Research and Innovation in Nutrition : A major health issue Milk products and innovative fermented ingredients for target populations : Focus on the PROLIFIC Project

Yves Le Loir, Charles Le Bras, Anna Lambert, Marine Mantel, Rodrigue Brossaud, Sarah Blanchet

Research and Innovation in Nutrition :
A major health issue

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CENTRE DE RECHERCHE
EN NUTRITION HUMAINE
OUEST

OUEST
VALORISATION
Ressources d'innovation

Milk products and innovative fermented ingredients for target populations : Focus on the PROLIFIC Project

Yves LE LOIR

STLO - INRAE Institut Agro Rennes

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Ressources d'innovation

PROLIFIC « May your diet be your first medicine »

ENS-
Central Nervous
System (CNS)
interactions

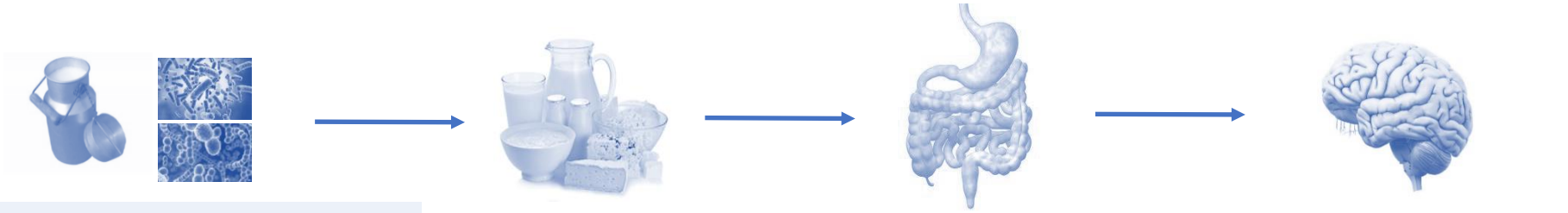
Lactic- and Propionic- Acid bacteria
Metabolites of interest
(SCFA, CLA, oligosaccharides, vitamins, etc.)

Food microbiota-intestinal
epithelial barrier (IEB)
interactions

IEB-Enteric nervous
system (ENS) interactions

STLO

Scientific continuum and expertise



Milk Components
Microbiology, Nutrition & Health

Foods
Biological activities

Gut & Microbiota
IBD, allergy

Gut-brain axis
Cognitive Functions

IRISA — LS2N/ComBI — *(bioinformatic)*

CIRM_BIA — STLO — *(microbiology, milk processing)*

bia — *(allergy)*

numecan — *(nutrition, cognition)*

UMR TENS — *(gut-brain axis)*

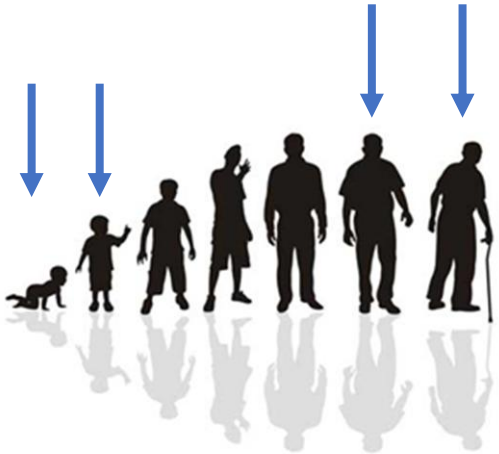
STLO

For which target populations?

The first **1000** days of life

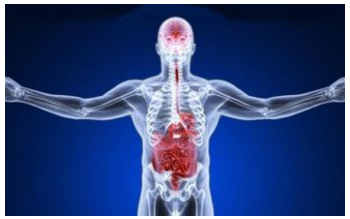
(0-6 months and 6 months-3 years)

The **seniors**



For which functionalities ?

Colonisation and homeostasis of gut microbiota



Cognitive development / Neurodegeneration

Inflammation
Tolerance / Allergy

STLO

Human ressources

5 **PhD projects** (*180 months*)

4 **post-docs** (*60 months*)

5 **Contracts Technicians and Engineers** (*90 months*)

Funding

13,9 **M€ Full cost**

7,55 **M€ funded by Bba Milk Valley**

1,86 **M€ funded by Regions BZH and PDL**

STLO

Thank you for your attention

Questions
& Answers

Two human milk synthetic bacterial community (SynCom) exhibited contrasted impacts *in vitro* on intestinal barrier and immune function

Charles LE BRAS

STLO & NuMeCan

Introduction

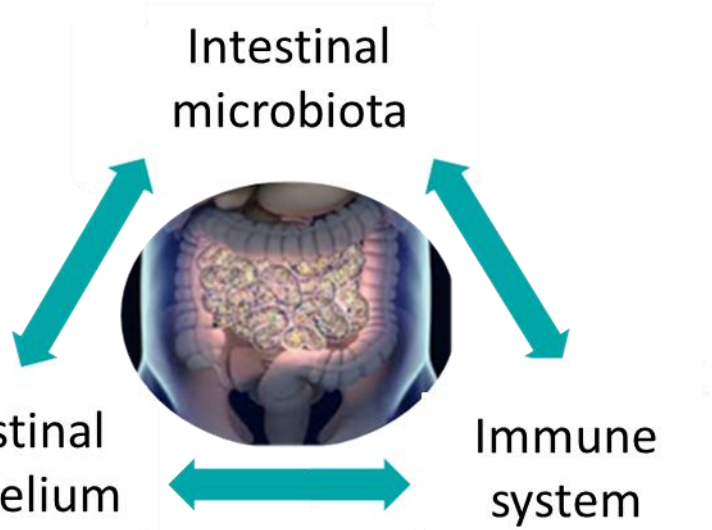
Breastfeeding

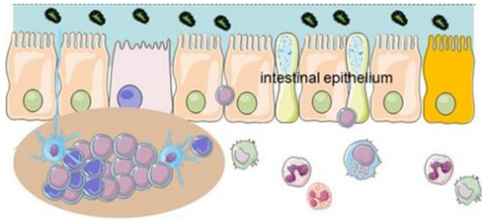
Health benefits

?

