Gut brain axis in neurodegenerative disorders: pathophysiological and pharmacological implications

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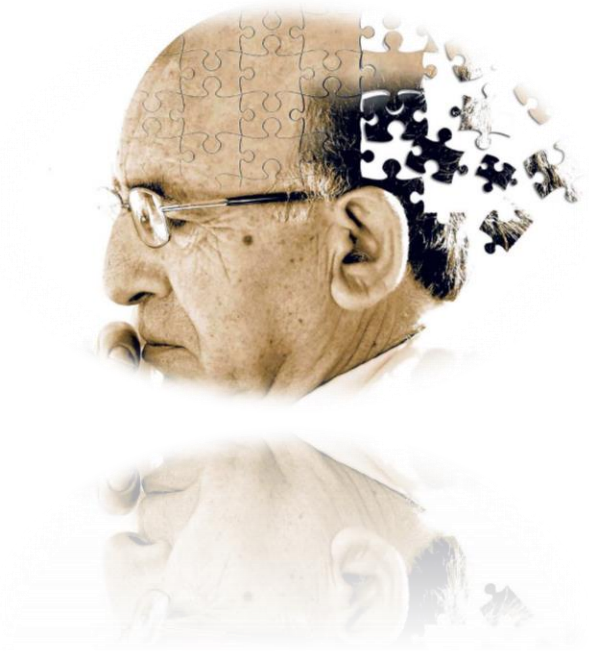
NEURODEGENERATIVE DISORDERS

- ALZHEIMER'S DISEASE (AD)
- PARKINSON'S DISEASE (PD)
- HUNTINGTON'S DISEASE (HD)
- AMYOTROPHIC LATERAL SCLEROSIS (ALS)
- MULTIPLE SCLEROSIS (MS)

ALZHEIMER'S DISEASE

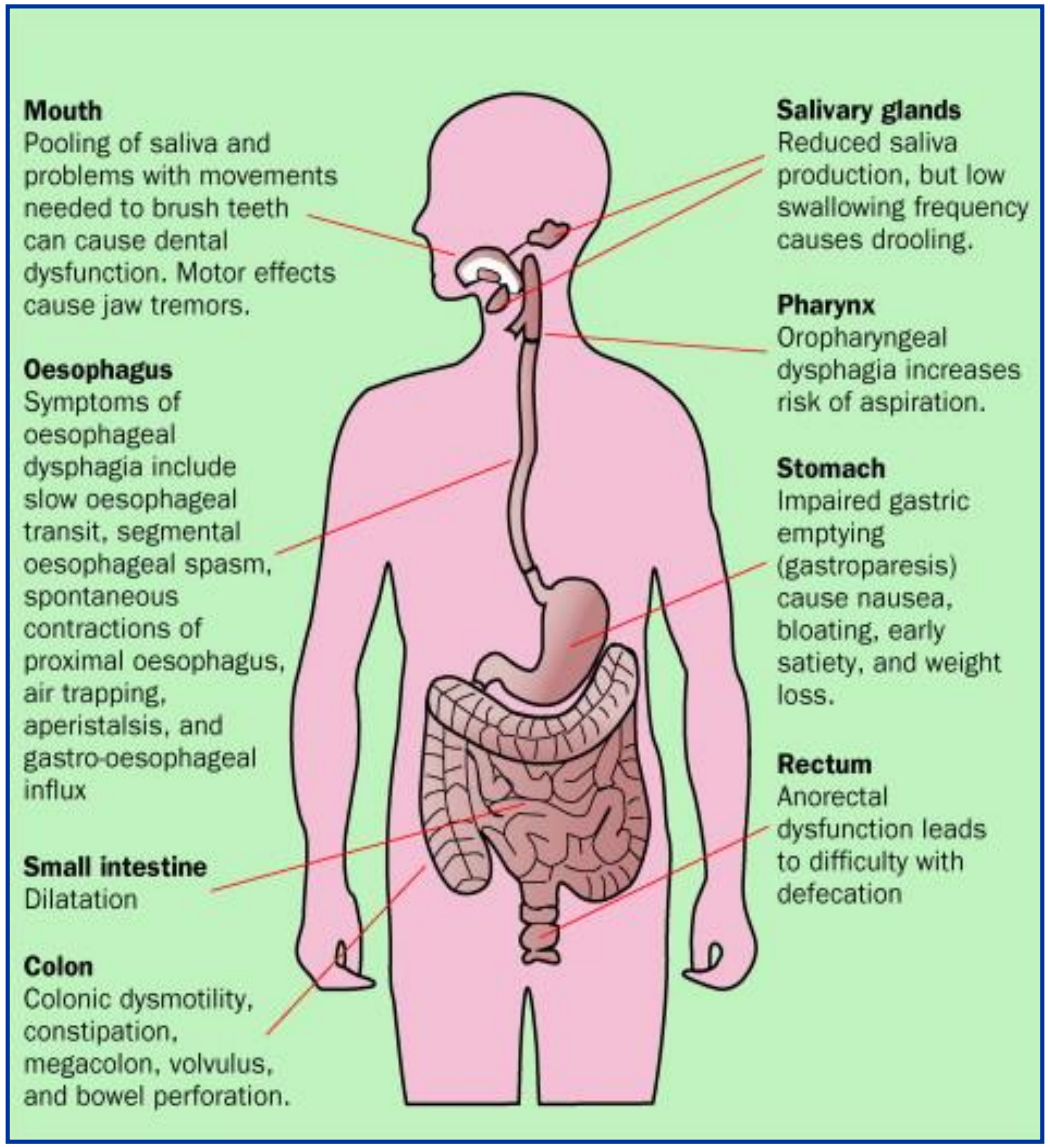


- ✓ **Alzheimer's disease (AD), the most common neurodegenerative disease, is characterized by memory loss and severe cognitive impairment, altered behavior, and impairment in activities of daily living**
- ✓ **AD patients commonly experience intestinal symptoms, including fecal incontinence and constipation, which contribute to AD morbidity and complicate its clinical management**

Wu JH, *et al.* (2011) J Am Geriatr Soc 59:2053-61

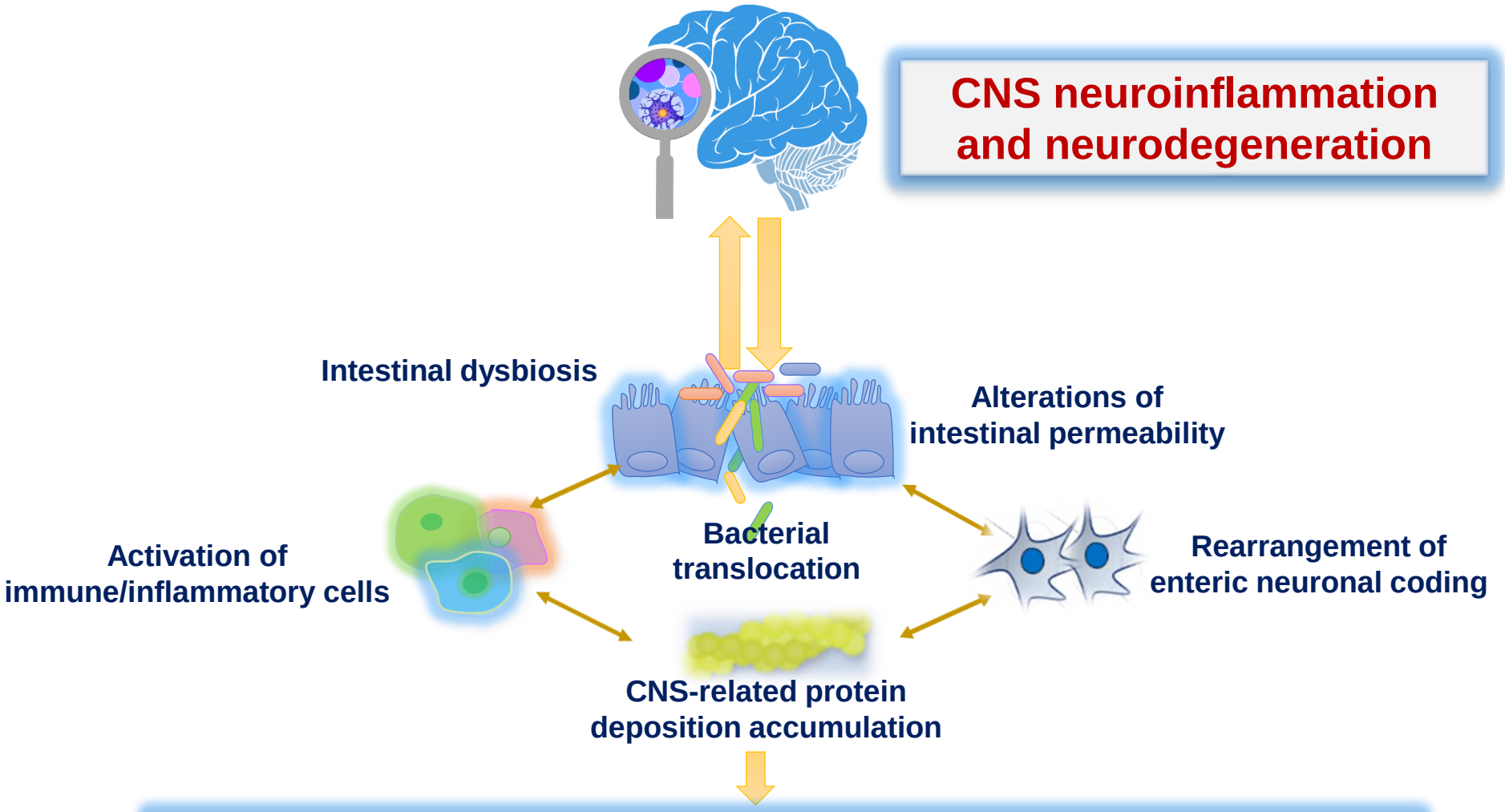
PARKINSON'S DISEASE

Symptoms	Frequency
Excessive salivation	70-80%
Dysphagia	30-80%
Gastro-oesophageal reflux	26%
Nausea, vomiting	20%
Dyspepsia (altered gastric emptying)	70-100%
Abdominal swelling	20-90%
Constipation	50-90%
Anorectal dysfunction	65%



Pfeiffer RF Parkinsonism Relat Disord 2011;17:10; Maeda T et al., Parkinsons Dis 2013;742128; Cersosimo MG et al. J Neurol 2013; 260:1332; Martinez-Martin P, et al. Mov. Disord. 2007;22:1623–1629; Pedrosa Carrasco AJ, et al., NPJ Parkinsons Dis. 2018 Mar 16;4:6

