	13.760	<0.0001	0.8166	<0.0001	0.8862	0.0002	0.9987	0.0001
GFAP - CA1sp	1.319	0.2854	0.8163	0.2618	0.9838	0.7561	0.9541	0.4444
GFAP - CA3sp	1.789	0.1691	0.419	0.1283	0.5397	0.8925	0.9969	0.7975
GFAP - LIIEC	1.576	0.2143	>0.9999	0.481	0.9156	0.4625	0.9261	0.1769
GFAP - DG/GCL	1.286	0.2961	0.8577	0.2528	0.9631	0.6937	0.9893	0.5028
GFAP - DG/Hilus	2.573	0.0712	0.9863	0.0872	0.3988	0.1703	0.6004	0.8232
GFAP - DG/ML	2.771	0.0575	0.9516	0.1255	0.1285	0.3175	0.3235	>0.9999
Iba1 - SNpc	9.657	0.0001	0.938	0.0008	0.8084	0.0001	0.4662	0.0085
Iba1 - CA1sp	14.970	<0.0001	0.9991	<0.0001	0.0944	<0.0001	0.1236	0.0085
Iba1 - CA3sp	3.961	0.0165	0.9947	0.028	0.9964	0.0495	>0.9999	0.0461
Iba1 - LIIEC	2.444	0.0821	0.9993	0.175	0.9851	0.2191	0.9639	0.0881
Iba1 - DG/GCL	1.357	0.2736	0.9891	0.5596	0.9553	0.3766	0.9971	0.2784
Iba1 - DG/Hilus	0.307	0.8196	>0.9999	0.8492	0.9937	0.829	0.9903	0.9458
Iba1 - DG/ML	2.321	0.0939	0.8541	0.0828	0.338	0.3511	0.8022	0.8657
S100B - SNpc	10.080	<0.0001	>0.9999	0.0002	0.3779	0.0002	0.3977	0.0175
HMGB1 - SNpc	7.570	0.0006	>0.9999	0.0015	0.901	0.0016	0.9093	0.0097
S100B - CA1sp	2.428	0.0835	0.9861	0.3075	0.8862	0.1723	0.9803	0.0804
HMGB1 - CA1sp	11.630	<0.0001	0.9978	0.0002	0.8554	<0.0001	0.7453	0.0013
TH - SNpc	1.271	0.3008	0.9524	0.401	0.9974	0.7156	0.8892	0.3021
α Syn - SNpc	25.340	<0.0001	0.9238	<0.0001	0.1131	<0.0001	0.3387	<0.0001

Table S2. Immunofluorescence statistics. Rats were treated intranasally with FPS-ZM1 for two weeks, starting 70 days after the LPS injection (2 mg/kg, i.p.). Brains were collected on day 90 post LPS injection, and were followed by immunofluorescence analysis. Tukey's *post hoc* tests were conducted following one-way ANOVA testing. All groups had an n = 9, where n indicates the number of animals (biological replicates). Degrees of freedom are represented as F (DFn,DFd). Abbreviations: SNpc – substantia nigra pars compacta; CA1sp – stratum pyramidale of CA1; CA3sp – stratum pyramidale of CA3; LIIEC – layer II of the entorhinal cortex; DG – dentate gyrus; GCL – granule cell layer; ML – molecular layer; GFAP – glial fibrillary acidic protein; Iba1 – ionized calcium-binding adapter molecule 1; S100B – S100 calcium-binding protein B; HMGB1 – high mobility group box 1 protein; TH – tyrosine hydroxylase; α Syn – α -synuclein.