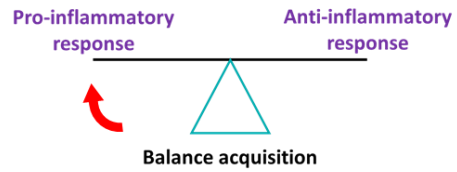
Human milk microbiota

Intestinal homeostasis





Intestinal Barrier maturation

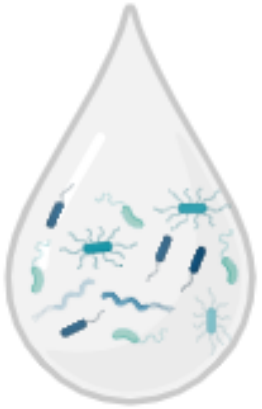


Immune maturation

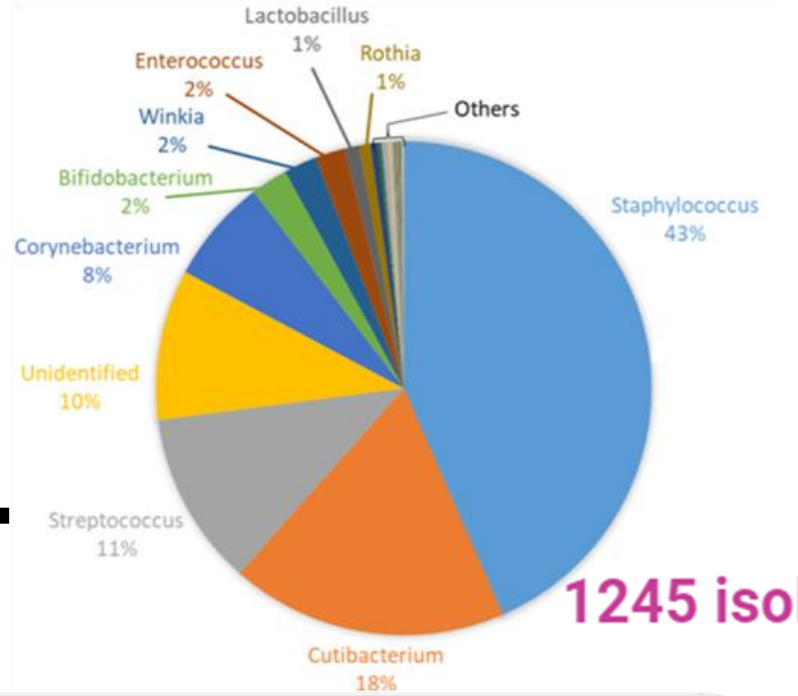
Our hypothesis is that the microbiota of human breast milk contributes to the health benefits of breast milk