PATHOPHYSIOLOGICAL HYPOTHESIS



Pellegrini et al., Acta Neuropathologica (2018) 136:345–361

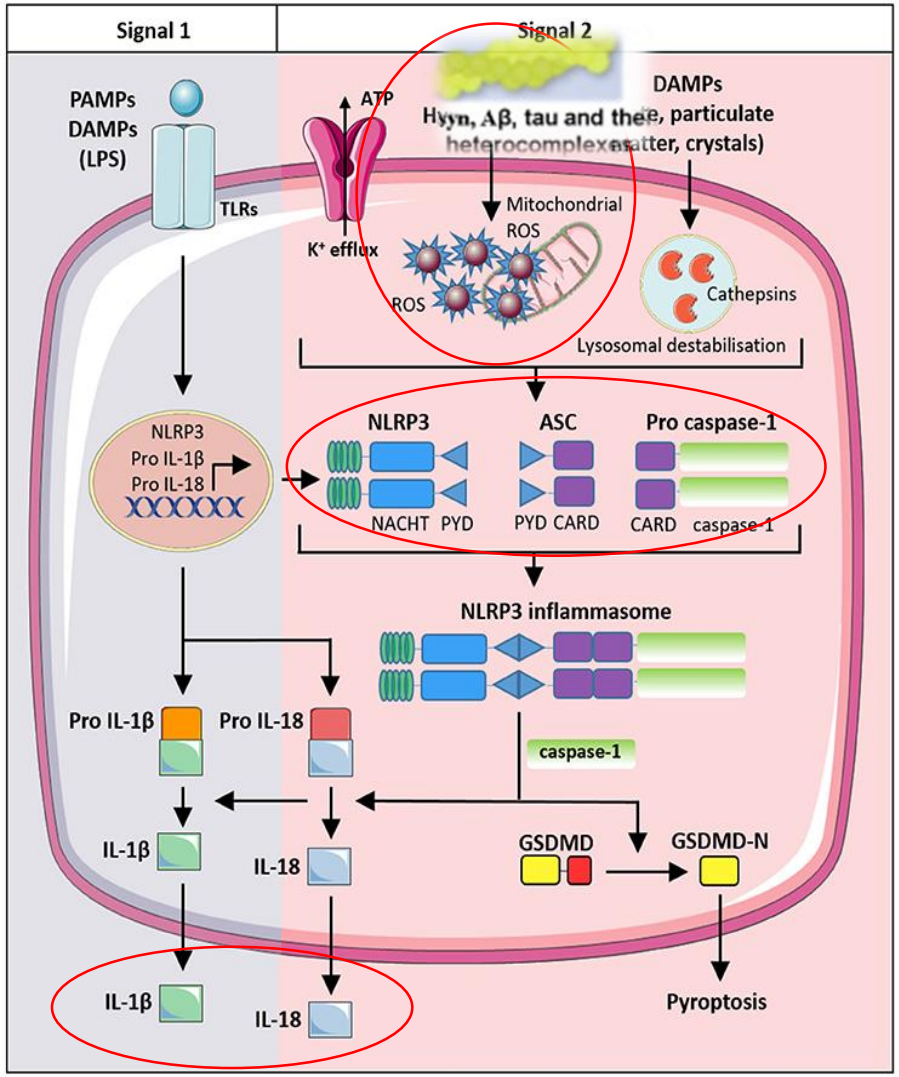
CLINICAL EVIDENCE IN CNS DISORDERS

Neurological disorder	Main changes in gut microbiota composition	Morphofunctional alterations of intestinal epithelial barrier	Intestinal neuro/immune inflammatory responses and CNS related protein deposition
PD	✓ *Bacteroidetes (conflicting evidence)* ✓ *= Firmicutes (conflicting evidence)* ✓↑ *Blautia, Coprococcus, Roseburia, Escherichia coli, Akkermansia, Bifidobacterium, Flavonifractor and Lactobacillus* ✓↓ *Ralstonia, Faecalibacterium prausnitzii, Clostridium coccoides and Bacteroides fragilis* ✓↑ *Enterobacteriaceae* ✓↓ *Prevotellaceae* ✓↓ Fecal SCFAs levels (butyrate, acetate, propionate)	✓ No functional alterations of intestinal permeability ✓↓ Occludin expression ✓↓ ZO-1 expression and distribution ✓↑ LPS serum levels	✓ Nitrotyrosine ✓ Enteric glia activation (GFAP, Sox-10,S100-beta) ✓ Enteric α-synuclein accumulation ✓ Colonic pro-inflammatory cytokines (TNF, IFN-γ, IL-6, IL-1β) ✓ Enteric glia activation (GFAP, Sox-10, S100-beta) ✓ Colonic TLR-4 expression ✓ Colonic CD3+ T cells ✓ Enteric α-synuclein accumulation
AD	✓↓ *Decreased Faith's Phylogenetic Diversity* ✓↓ *Firmicutes, Actinobacteria, Ruminococcaceae, Turicibacteraceae, Peptostreptococcaceae, Clostridiaceae, Mogibacteriaceae, Bifidobacteriaceae, Erysipdotrichaceae CC115, Clostridiaceae SMB53, Dialister, Clostridium, Bifidobacterium, Turicibacter, Bifidobacterium, Adlereutzia* ✓↑ *Bacteroidetes, Proteobacteria, Bilopila, Bacteroidaceae, Gemellaceae, Rikenellaceae, Bacteroides, Phascolarctobacterium, Gemella, Alistipes, Blautia*	✓ ↑ circulating ZO-1, LPS, claudin-5, FABP2 levels	✓ Colonic CD68 macrophages ✓ Fecal calprotectin levels ✓ Enteric Aβ and tau accumulation

Abbreviations: SCFAs: short chain fat acids; TNF: tumor necrosis factor, IFN-γ: interferon gamma, IL-6: interleukin-6; IL-1β interleukin-1 beta; GFAP: glial fibrillary acidic protein; SCFAs: short chain fat acids; ZO-1: zonulin-1; LPS: lipopolysaccharides, S100β: S100 calcium-binding protein β; TLR: toll like receptor

Pellegrini et al., Acta Neuropathologica 2018; 136:345-361 Perez-Pardo et al. Gut 2019; 68:829-843

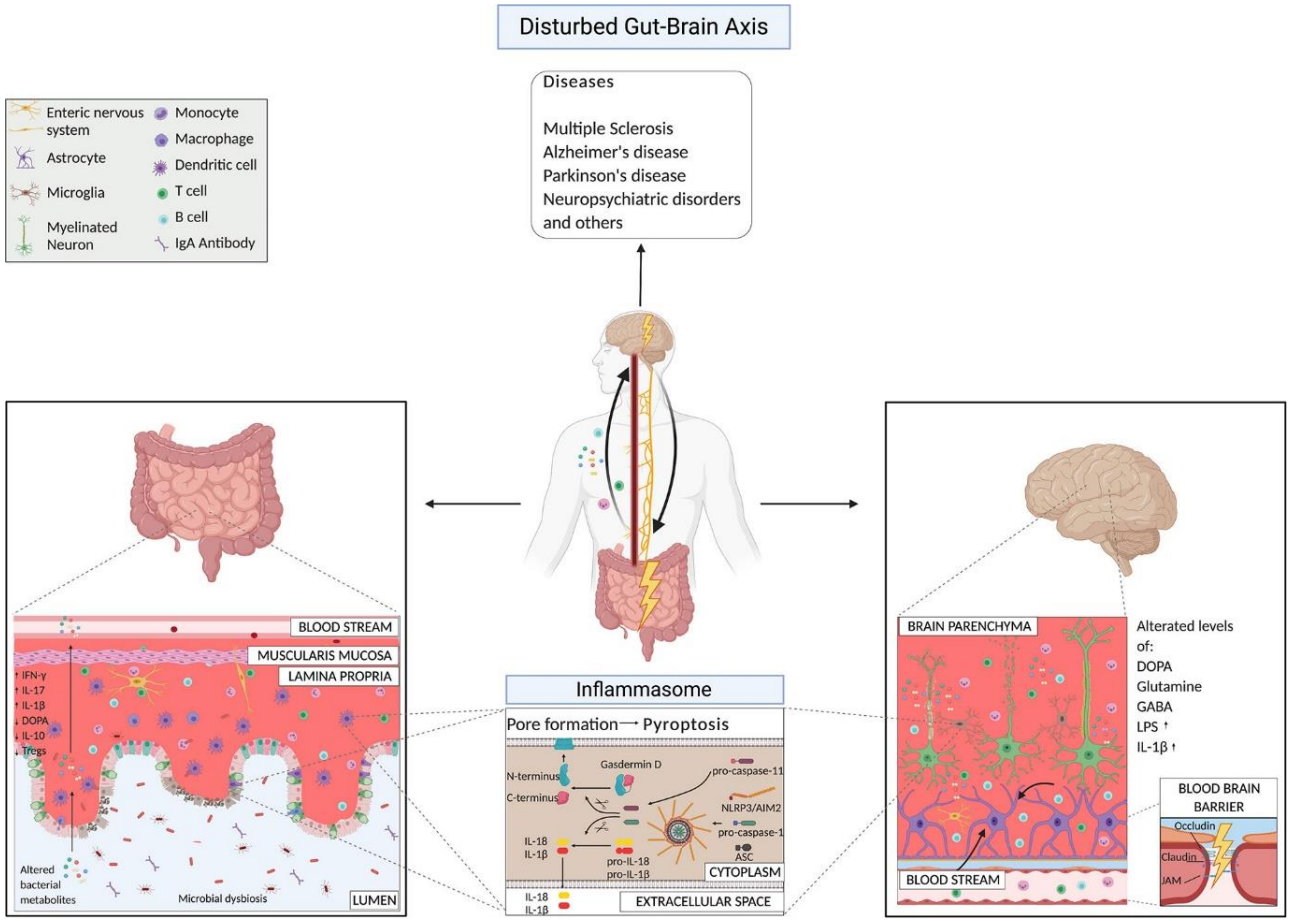
THE NLRP3 INFLAMMASOME MULTIPROTEIN COMPLEX



- Leading role in the release of IL-1β and IL-18
- Pivotal in the maintenance of cytosolic surveillance and the pathophysiology of immune-inflammatory responses
- NLRP3 senses changes in enteric bacteria and CNS-related proteins deposition shaping immune/inflammatory responses
- Increasing evidence suggest relevant roles in the pathophysiology of CNS disorders

Pellegrini et al., Progress in neurobiology (2020) 191:101806.

MICROBIOTA-GUT-INFLAMMASOME-BRAIN AXIS



- There is pioneering evidence supporting that enteric NLRP3 activation is at the crossroads among changes in enteric bacteria, intestinal CNS-related protein accumulation and immune/inflammatory responses in brain disorders that can contribute to central pathology via gut-brain signalling.
- This pathway is referred as **'microbiota-gut-inflammasome-brain axis'.**

Rutsch et al., Frontiers in immunology 2020

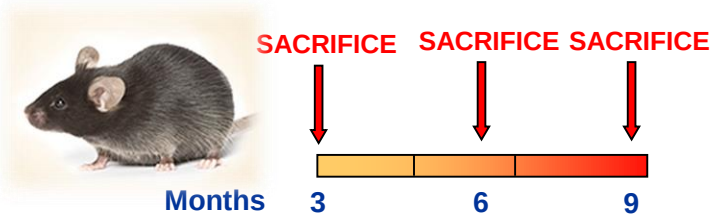
Pellegrini et al., Progress in neurobiology (2020) 191:101806.

OUR EXPERIENCES

MOUSE MODEL OF SPONTANEOUS PARKINSON'S DISEASE

Animals: Transgenic mice expressing high levels of WT or mutant (A53T and A30P) α-synuclein

A53TαS mice develop a severe disease at ~12 months of age, with rapid progression to end-stage within 14-21 days from the onset



- Neuronal α-synuclein and ubiquitin aggregates/inclusions
- Axonal degeneration
- Neuronal loss
- Enteric pathological α-syn deposition before brain pathology

Lee MK et al., Proc Natl Acad Sci USA 2002;99:8968 Rota et al. Translational Neurodegeneration (2019) 8:5

✓Expression of colonic pathological α-synuclein (Western blot)

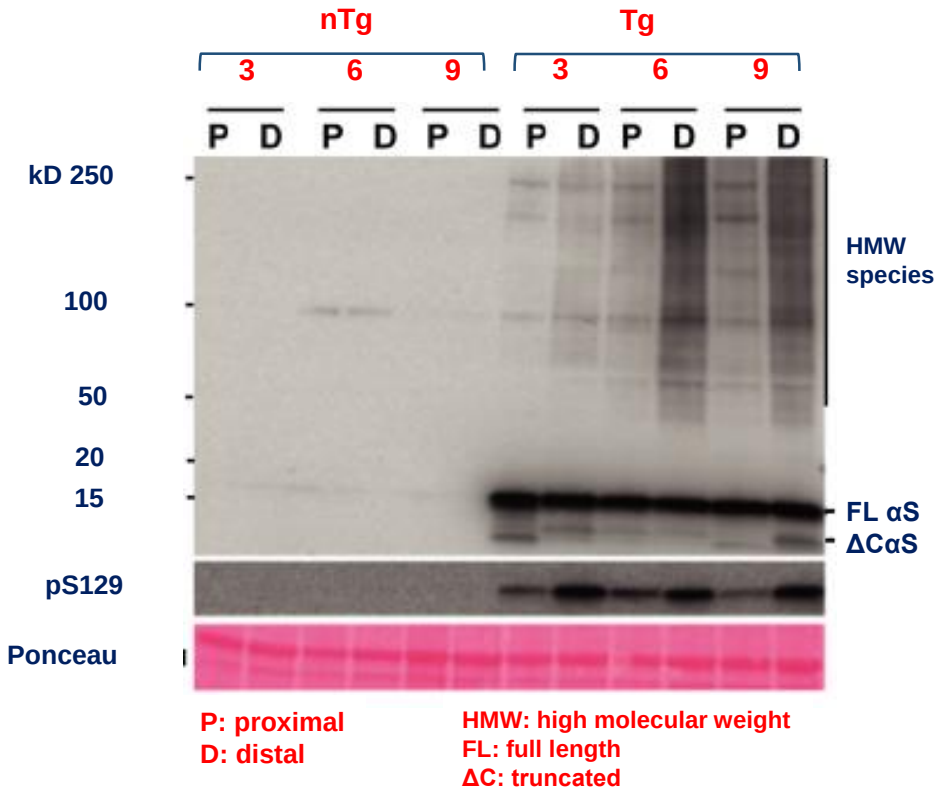
✓Microglia activation in the brain (immunofluorescence of Iba-1)