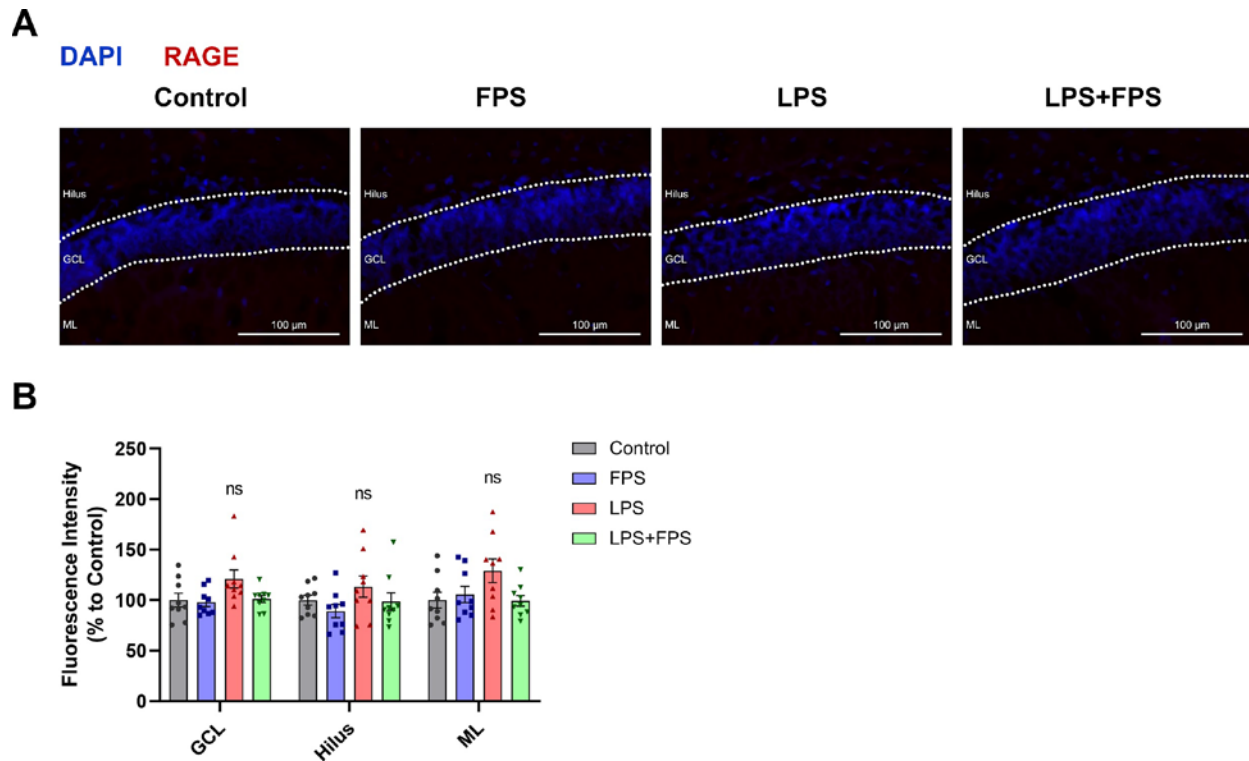
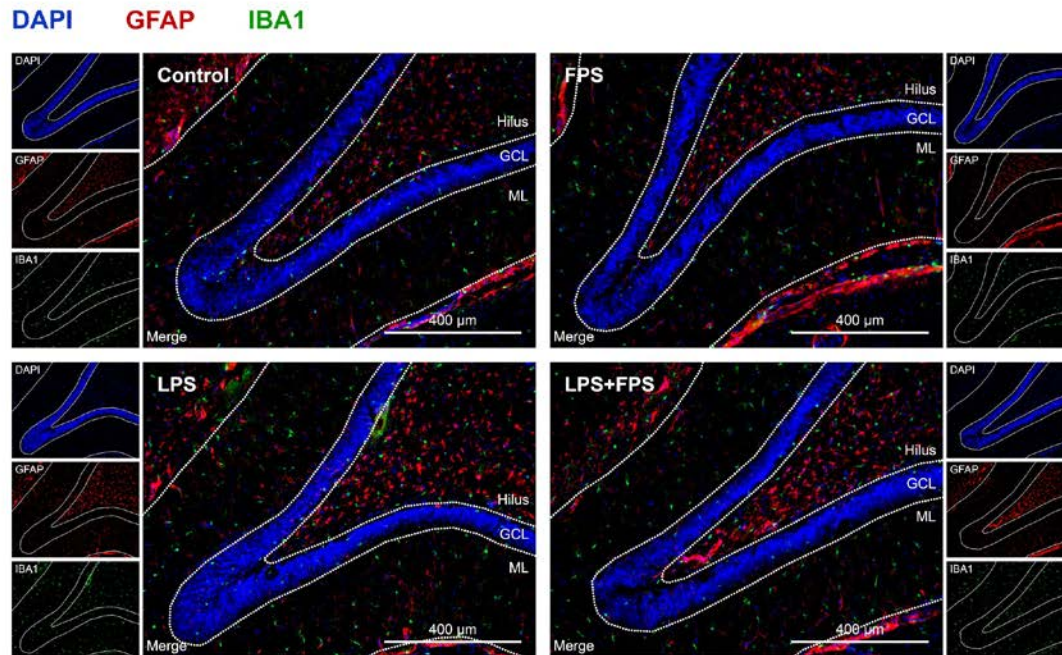