Strategy

28
Donors



Bacterial collection

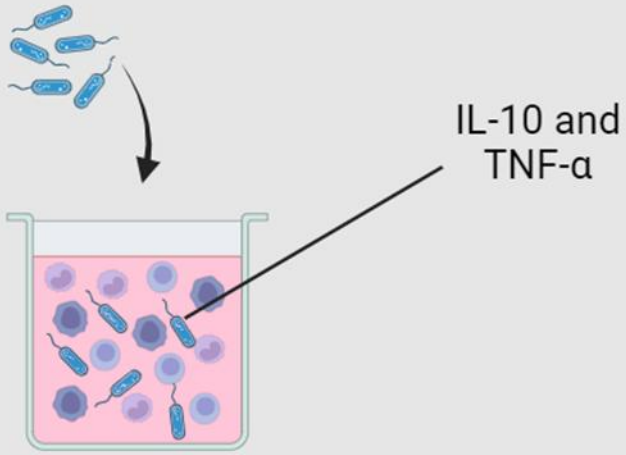


1245 isolates

Preselection

Human PBMC model

84 isolates

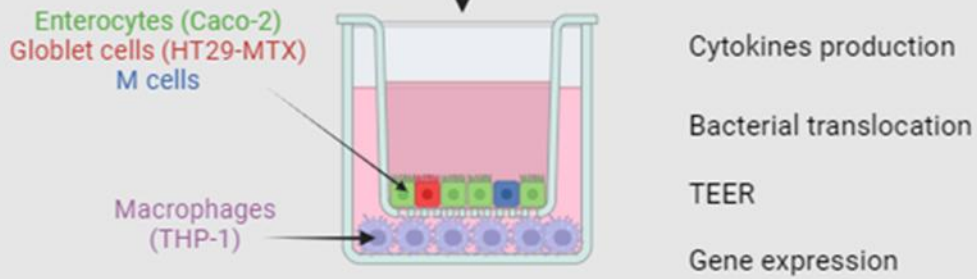


Quadricellular model

29 isolates

OR

2 SynComs



Cytokines production

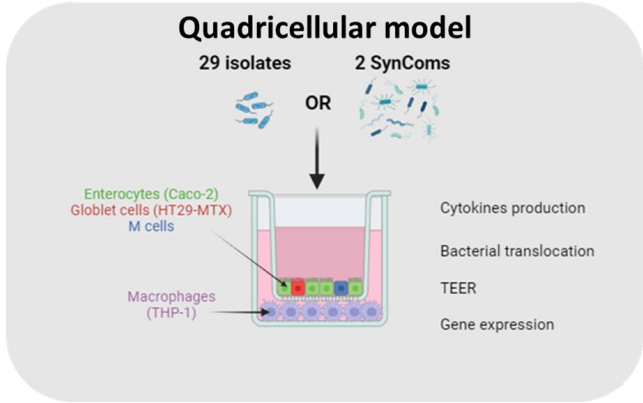
Bacterial translocation

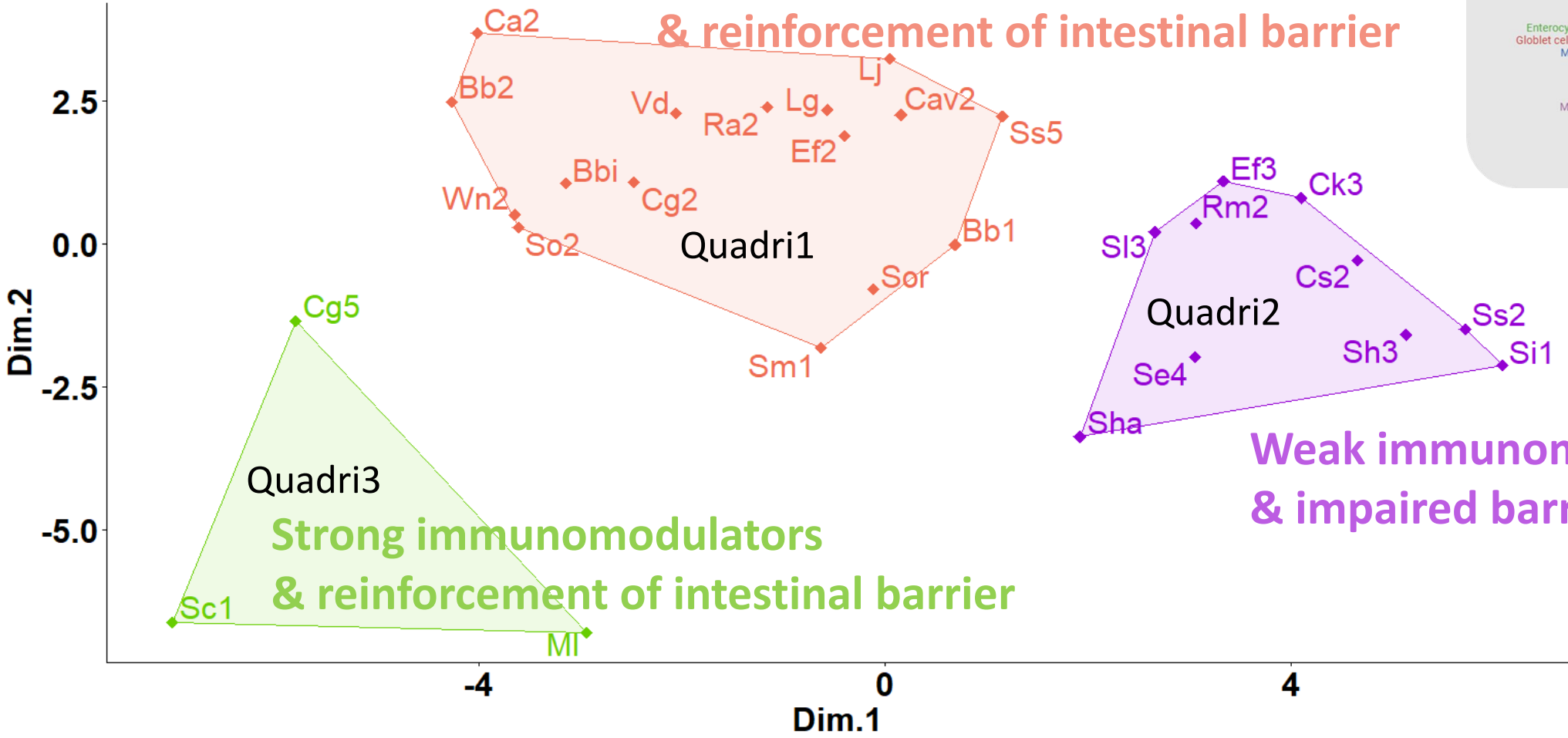
TEER

Gene expression

Individual impact of HM bacteria on immune and barrier functions

Classification by *MultiDimensionate Scaling*





Great variability of functional profiles of HM bacteria

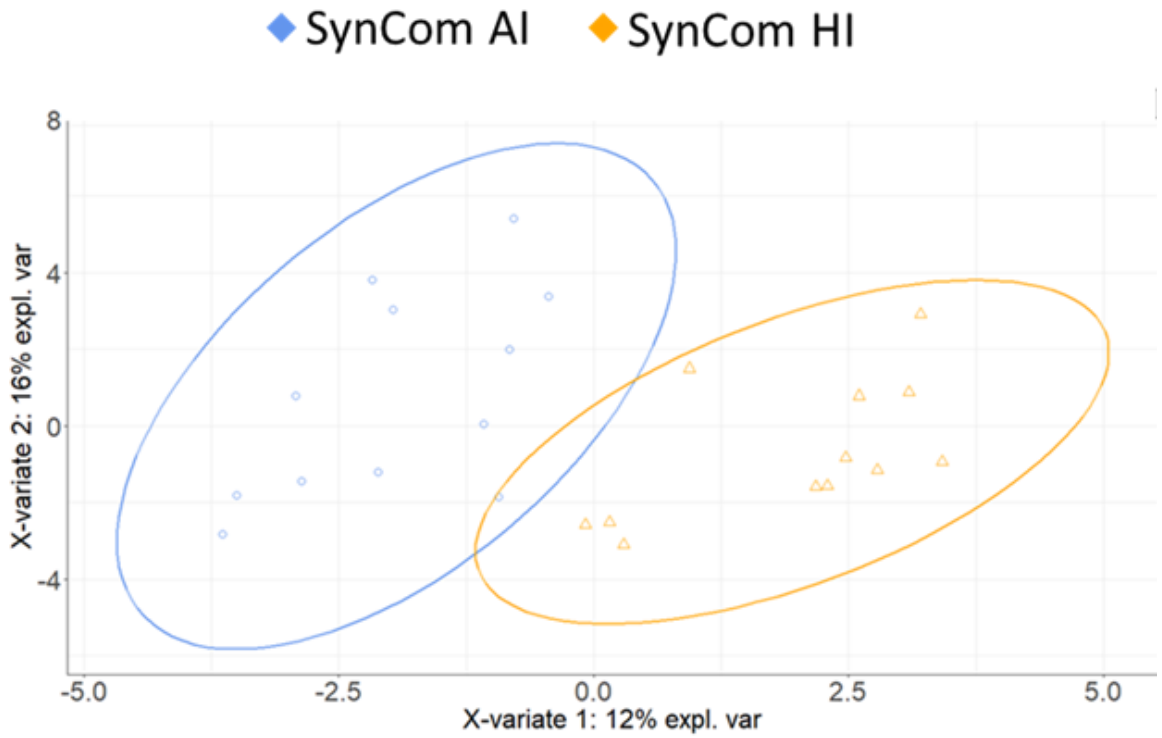
Immune and barrier properties of the 2 SynComs

Design of 2 Synthetic Communities :

- ➔ **Mimicking HM microbiota**
- ➔ **With different functional properties**

	SynCom AI	SynCom HI	
Anti-inflammatory	Cutibacterium granulosum (Cg2)	Cutibacterium granulosum (Cg5)	Strong immunomodulatory
Anti-inflammatory	Staphylococcus haemolyticus (Sha)	Staphylococcus capitis (Sc1)	Strong immunomodulatory
Anti-inflammatory	Winkia neuii (Wn2)	Micrococcus luteus (Ml)	Strong immunomodulatory
Anti-inflammatory	Streptococcus infantis (Si1)	Staphylococcus epidermidis(Se4)	Anti-inflammatory
	Cutibacterium acnes (Ca2)	Streptococcus salivarius(Ss2)	
	Corynebacterium simulans (Cs2)	Corynebacterium kroppenstedtii (Ck3)	
	Bifidobacterium breve (Bb1)	Bifidobacterium breve (Bb2)	
	Lactobacillus gasseri (Lg)	Lactobacillus jensenii (Lj)	
Common strains	Rothia mucilaginosa (Rm2)		
Common strains	Veillonella dispar (Vd)		
Common strains	Bifidobacterium bifidum (Bbi)		

sPLS-DA analysis



- **Contrasted immunomodulatory properties as expected from the assembly**
- **New properties that differed from those of the individual bacteria**

Conclusion

- The profile of HM bacterial SynComs reflected the coexistence of specific bacterial profiles that comprise the HM microbiota, leading to its role in driving gut homeostasis

- The functional properties of the HM bacterial community relied on a combination of strain-specific features rather than on the taxonomic composition itself

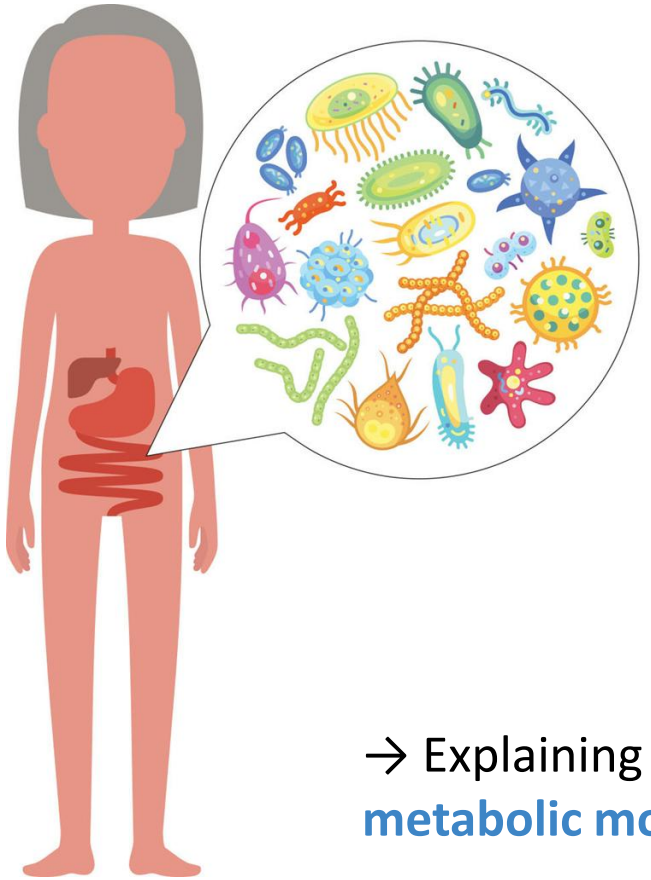
Questions
& Answers

In silico modeling of host-lactic acid bacteria interactions at the Epithelial Intestinal Barrier