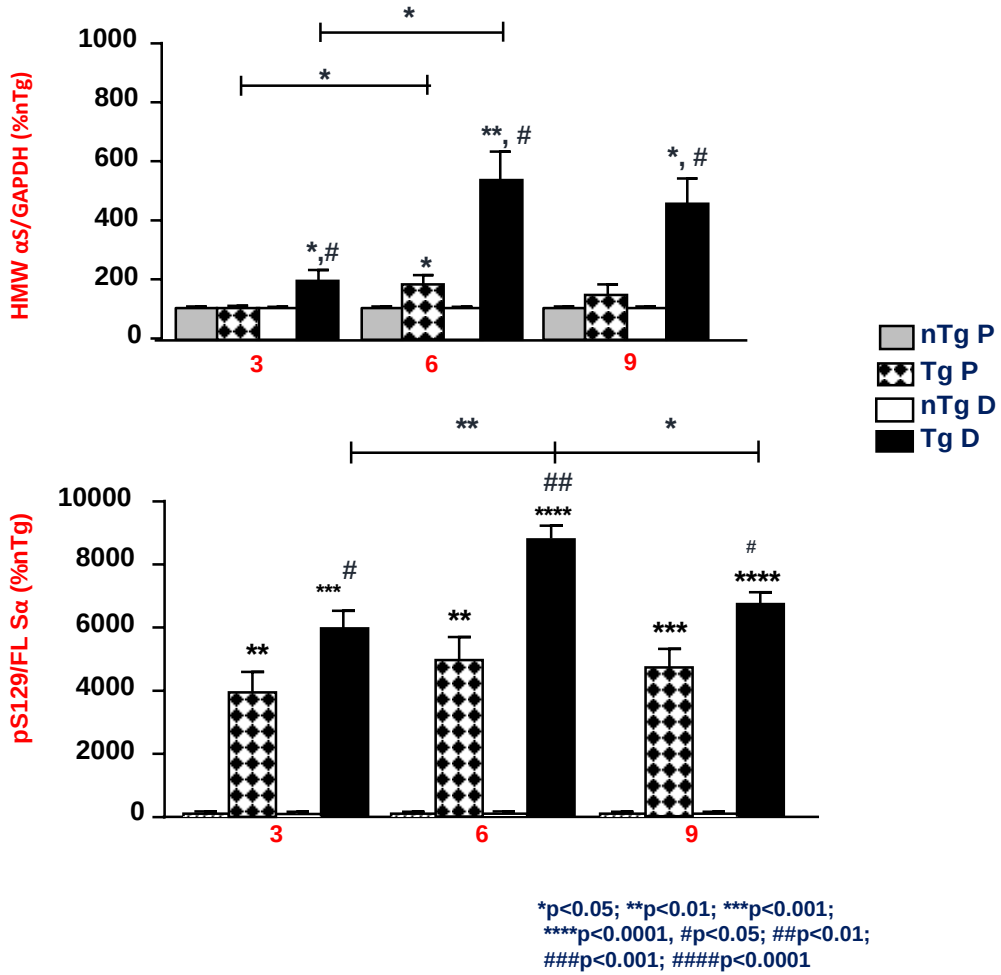
✓Alterations of intestinal barrier (colonic expression of zonulin-1 and occludin by Western blot)

✓Colonic inflammasome signalling activation (IL-1β levels and caspase-1 activity by ELISA)

✓Colonic motility (electrically evoked colonic contractions *in vitro*)

Early colonic α-synuclein (αS) accumulation in PD mice

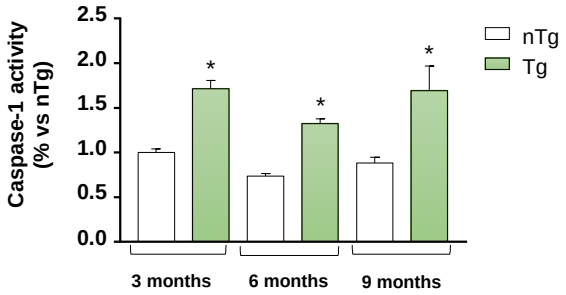




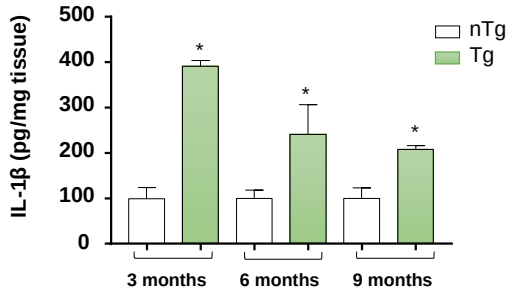
Rota L. et al., Transl Neurodegener. (2019) 6;8:5

Enteric inflammatory responses in pre-symptomatic transgenic (Tg) mice precede brain inflammation

Colonic caspase-1 activity

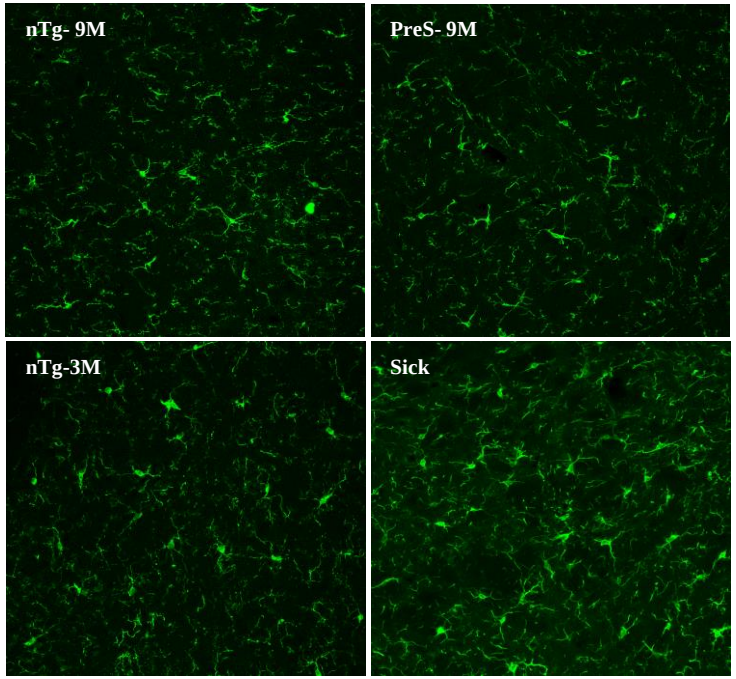


Colonic IL-1β levels



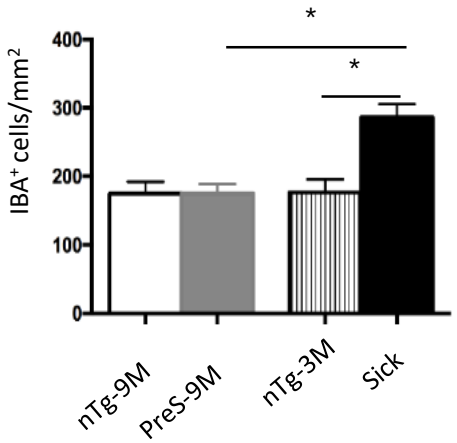
*P<0.05 vs respective nTg

Immunofluorescence of Iba-1 (microglia)