Figure S1. Immunofluorescence analysis of RAGE in the granule cell layer (GCL), hilus and molecular layer (ML) of the hippocampal dentate gyrus. Rats were treated intranasally with FPS-ZM1 for two weeks, starting 70 days after the LPS injection (2 mg/kg, i.p.). Brains were collected on day 90 post LPS injection. (A) Representative micrographs of RAGE (red) and DAPI (blue) immunostainings in different regions of the dentate gyrus. Color channels, groups and scale bars are embedded in the figures. (B) Representative graphs of the quantification of RAGE fluorescence intensity. All groups had an $n = 9$, where n indicates the number of animals (biological replicates). Values are represented as mean $\pm$ SEM. Statistical analyses were carried out through one-way ANOVAs, followed by Tukey's *post-hoc* test. ns - not significant.

A



B

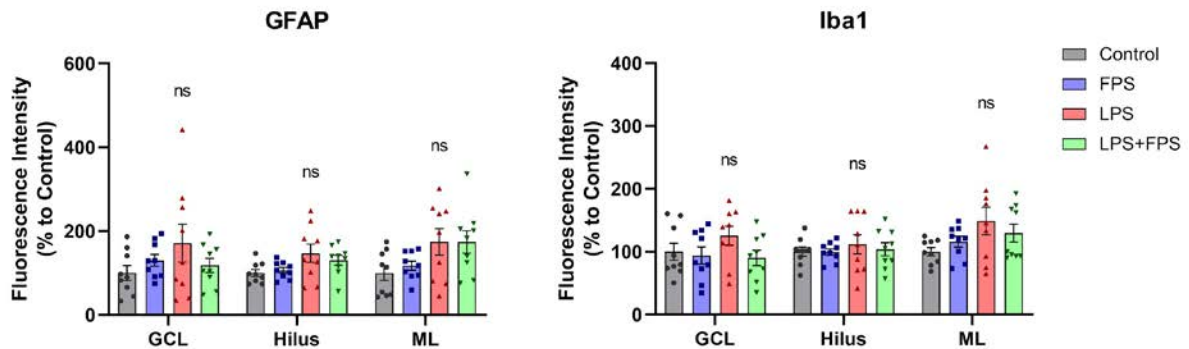


Figure S2. Immunofluorescence analysis of GFAP and Iba1 in the granule cell layer (GCL), hilus and molecular layer (ML) of the hippocampal dentate gyrus. Rats were treated intranasally with FPS-ZM1 for two weeks, starting 70 days after the LPS injection (2 mg/kg, i.p.). Brains were collected on day 90 post LPS injection. (A) Representative micrographs of GFAP (red), Iba1 (green) and DAPI (blue) immunostainings in different regions of the dentate gyrus. Color channels, groups and scale bars are embedded in the figures. (B) Representative graphs of the quantification of GFAP and Iba1 fluorescence intensity. All groups had an $n = 9$, where n indicates the number of animals (biological replicates). Values are represented as mean $\pm$ SEM. Statistical analyses were carried out through one-way ANOVAs, followed by Tukey's *post-hoc* test. ns - not significant.