Anna LAMBERT

LS2N & TENS laboratories

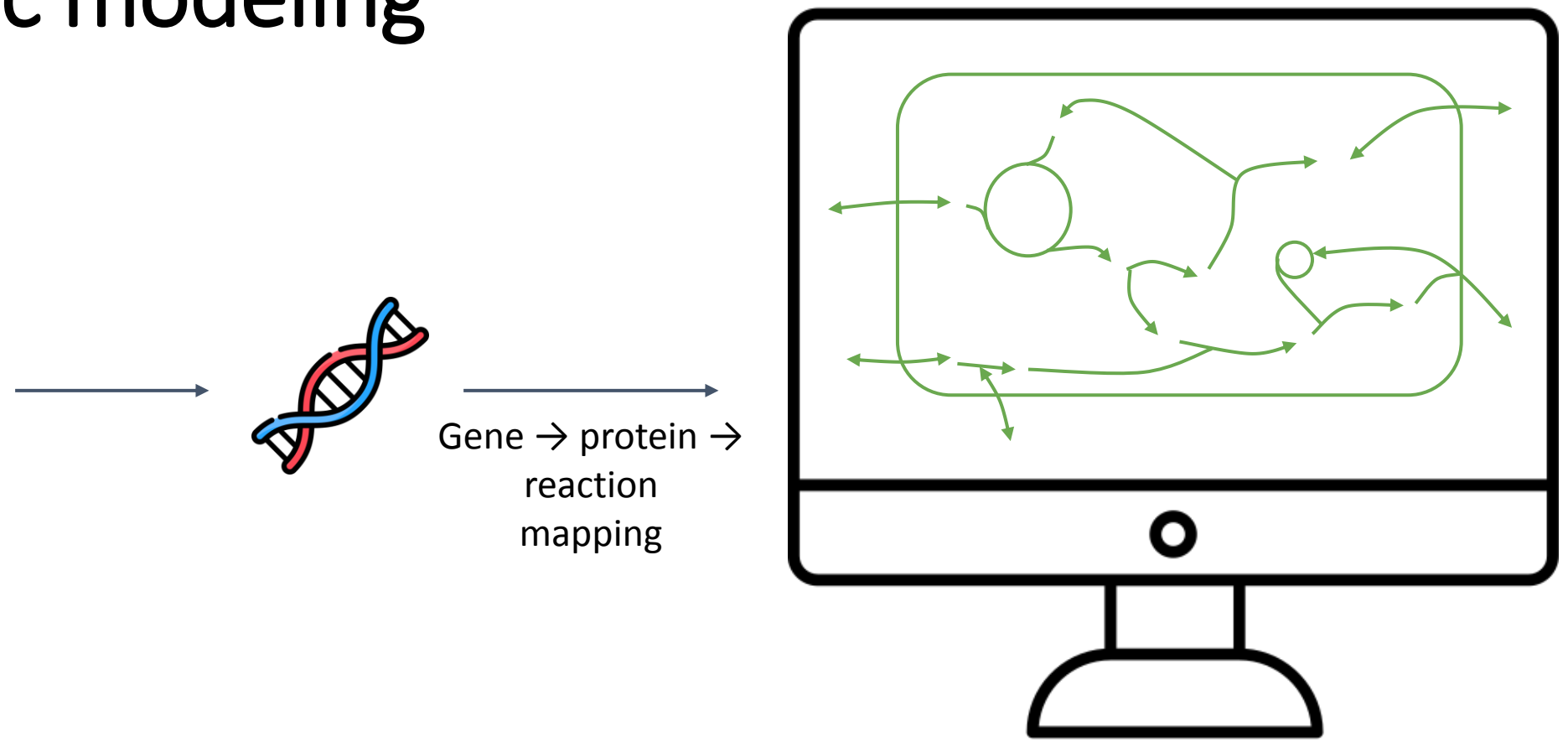
The host - microbiota ecosystem



- The gut microbiota is a complex ecosystem
- It impacts our health in several ways, notably through metabolism (e.g. production of vitamins or butyrate)
- Mechanisms of action of many bacteria remains unclear

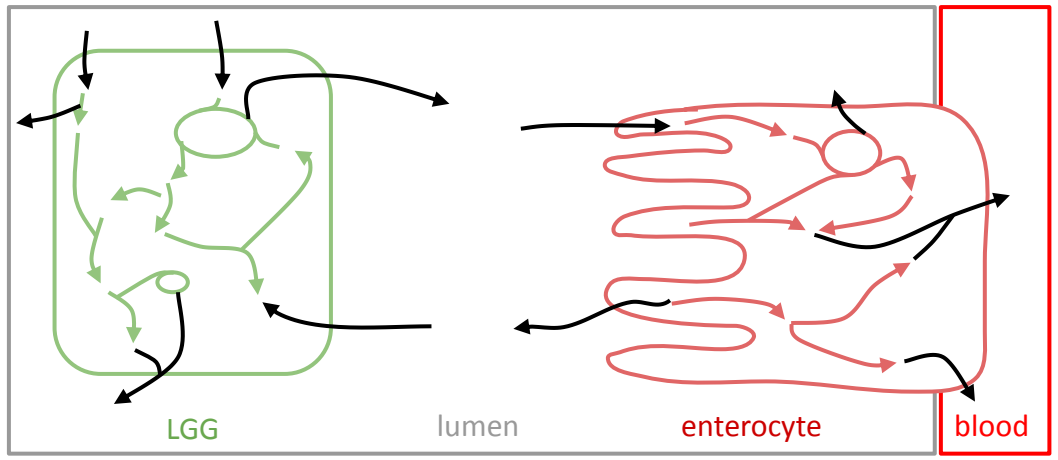
→ Explaining the influence of gut bacteria on the host using **computational metabolic models**

Metabolic modeling



Metabolic model : Informatic reproduction of the metabolic network of an organism

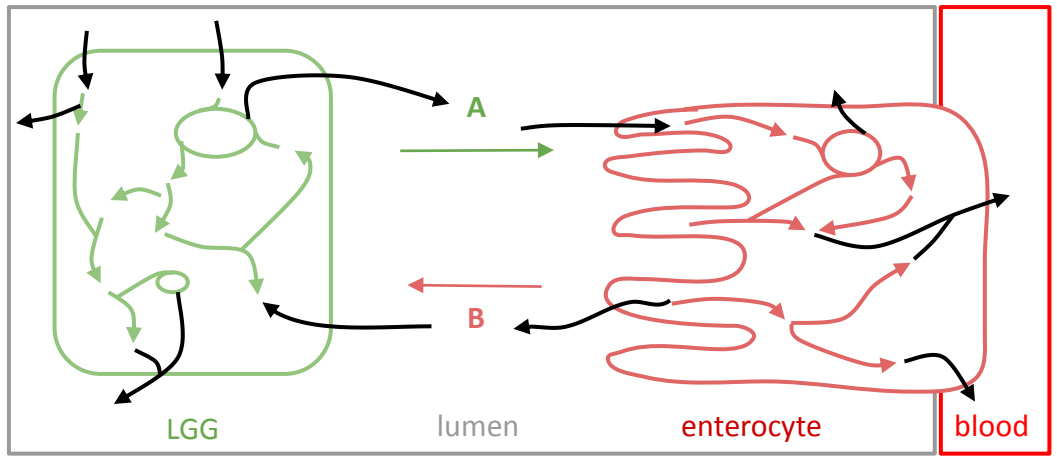
Exploration of ecosystem interaction



- Interaction score
  (competition, neutrality, mutualism)