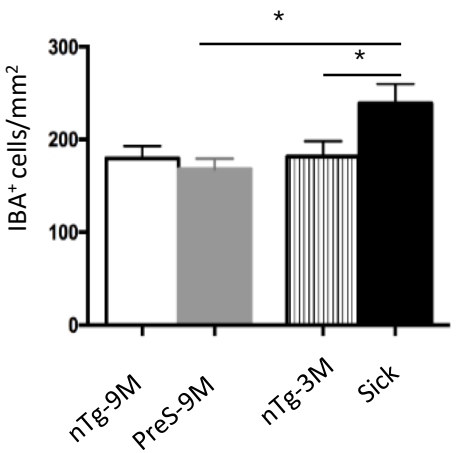


Pellegrini et al., 2022 NPJ Parkinsons Dis. 2022 Jan 12;8(1):9

SpC

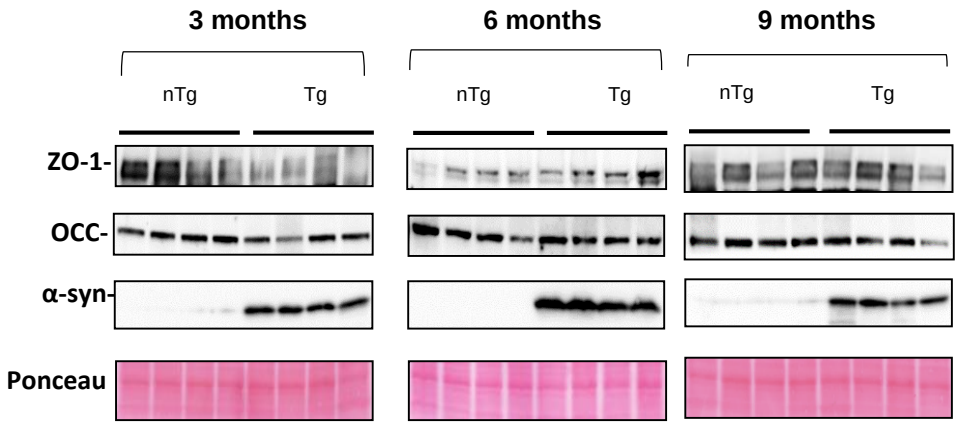


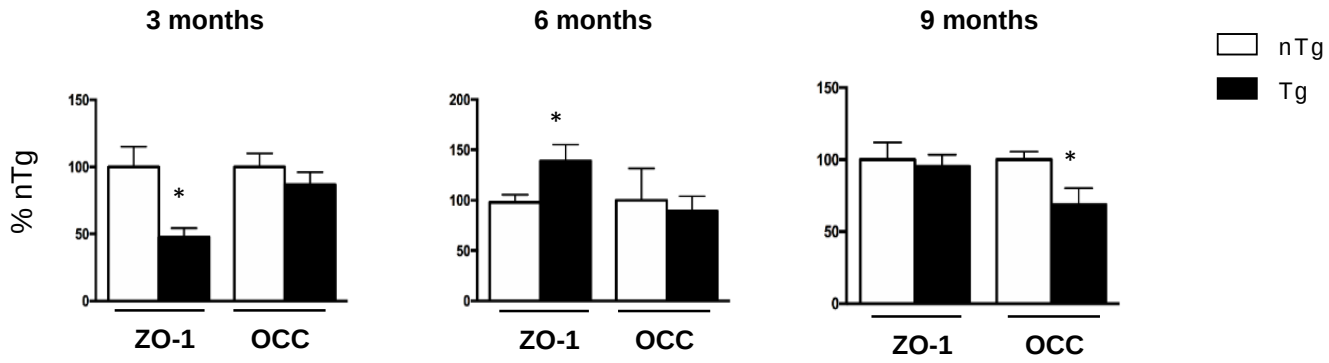
MB



Pre-symptomatic Tg mice displayed impairments of gut barrier integrity

Tight junction protein expression



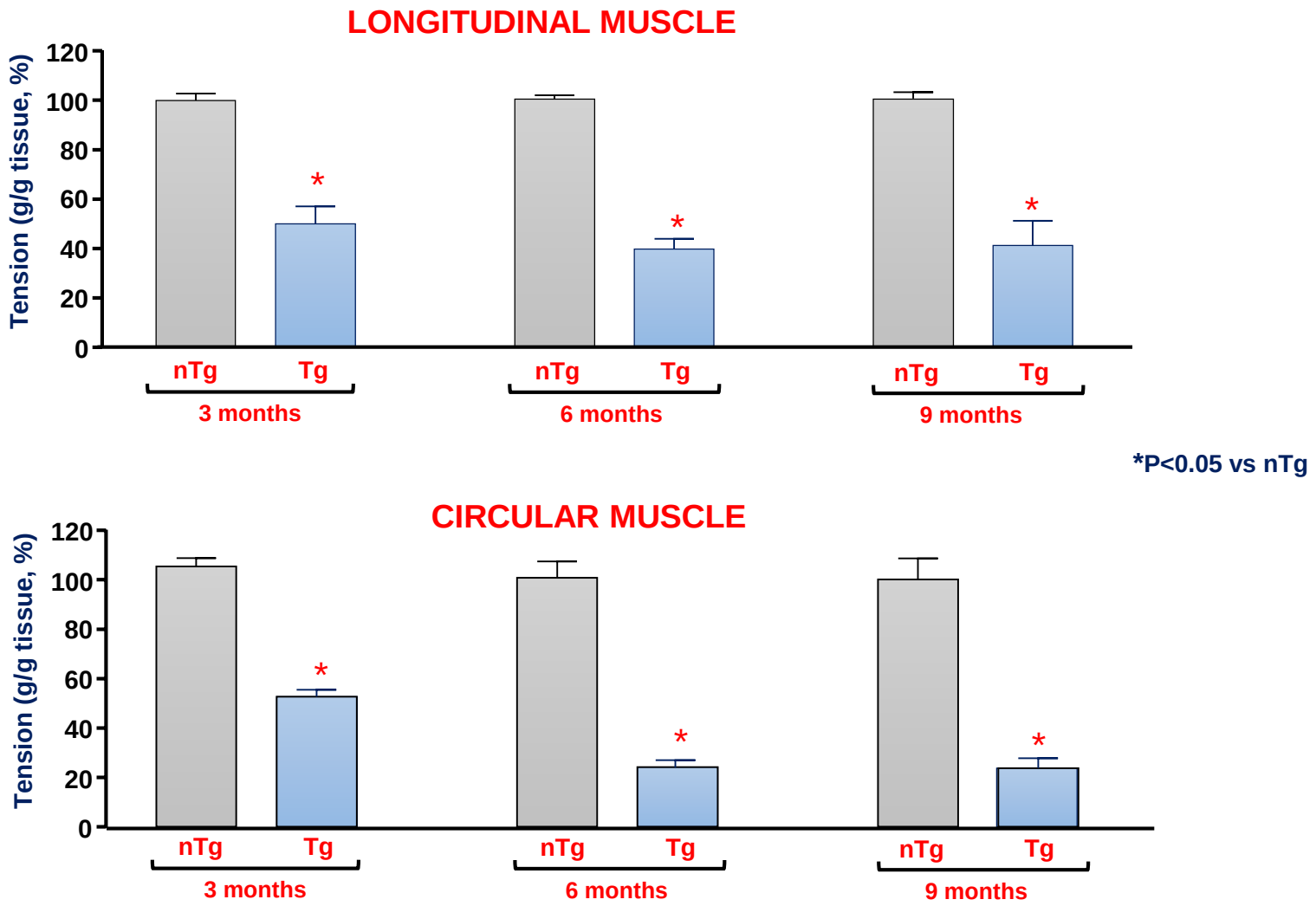


*P<0.05 vs respective nTg

Pellegrini et al., 2022 NPJ Parkinsons Dis. 2022 Jan 12;8(1):9

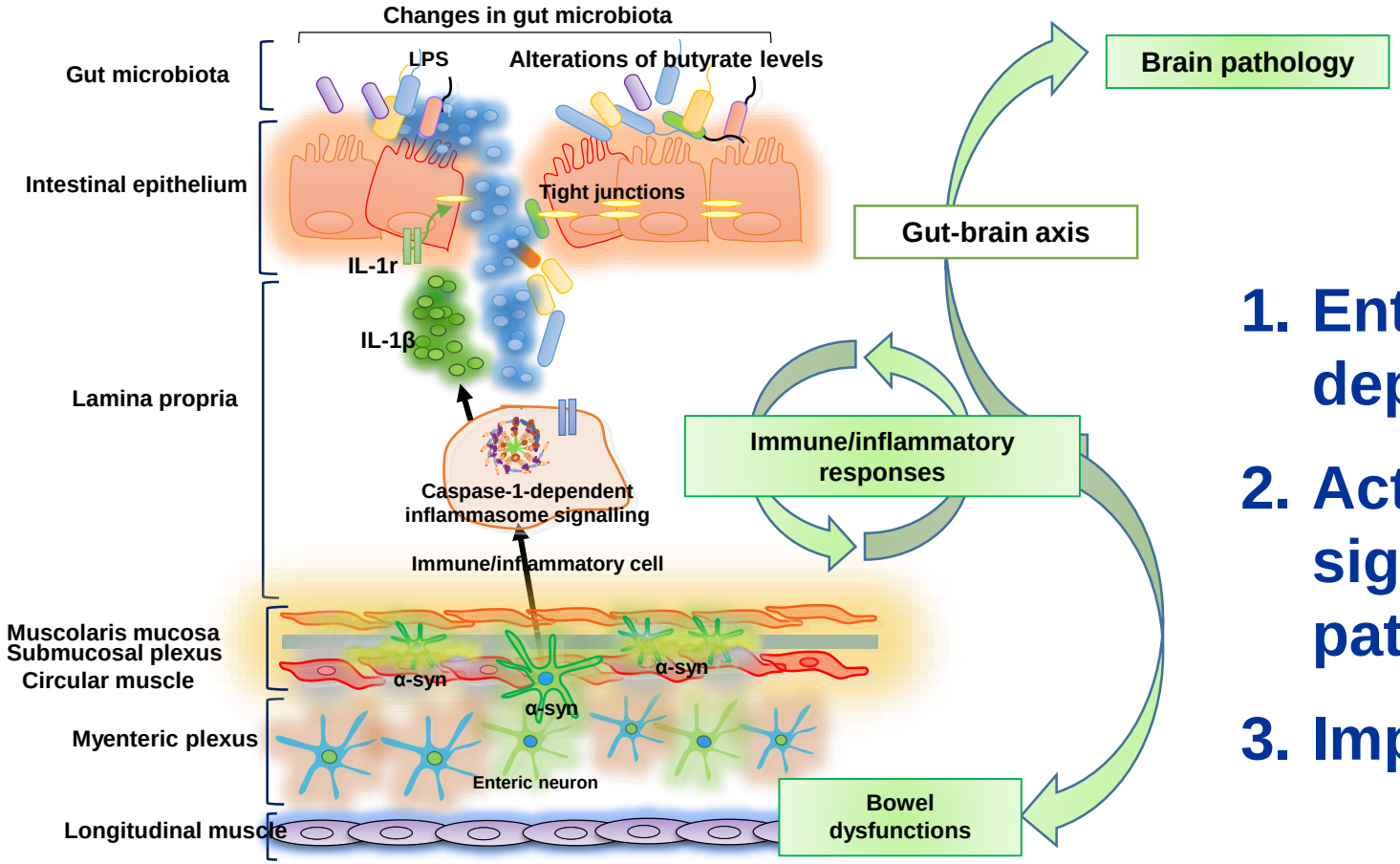
Tg mice display altered colonic motility in vitro

Electrically evoked colonic contractions in tissues maintained in standard Krebs solution



Rota L. et al., Transl Neurodegener. 2019;8:5

Pathophysiological intestinal paths in presymptomatic PD mice

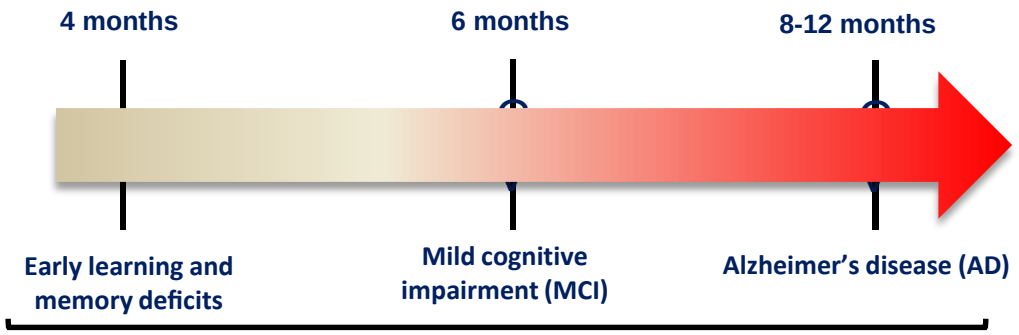


1. Enteric pathological α-syn deposition
2. Activation of immune/inflammatory signaling, including inflammasome pathways
3. Impairment of gut barrier

Pellegrini et al., 2022 NPJ Parkinsons Dis. 2022 Jan 12;8(1):9

MOUSE MODEL OF AD

SAMP8 mouse



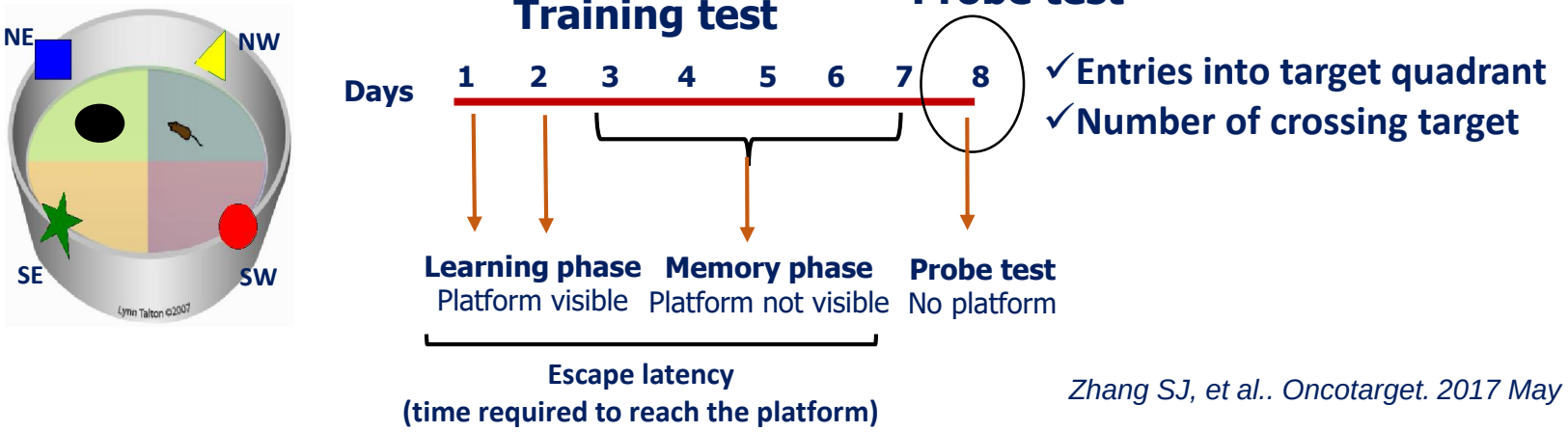
Canudas A.M. et al. 2005 Mech. Ageing Dev. 126:1300–1304.
D.A. Butterfield, H.F. Poon 2005 Exp. Gerontol. 40:774–783.
R. Strong et al. 2003 Brain Res. 966:150–156.

- SAMP8 (Senescence-Accelerated Mouse-Prone 8) mouse develops spontaneously early learning and memory deficits, with similar features to those observed in AD patients.
- The SAMP8 mouse develops a severe disease after eight months of age, characterized by an impairment of cognitive and motor functions along with intestinal symptoms, starting from six months of age before the full development of CNS pathology.
- Of note, the SAMP8 mouse displays the main clinical and pathophysiological features to those observed in AD patients, including Aβ1-40 or 1-42 proteins in hippocampal granules, hyperphosphorylation of tau protein, increase in α-syn, presenilin, oxidative damage, glutamate and nNOS levels, decrease in ChAT activity, central and systemic neurogenic/inflammatory responses

MOUSE MODEL OF MCI AND AD

✓Cognitive functions were assessed in animals at 4, 6 and 8 months of age in order to evaluate alterations from the initial phases of early learning and memory deficiencies until the full development of AD

✓Cognitive functions (Morris Water Maze test)



Zhang SJ, et al.. Oncotarget. 2017 May 4

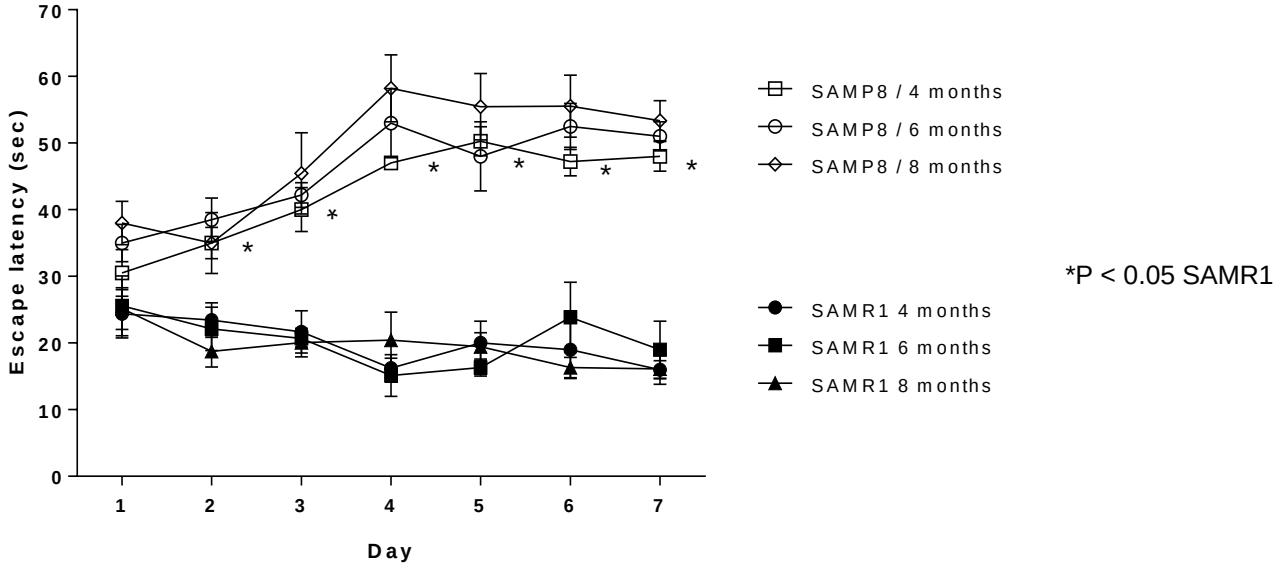
✓Colonic interleukin(IL)-1β and AD-related protein levels (ELISA)

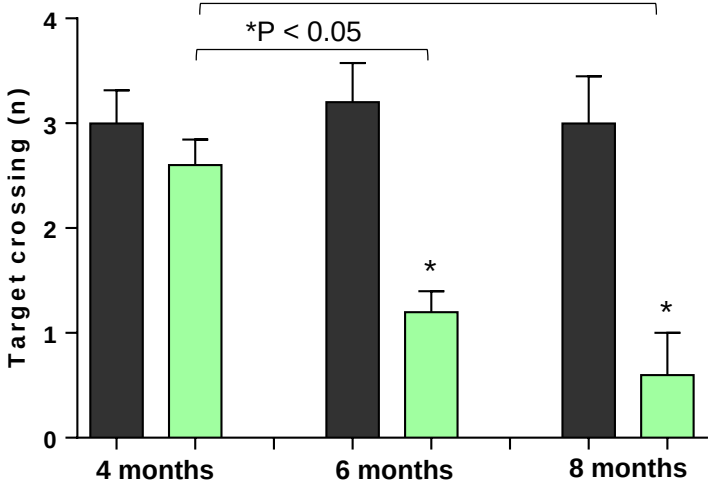
✓NLRP3, ASC and caspase-1 expression in colonic tissues (western blot)