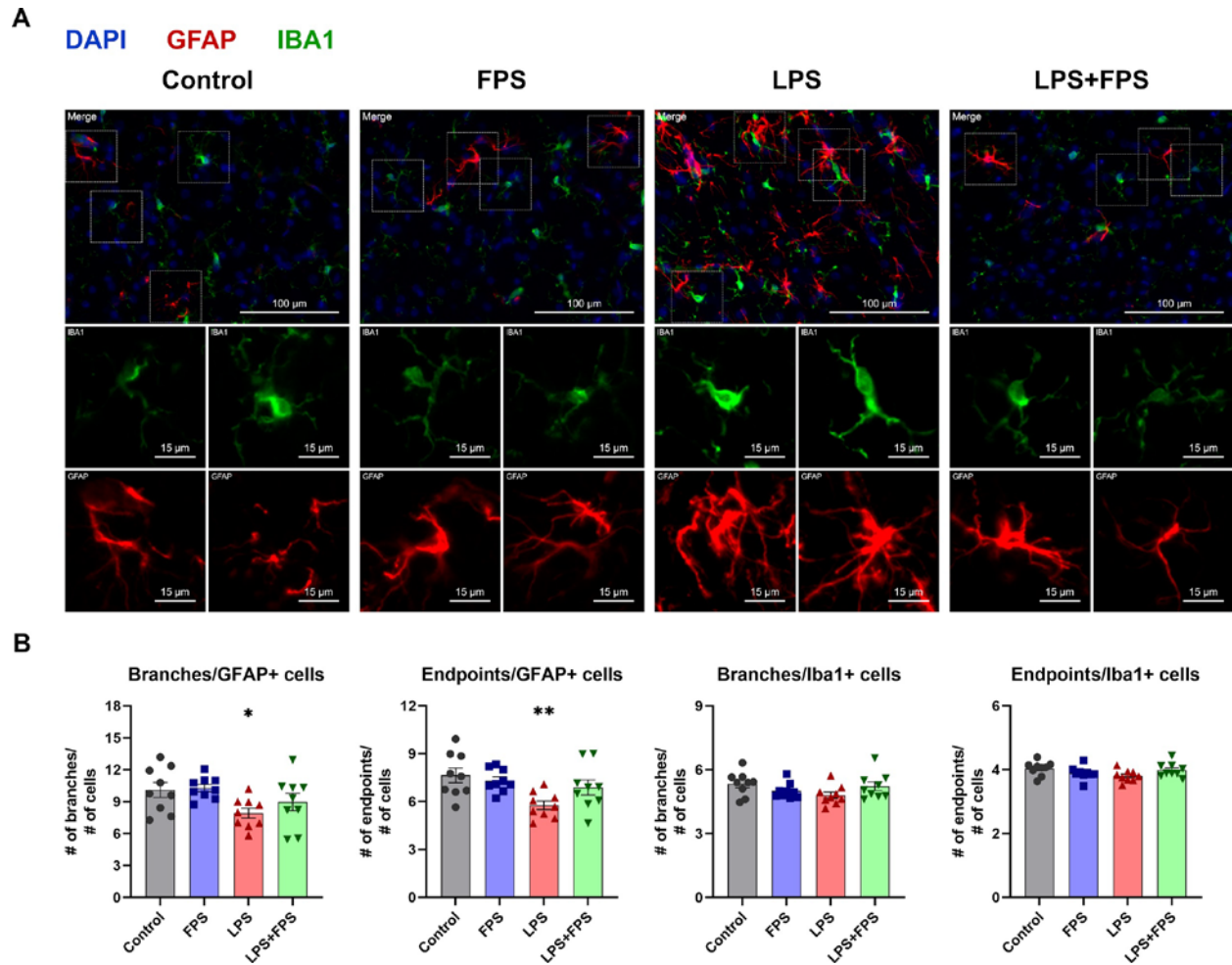


Figure S3. Chronic neuroinflammation promoted by LPS is suppressed by encephalic RAGE blockade – cell detail and morphology analysis. Immunofluorescence analysis of glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1) in the substantia nigra pars compacta (SNpc). Rats were treated intranasally with FPS-ZM1 for two weeks, starting 70 days after the LPS injection (2 mg/kg, i.p.). Brains were collected on day 90 post LPS injection. All groups had an $n = 9$, where n indicates the number of animals (biological replicates). (A) Representative micrographs of GFAP (red), Iba1 (green) and DAPI (blue). Color channels, groups and scale bars are embedded in the figure. Dotted boxes indicate the magnified cells. (B) Representative graphs of the quantification of GFAP and Iba1 cell morphology. Values are represented as mean $\pm$ SEM. Statistical analyses were carried out through one-way ANOVAs, followed by Dunnet's *post-hoc* test. * p -value < 0.05 ; ** p -value < 0.01 .