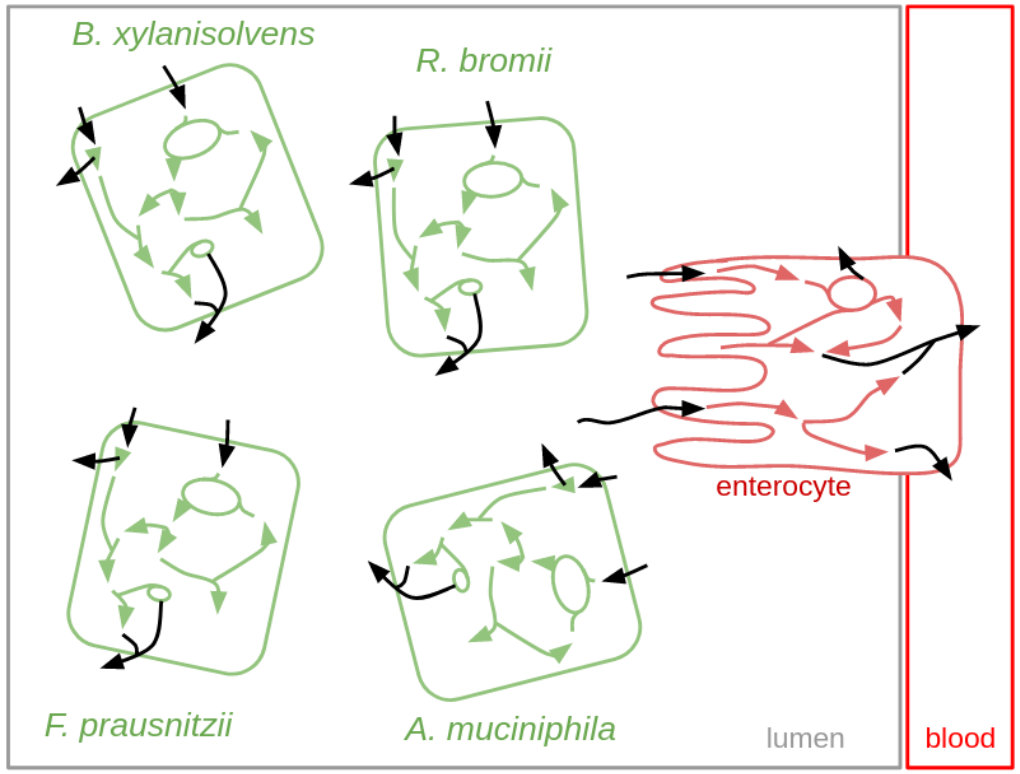


Exploration of ecosystem interaction



- Interaction score
  (competition, neutrality, mutualism)
- Cross-fed metabolites

Exploration of ecosystem interaction



- Interaction score
  (competition, neutrality, mutualism)
- Cross-fed metabolites
- Applied in larger ecosystems

Questions
& Answers

Combined effects of propionic acid bacteria and n-6 polyunsatured fatty acids on the intestinal barrier

Marine MANTEL

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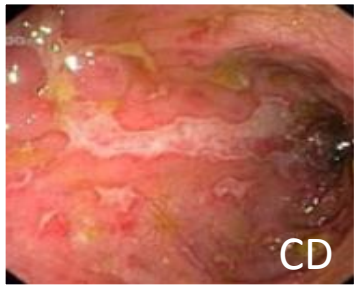
Intestinal bowel disease (IBD)

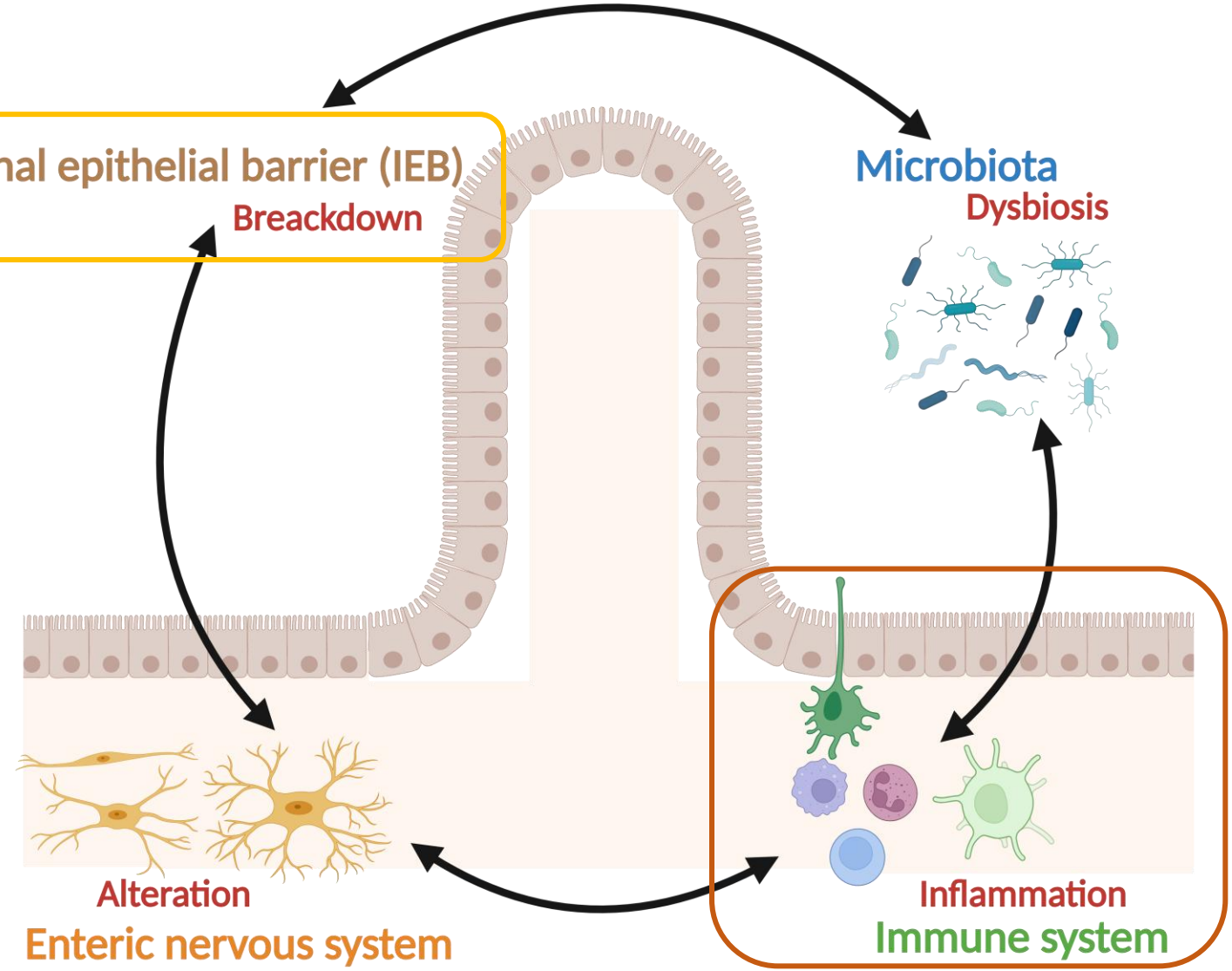
Crohn’s disease (CD) and Ulcerative colitis (UC)



Chronic inflammation.