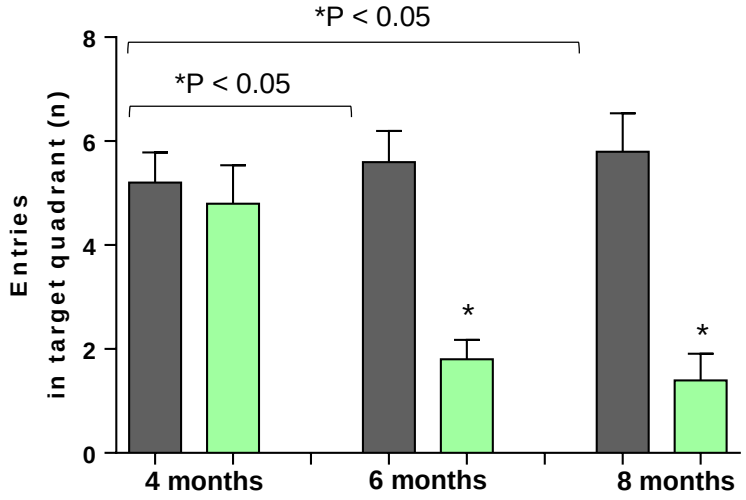
✓Claudin-1 expression and distribution in colonic tissues (immunofluorescence)

✓Fecal pellet output and in vitro colonic contractile activity

SAMP8 mice displayed an impairment of cognitive functions in the prodromal phase of AD, before the full development of CNS pathology

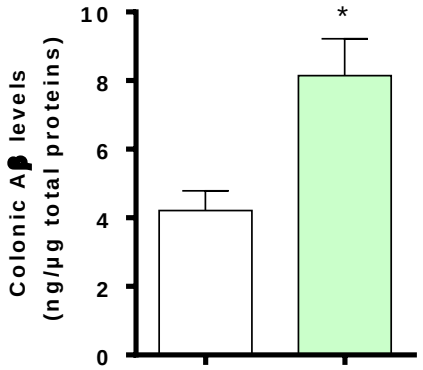




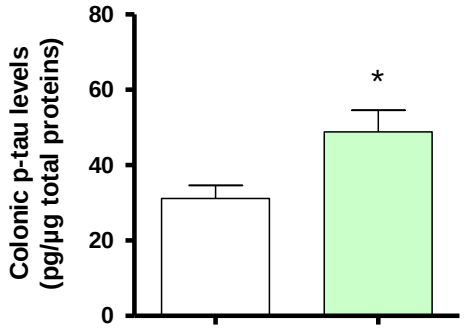


SAMP8 mice displayed colonic accumulation of AD-related proteins and inflammasome signalling activation

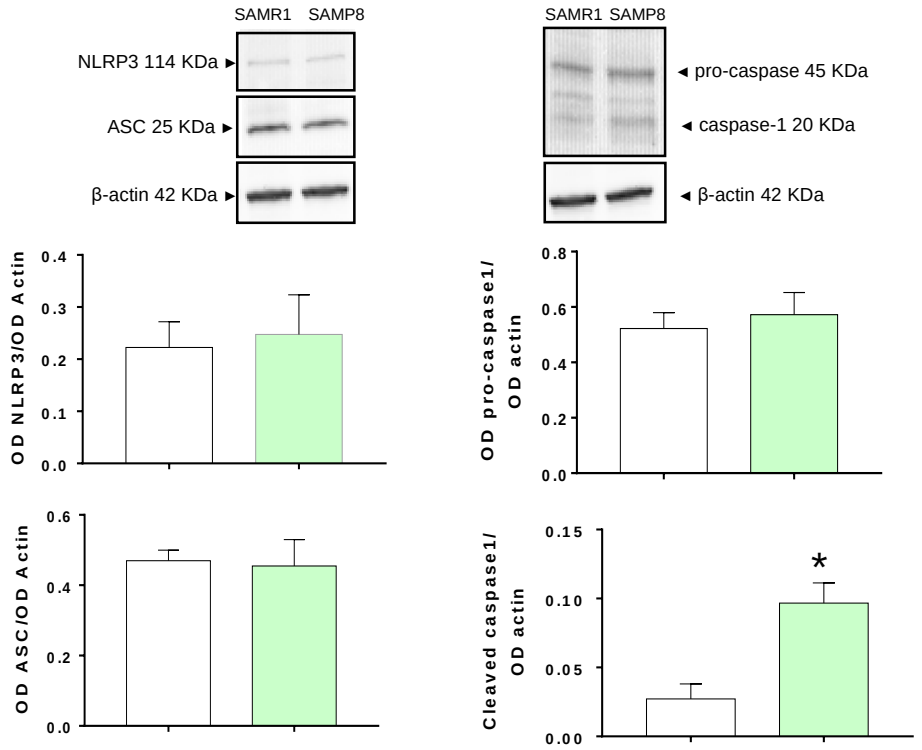
Aβ protein



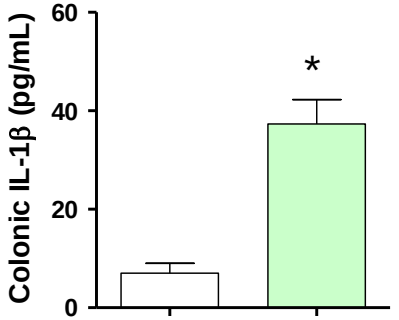
Tau protein



NLRP3, ASC, caspase-1 expression



IL-1β levels



SAMR1
SAMP8

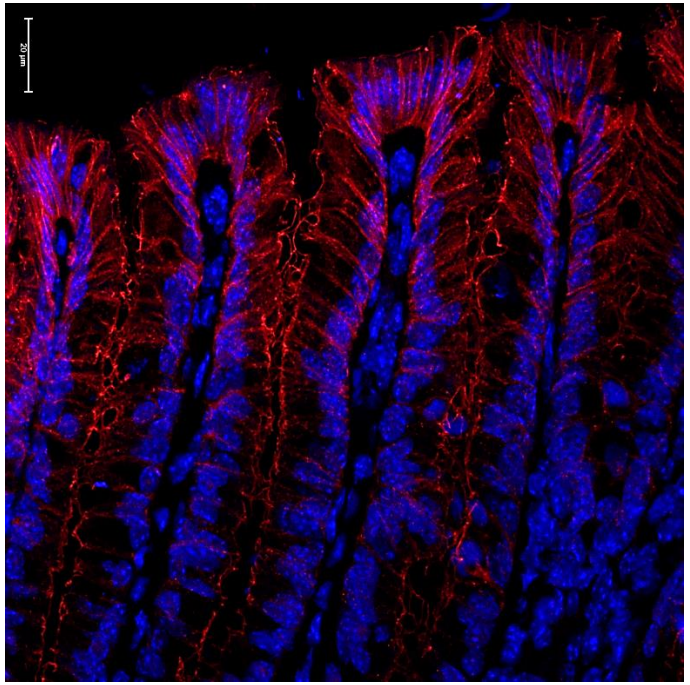
*P < 0.05 vs SAMR1

Pellegrini et al., 2020 Int J Mol Sci. 2020 15;21(10):3523.

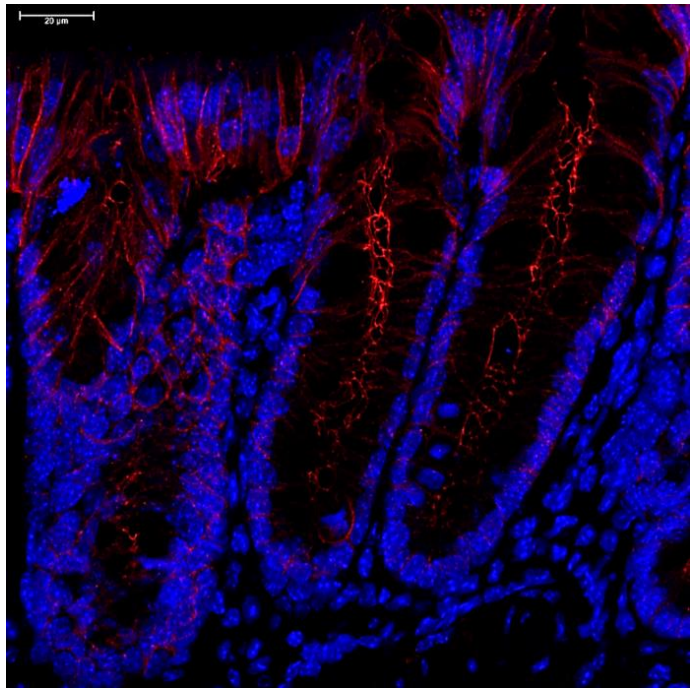
SAMP8 mice displayed impaired intestinal epithelial barrier

Claudin-1 immunofluorescence

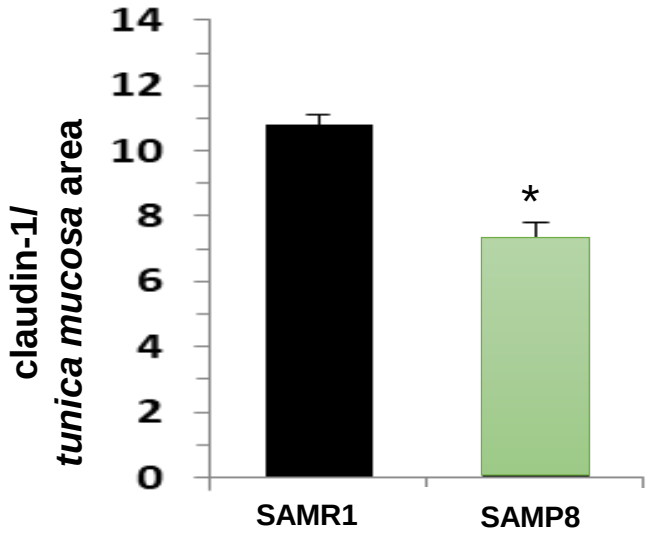
SAMR1



SAMP8



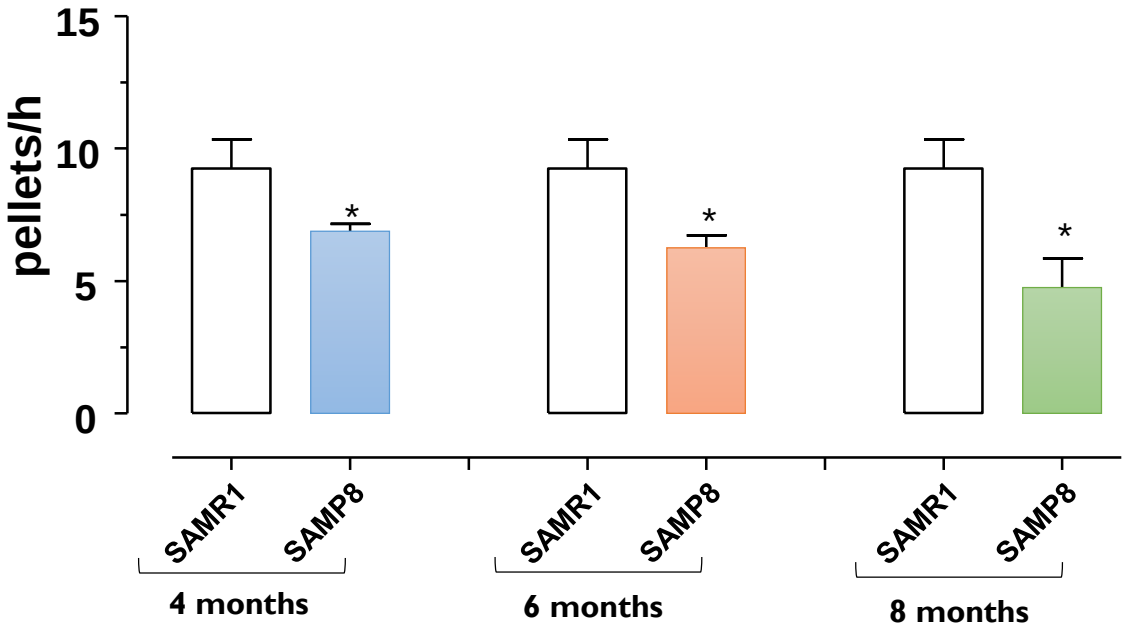
*P < 0.05 vs SAMR1



Pellegrini et al., 2020 Int J Mol Sci. 2020 15;21(10):3523.

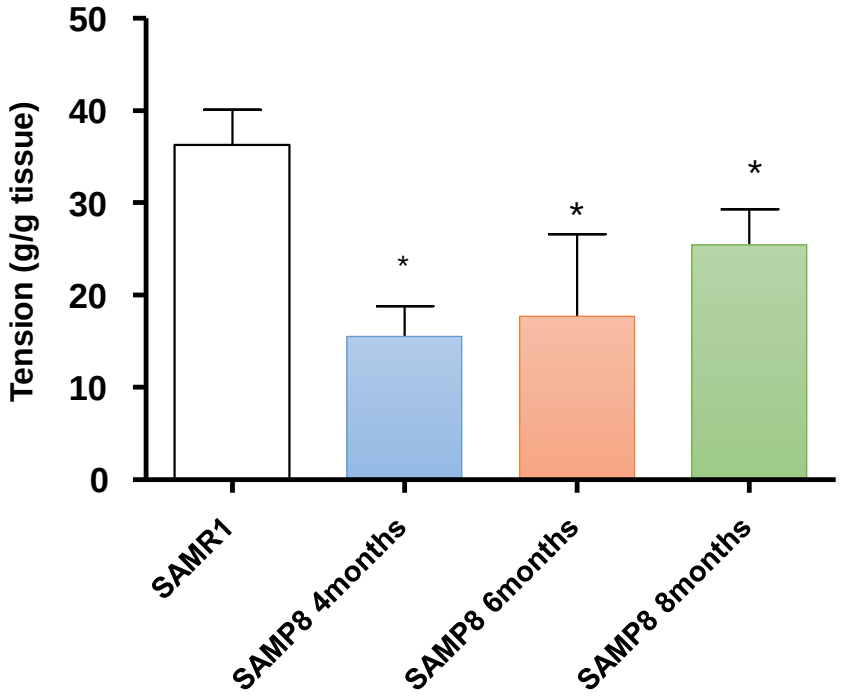
SAMP8 mice displayed impaired colonic motility

Fecal output



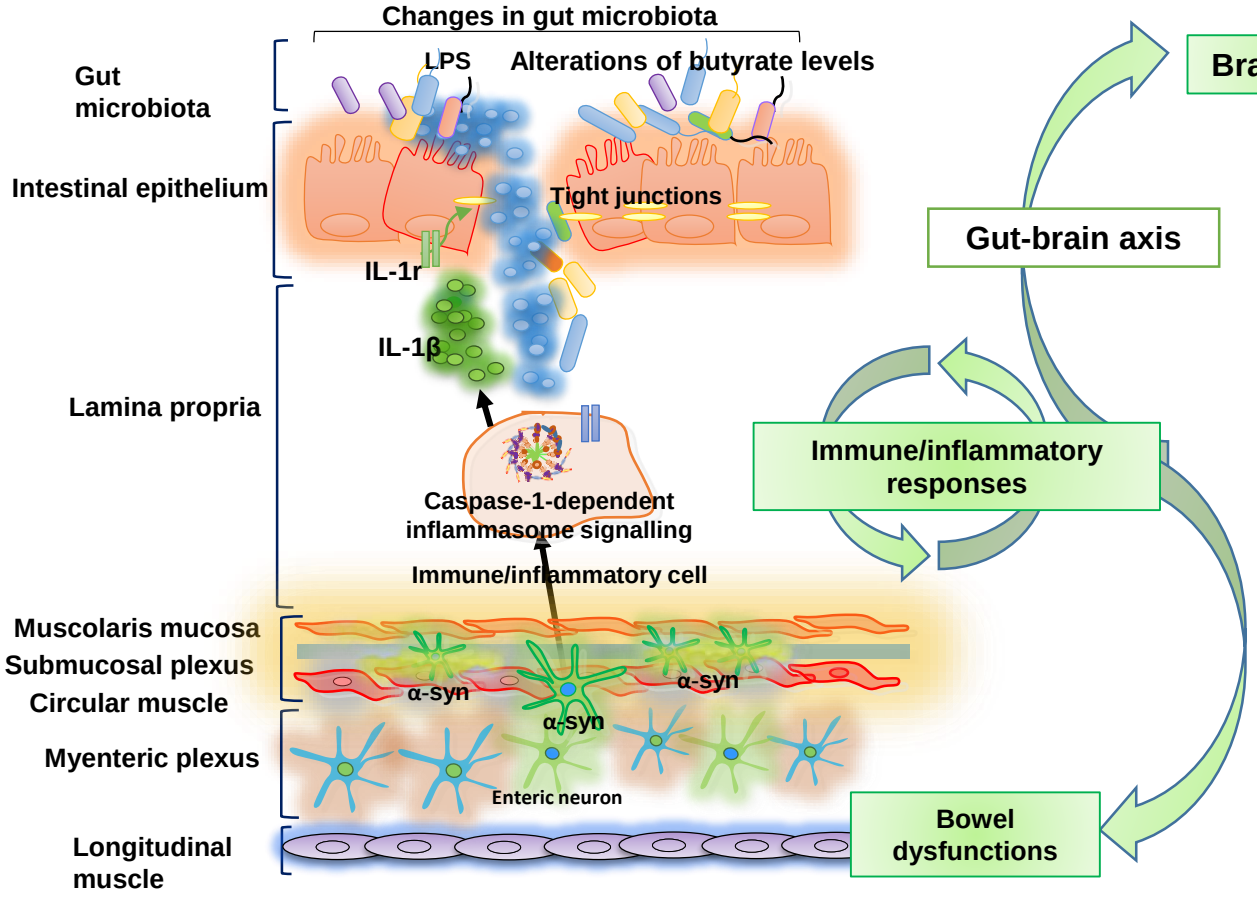
*P < 0.05 vs SAMR1

Electrically evoked colonic contractions (Standard Krebs solution)



*P < 0.05 vs SAMR1

Pathophysiological intestinal paths in presymptomatic SAMP8 mice



1. Enteric Aβ and p-tau accumulation
2. Activation of immune/inflammatory signaling, including inflammasome pathways
3. Impairment of gut barrier

Pellegrini et al., 2020 Int J Mol Sci. 2020 15;21(10):3523.

Can a gut-directed anti-inflammatory therapy represent a pharmacological tool to target gut-brain axis and treat brain diseases?

OUR EXPERIENCES

We examined the effects of a novel gut-directed locally acting NLRP3 inhibitor (INF, PCT/IB2022/054072), in a spontaneous model of Alzheimer's disease (AD)

METHODS: *IN VITRO, EX VIVO* AND *IN VIVO* STUDIES ON INF AS A GUT-DIRECTED ANTI-INFLAMMATORY DRUG

✓ ***In vitro*** **evaluation of IL-1β inhibition**

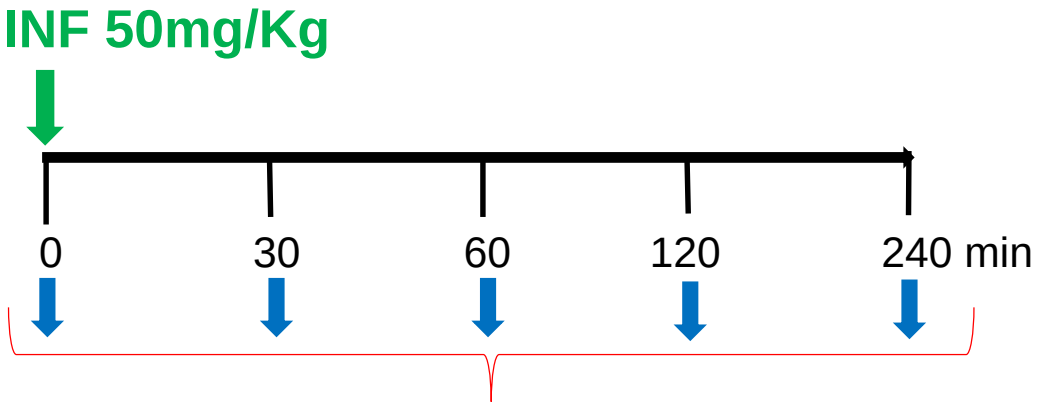
Evaluation of the inhibition of NLRP3-dependent IL-1β release in stimulated THP-1 cells

✓ ***Ex vivo*** **evaluation of intestinal absorption (non-everted sac method)**

INF was syringed into intestinal sacs from different segments of rat intestine. After 2h intestinal absorption and tissue accumulation of INF and its main metabolite were assessed

✓ ***In vivo*** **studies**

Animals: **C57BL/6 mice (28-30 g)**

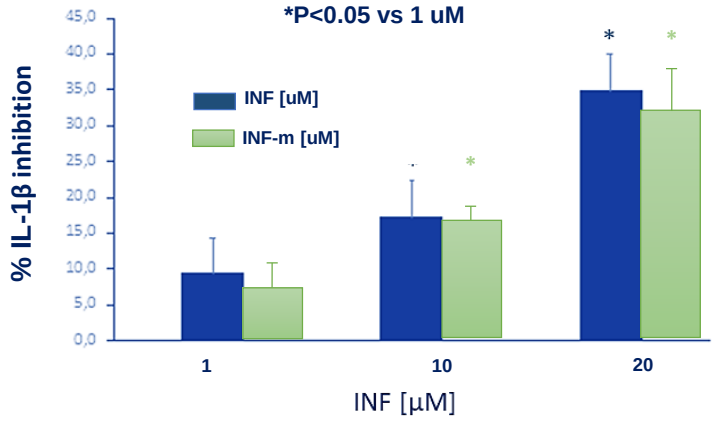


✓**Animals (n=5) received INF 50 mg/kg/day**

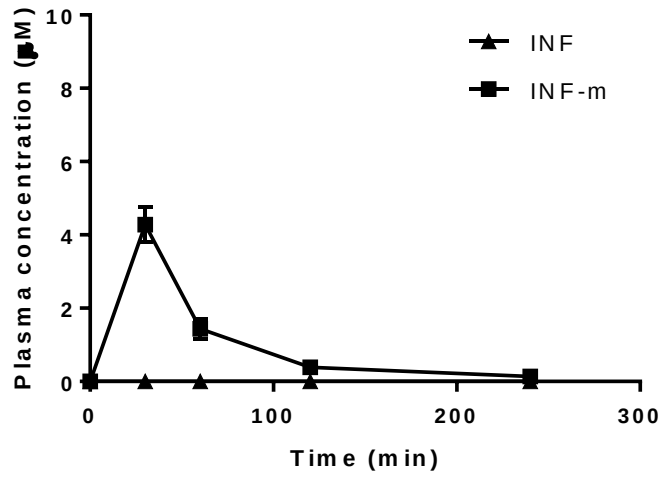
✓**Measurement of INF and INF-m concentration in plasma and brain samples**

RESULTS

IL-1β inhibition in stimulated THP-1 cells



Plasma concentration



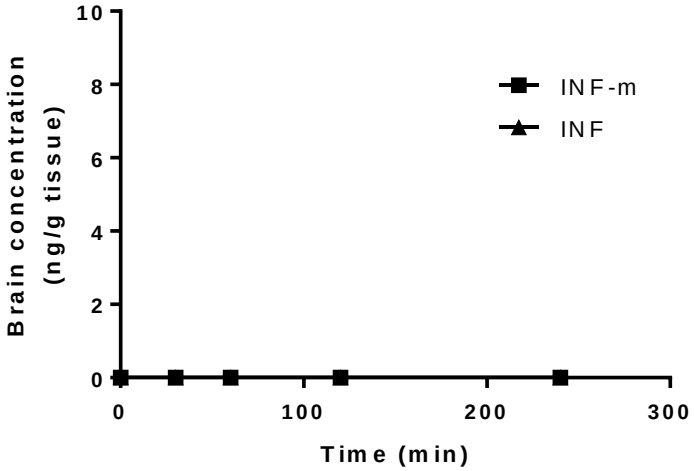
INF-m: INF active metabolite

Intestinal absorption and tissue accumulation (2h)

	Fraction absorbed (Fa) ± SE		
	Duodenum	**Jejunum**	**Ileum**
INF	Not detected	Not detected	Not detected
INF-m	0.0078 ± 0.0037	0.0046 ± 0.0007	0.017 ± 0.007

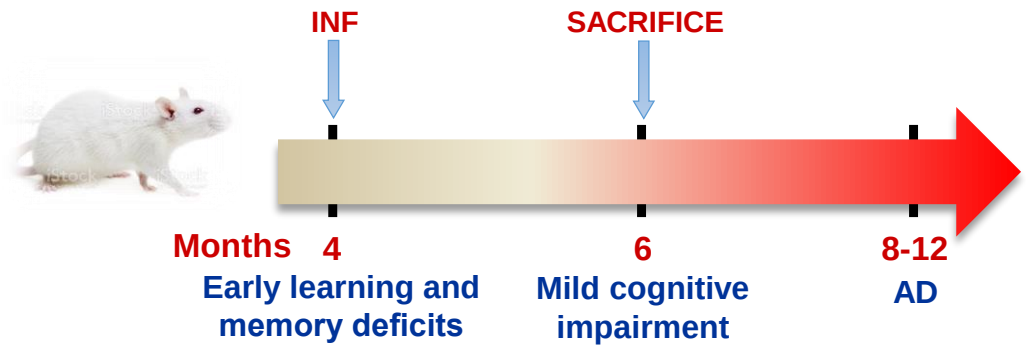
Tissue accumulation			
	Duodenum µg / g tissue	**Jejunum µg / g tissue**	**Ileum µg / g tissue**
INF	Not detected	Not detected	Not detected
INF-m	98 ± 16	80 ± 7	82 ± 7

Brain accumulation



METHODS

Animals: SAMP8 mouse (age 8 weeks; 25-30 g) a spontaneous model of AD; SAMR1 (control strain)