No curative treatment!

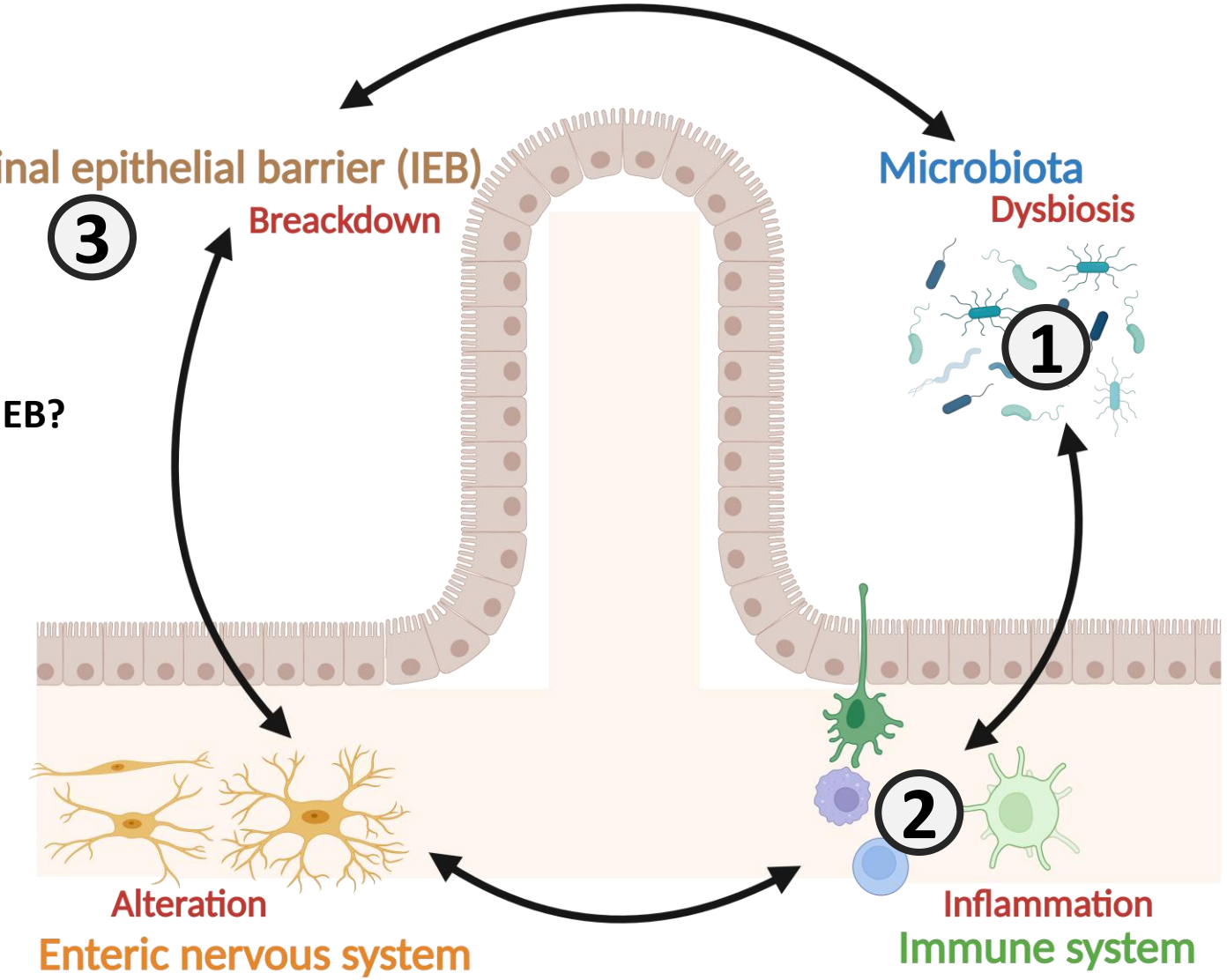
Intestinal bowel disease (IBD)

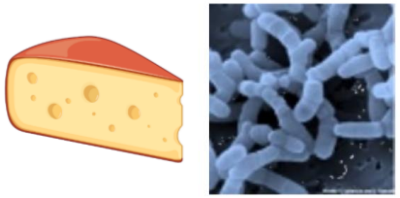


1st line of defense
Target of interest

Current treatments

How to reinforce IEB?





Propionibacterium freudenreichii (Pf)

3 **Does *Pf* can reinforce IEB?**

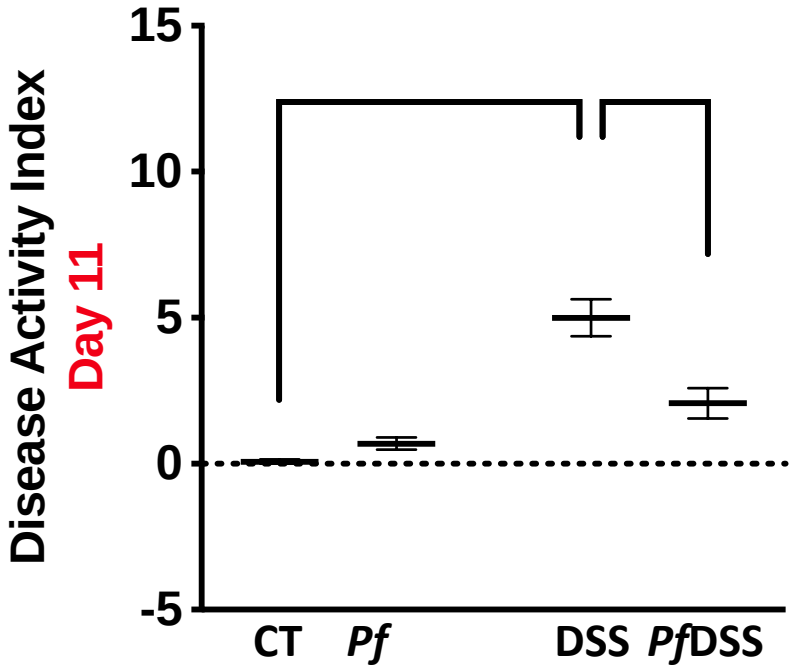
1 **Improved gut microbiota**

2 **Anti-inflammatory**

Pf supplementation

DSS-induced colitis

Colitis severity

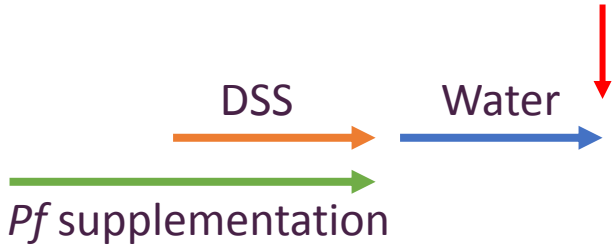


Pf prevents colitis severity induced by the DSS.