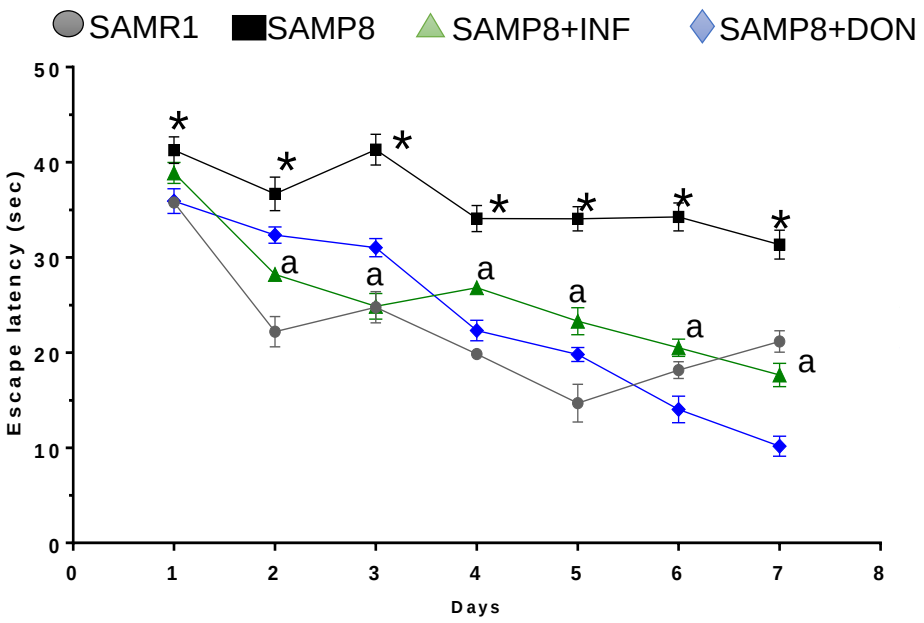
Pellegrini et al. Int. J. Mol. Sci. 2020, 21(10), 3523
Li et al., Exp. Brain Res. 2020, 238(11), 2603-2614
Canudas A.M. et al. 2005 Mech. Ageing Dev. 126:1300–1304
D.A. Butterfield, H.F. Poon 2005 Exp. Gerontol. 40:774–783

- ✓ Decrease in choline acetyl transferase Accumulation of Aβ, p-tau protein in colon and brain
- ✓ Increase in oxidative damage
- ✓ Increase in glutamate and neuronal nitric oxide synthase and decrease in choline acetyl transferase activity in the CNS
- ✓ Intestinal symptoms
- ✓ Central and systemic inflammation, characterized by NLRP3 activation
- ✓ Alterations of IEB and BBB

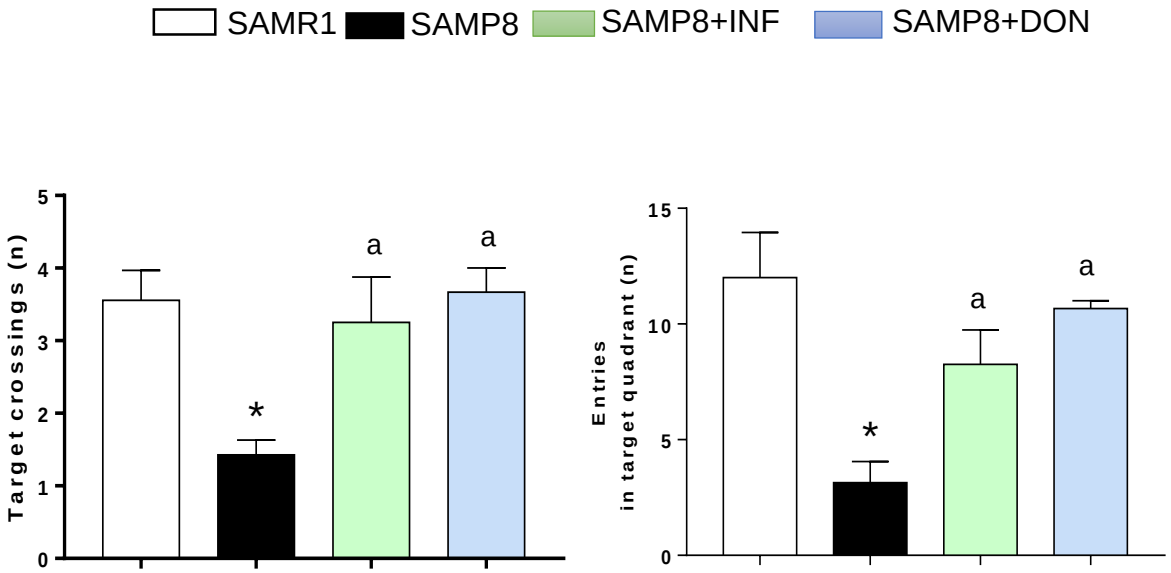
- ✓ Animals were treated orally with INF 50 mg/kg/day, donepezil (DON, as a standard comparator) 3 mg/kg/day or vehicle for two months (n=6/group) in order to evaluate the effects of a gut-directed anti-inflammatory therapy in the early phases of AD
- ✓ Morris water maze test
- ✓ Levels of phosphorylated (p)-tau and β-amyloid (Aβ1-42) in brain tissues (western blot and ELISA)
- ✓ Inflammasome signalling activation in colonic tissues (western blot and ELISA for NLRP3, ASC and caspase-1 and IL-1β)
- ✓ Expression of claudin-1, zonulin-1 and claudin-5 tight junction proteins in colonic and brain tissues (western blot)

INF COUNTERACTED COGNITIVE DECLINE IN EARLY AD

Training test



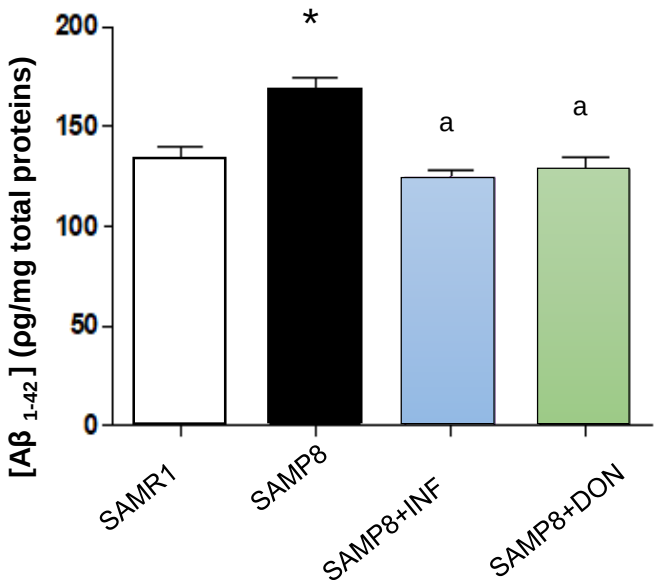
Probe test



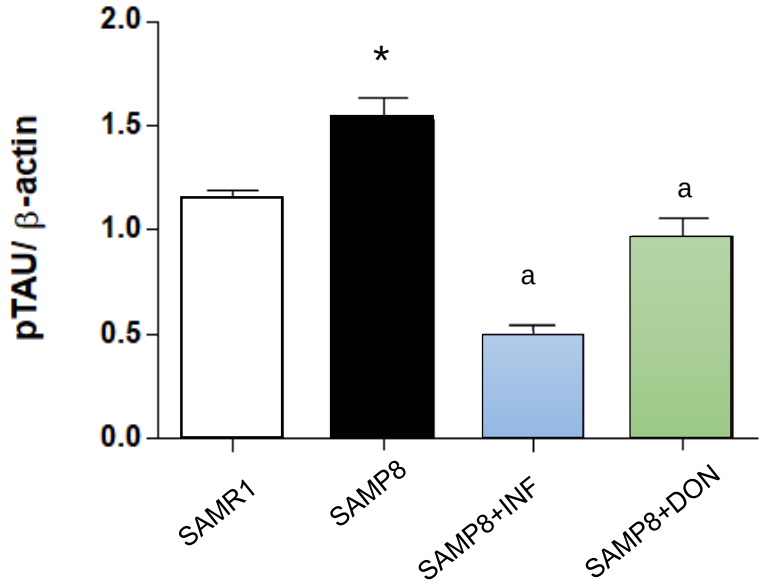
*P<0.05 vs SAMR1; ᵃP<0.05 vs SAMP8

INF DECREASED AD-RELATED BIOMARKERS (AB1-42 AND P-TAU) LEVELS IN BRAIN TISSUES FROM SAMP8 MICE

Brain Amyloid-β1-42 levels



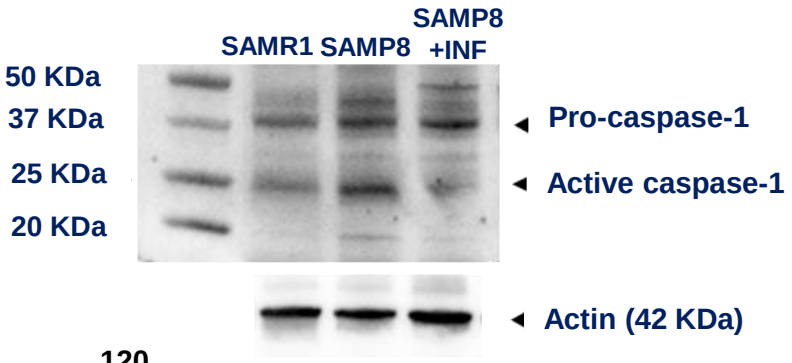
Brain p-Tau expression

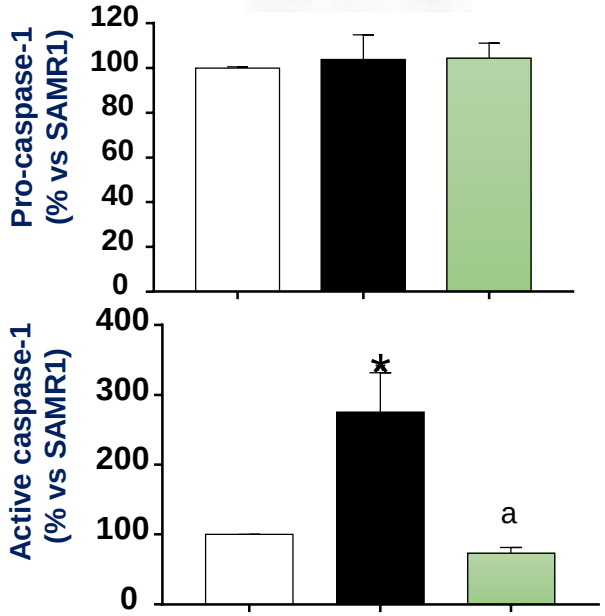


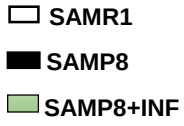
*P<0.05 vs SAMR1; ᵃP<0.01 vs SAMP8

INF COUNTERACTED COLONIC CASPASE-1 ACTIVATION AND IL-1β RELEASE IN SAMP8 MICE

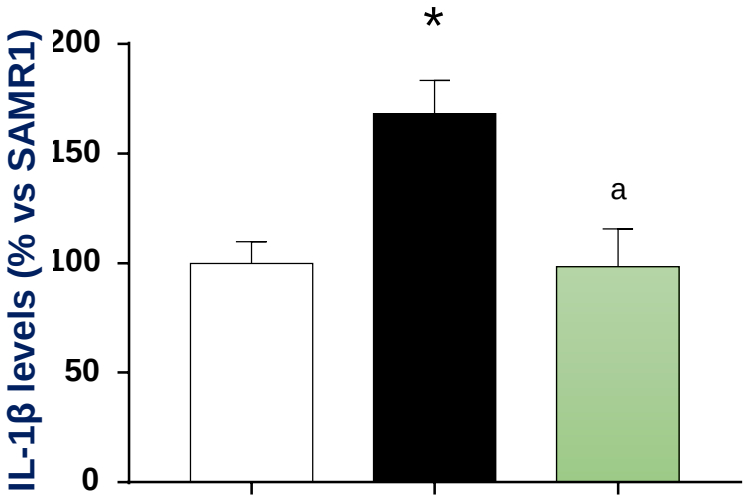
Caspase-1 expression







IL-1β levels

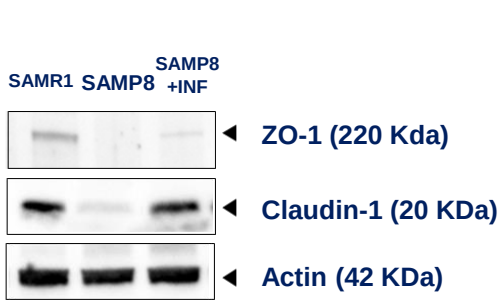


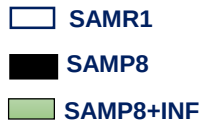
*P<0.05 vs SAMR1
[a]P<0.01 vs SAMP8

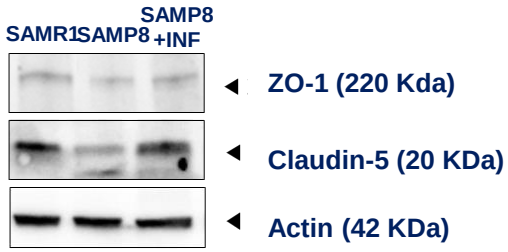
INF COUNTERACTED THE DECREASE IN CLAUDIN-1 EXPRESSION IN COLON AND BRAIN FROM SAMP8 MICE