Pf supplementation

DSS-induced colitis

Recovery protocol

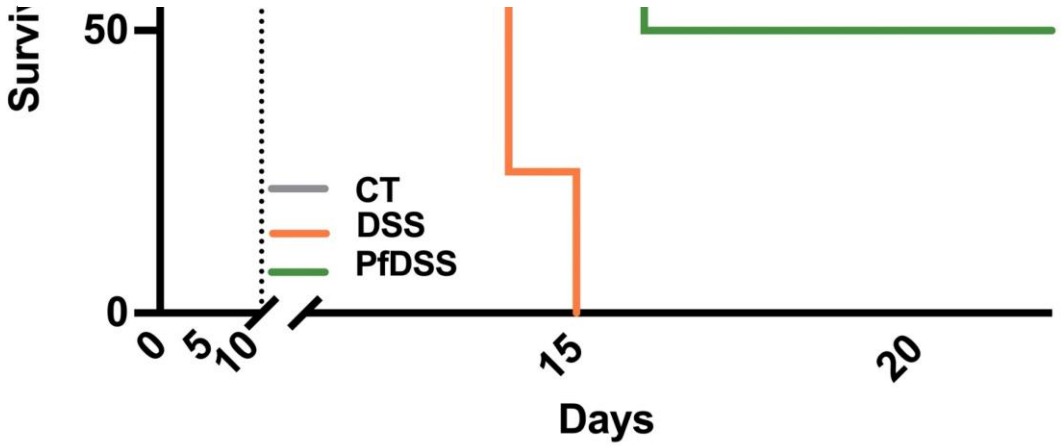


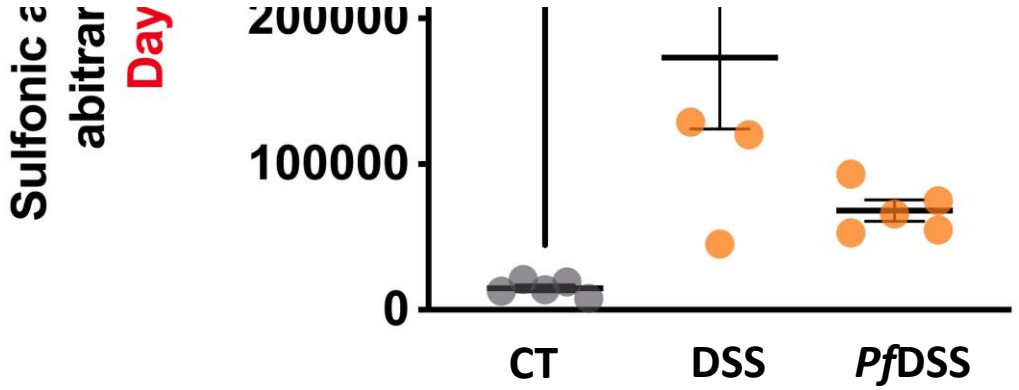
Day 11

Survival

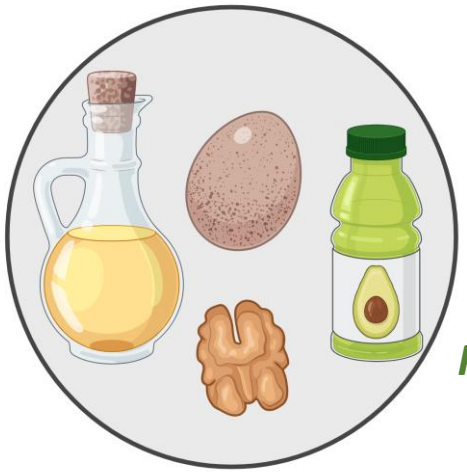
Intestinal permeability

Pf decreases intestinal permeability and improves recovery.

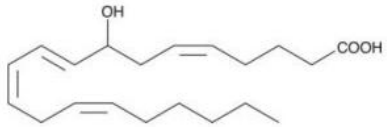




N-6 diet: good for the gut?



Enzymes



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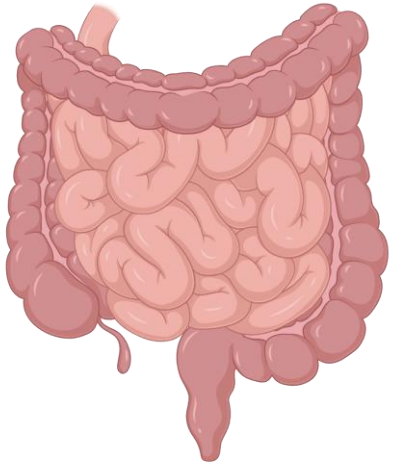


Pf: lipolytic activity

Metabolites

?

Disruption of n-6 metabolism in IBD patients.

Combine protective effects of n-6 enriched diet and *Pf*?

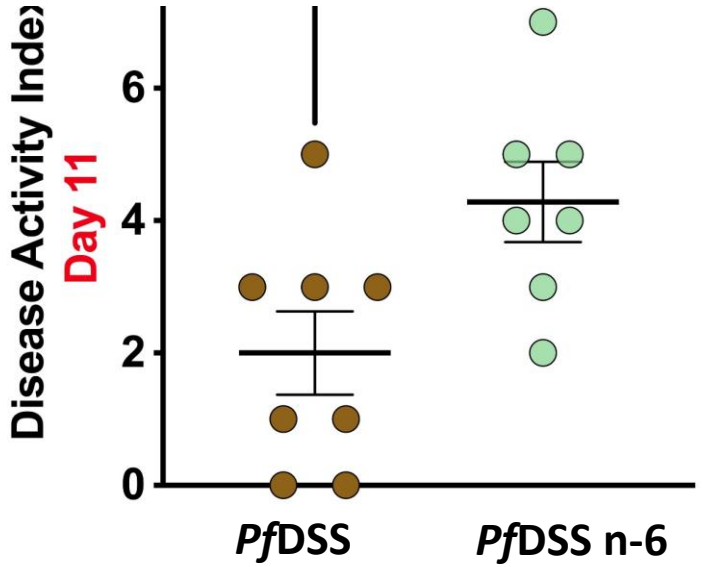
Pf supplementation
N-6 enriched diet

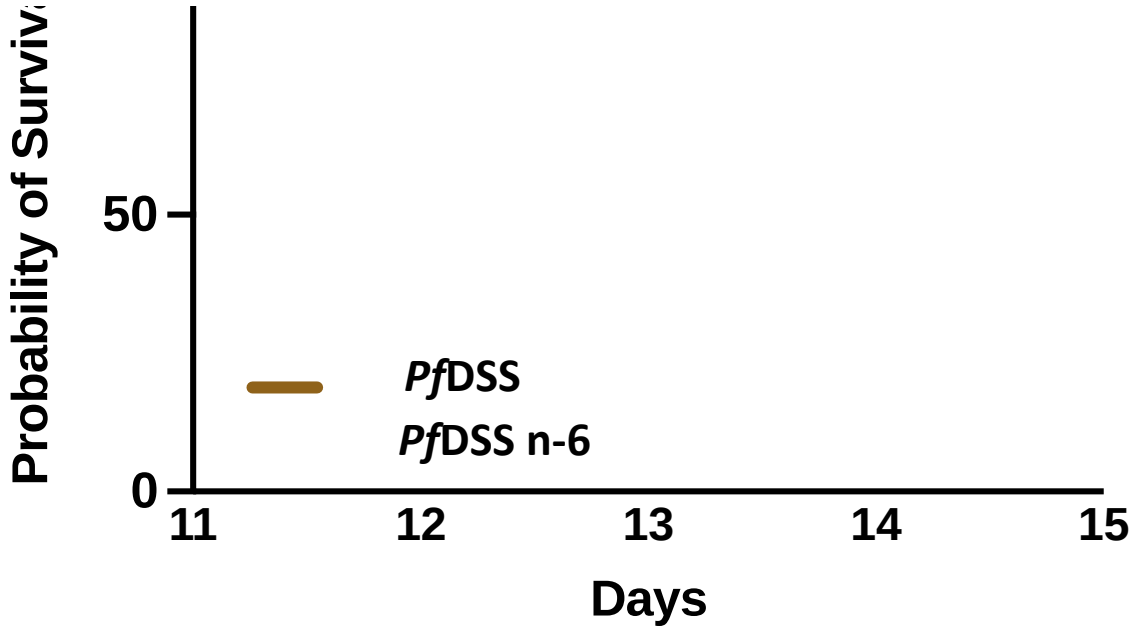
Recovery protocol

Colitis severitv

Survival

N-6 enriched diet abrogates *Pf* protective effects.