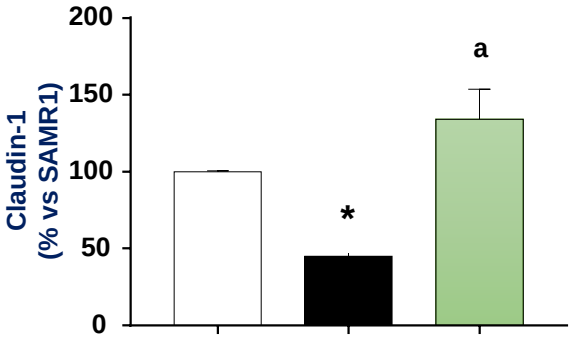
Colon

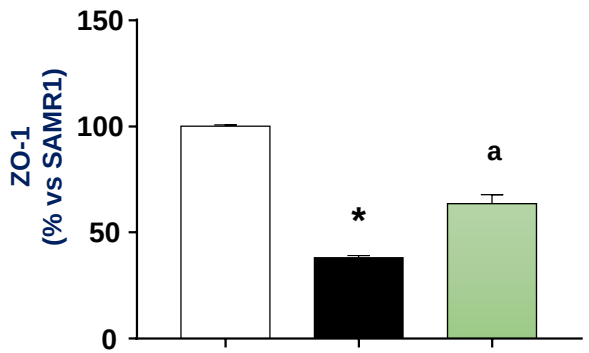
Brain

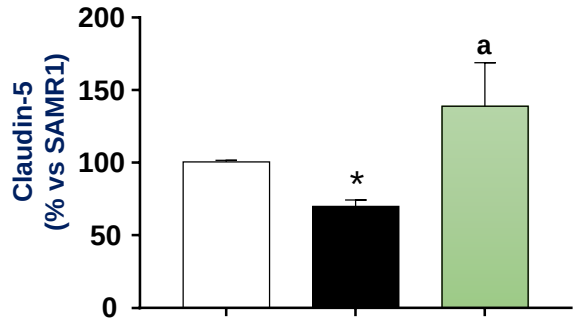


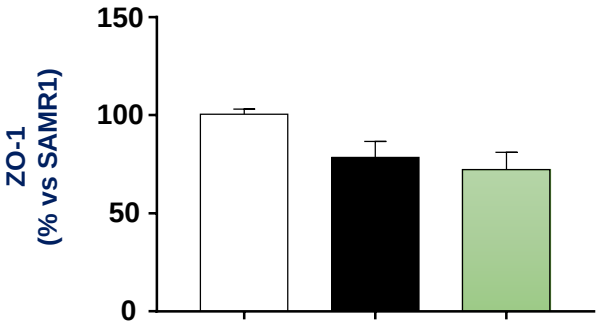












*P<0.05 vs SAMR1
aP<0.01 vs SAMP8

CONCLUSIONS

✓The novel gut-directed NLRP3 inflammasome inhibitor INF exerts beneficial effects on early AD, through reduction of colonic caspase-1 activation and consequent IL-1β release.

OUR CLINICAL STUDY

STUDY SUBJECTS

- 19 (14 M, 5 F) PD patients
  Age: 64-77 years; mean: 70.7 ± 1.2
  Disease duration: 5.8 ± 3.6 years
  Disease severity score (UPDRS III): 16.40 ± 6.06
- 19 age-matched healthy controls without gastrointestinal symptoms

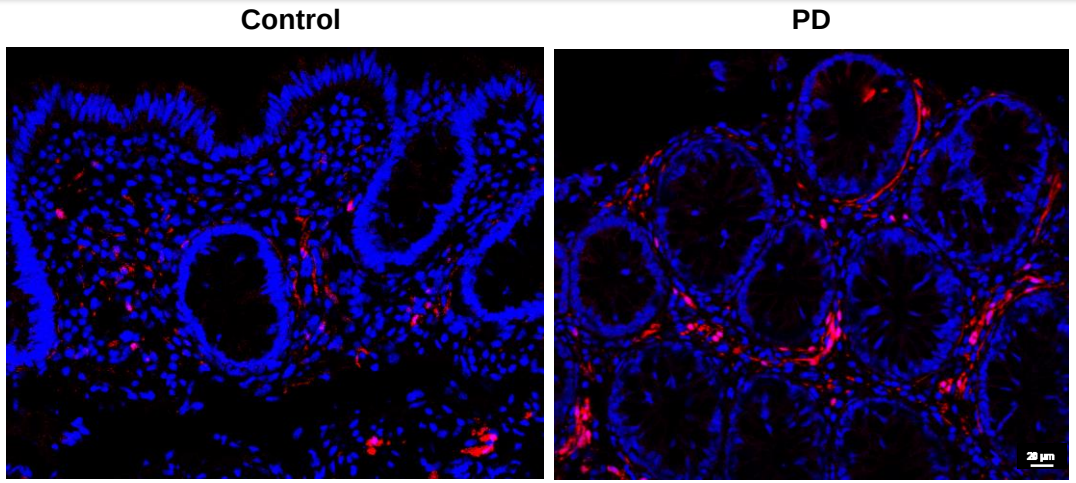
Selection of investigated parameters

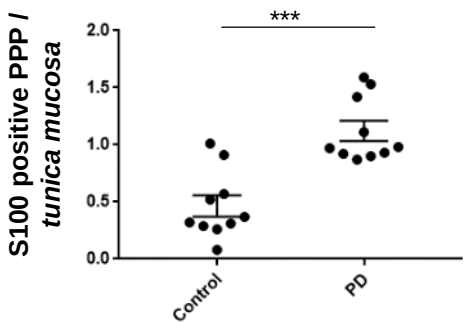
- ✓ Fecal IL-1β levels
- ✓ Circulating lipopolysaccharide Binding Protein (LBP) levels
- ✓ Mucosal biopsies from the descending colon were obtained from PD patients (n=10) and controls (n=10) formalin-fixed and paraffin-embedded for the evaluation of epithelial tight junction claudin-1 expression and glial cell activation (S-100).

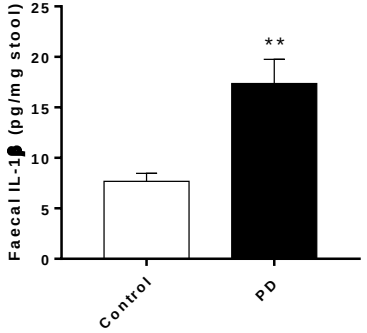
Bellini G et al., Eur J Neurol. 2022;00:1–11.

PD patients displayed enteric inflammation and altered intestinal barrier integrity and permeability

S-100 immunofluorescence and fecal IL-1β levels

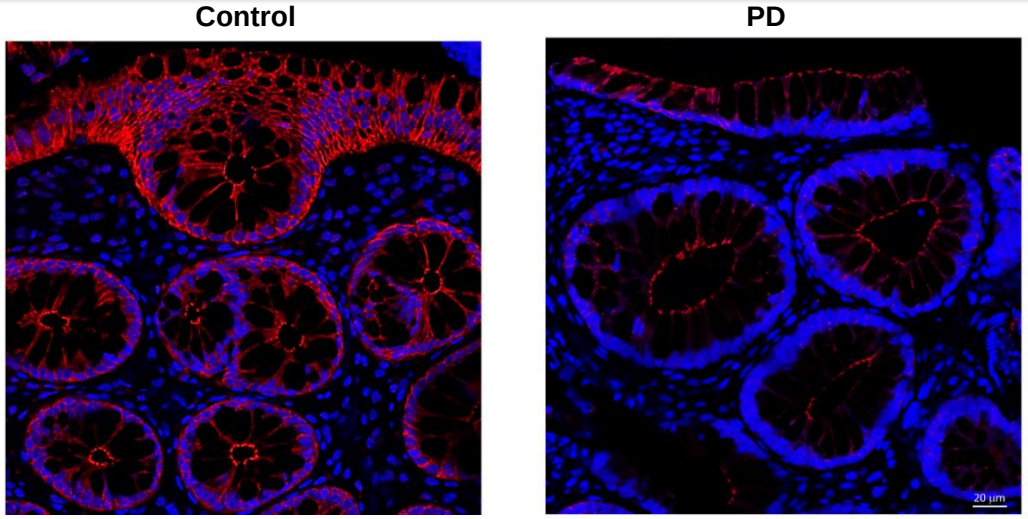


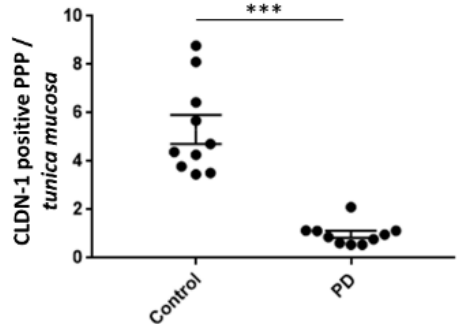


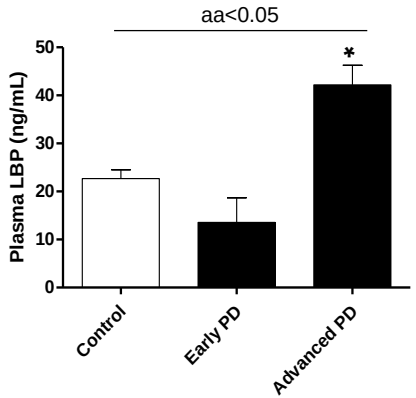


**P<0.05 vs control
***P<0.001 vs control

Claudin-1 immunofluorescence and plasma LBP levels

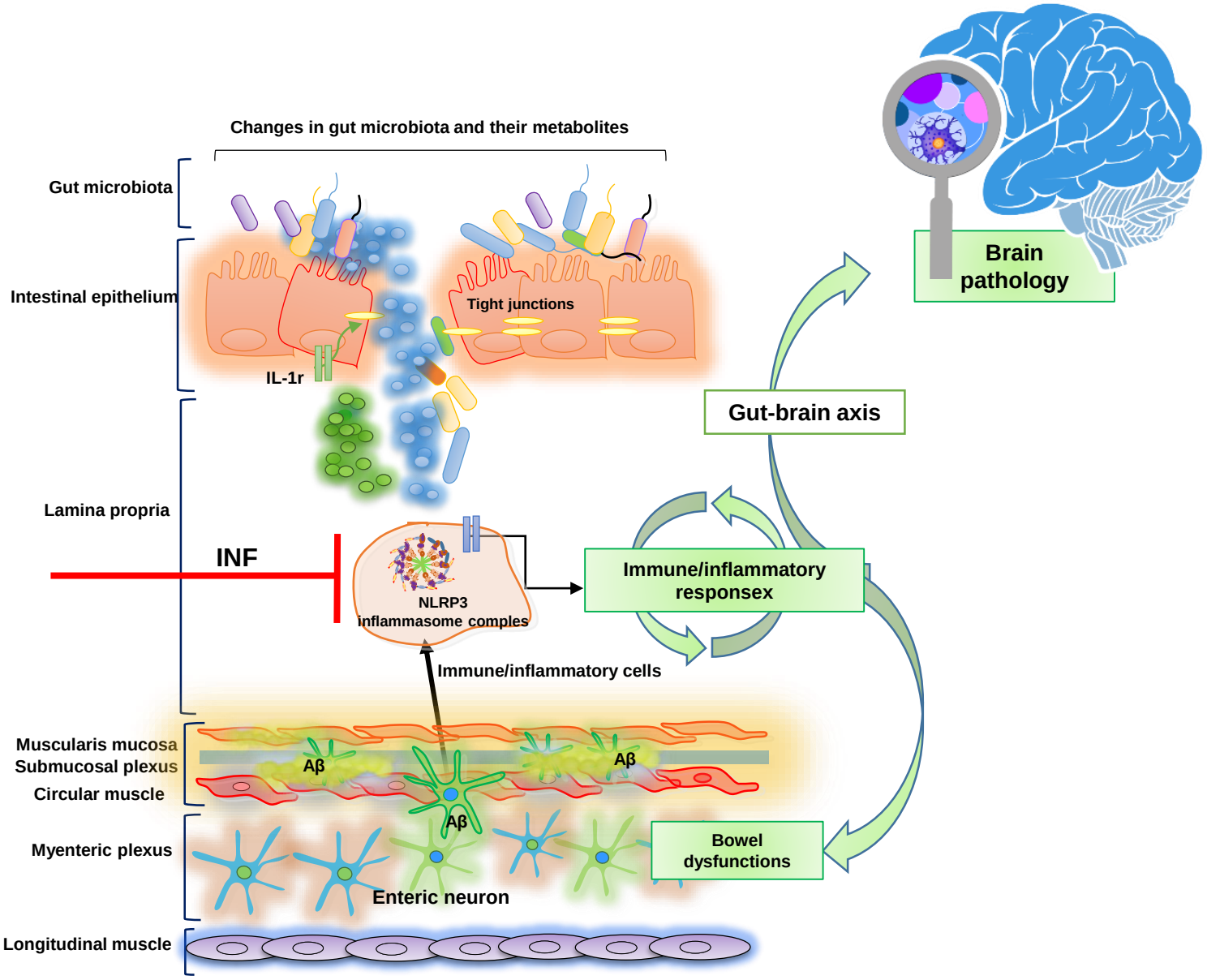






Bellini G et al., Eur J Neurol. 2022;00:1–11.

OVERALL CONCLUSIONS



- ✓In the very early stages of brain diseases, enteric CNS-related protein deposition, impairments of intestinal barrier and changes in gut microbiota, can trigger immune/inflammatory responses, characterized by overactivation of NLRP3 inflammasome pathways.
- ✓In the subsequent stages of the diseases, the enteric/immune inflammatory activation, besides determining bowel dysfunctions, can contribute to brain pathology through gut–brain axis
- ✓In this setting, a gut-directed anti-inflammatory therapy can represent a pharmacological tool to inhibit gut-brain communication and treat CNS disorders

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