Take home message

1)Pf reinforces IEB

2)Pf improves recovery and inhibits colitis

3)N-6 enriched diet abrogates *Pf* protective effects

Taking the patient's nutritional profile into account is essential when integrating probiotics !

Questions
& Answers

What are the beneficial effects of bacterial metabolites on cognitive and digestive functions in Alzheimer's disease ?

Rodrigue BROSSAUD

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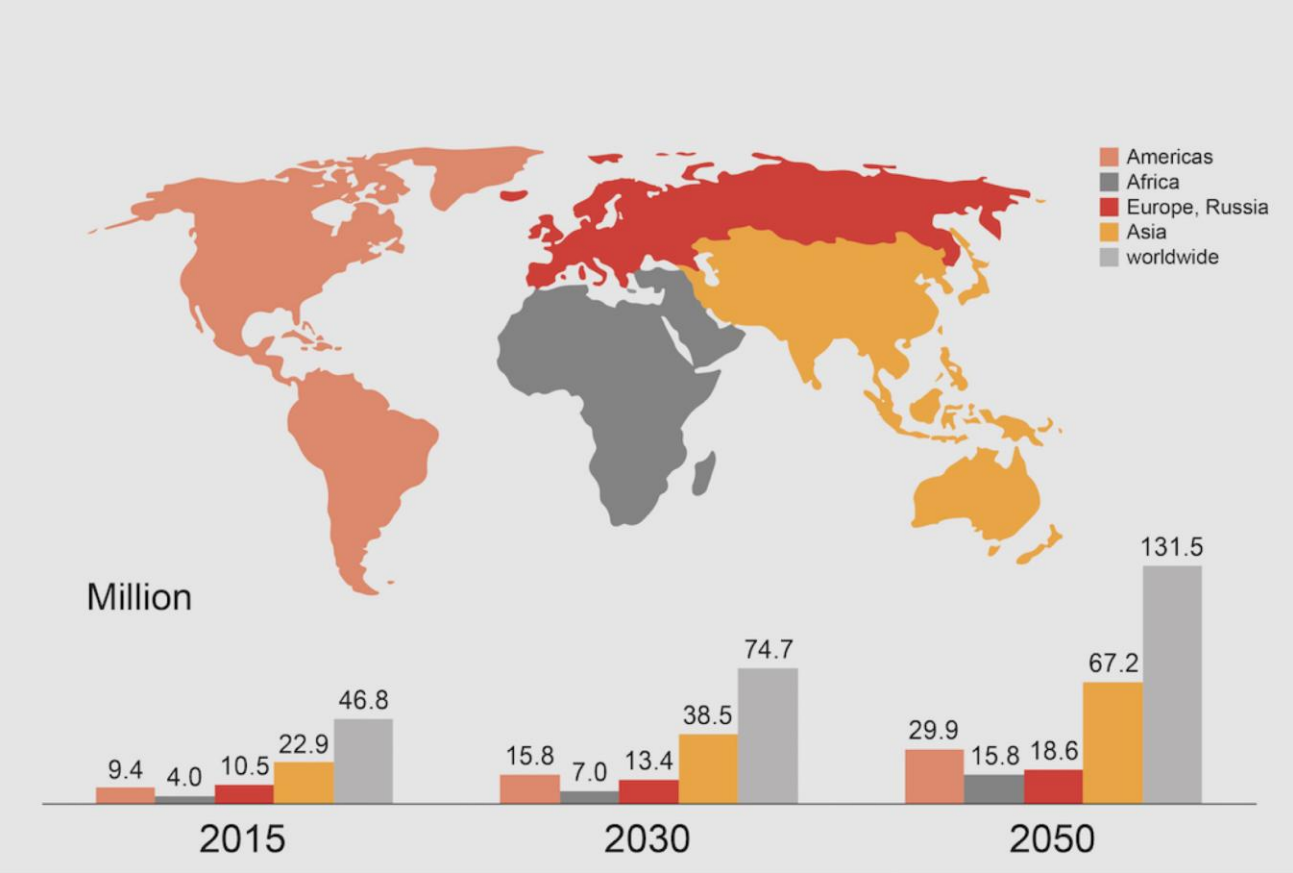
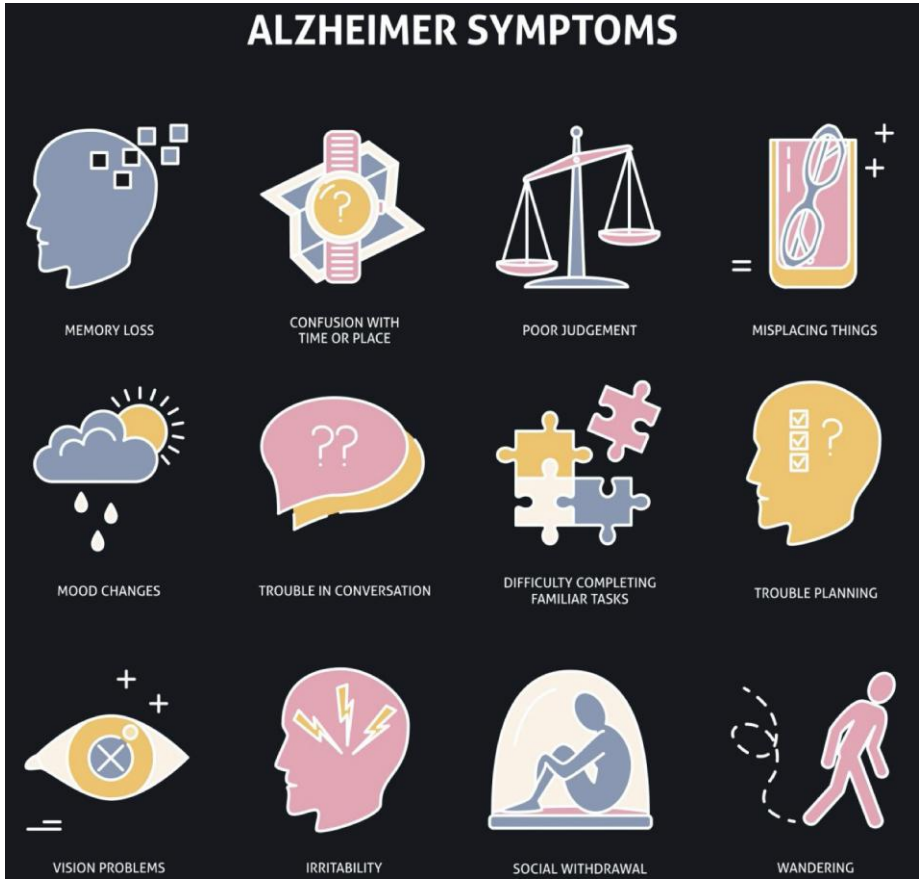
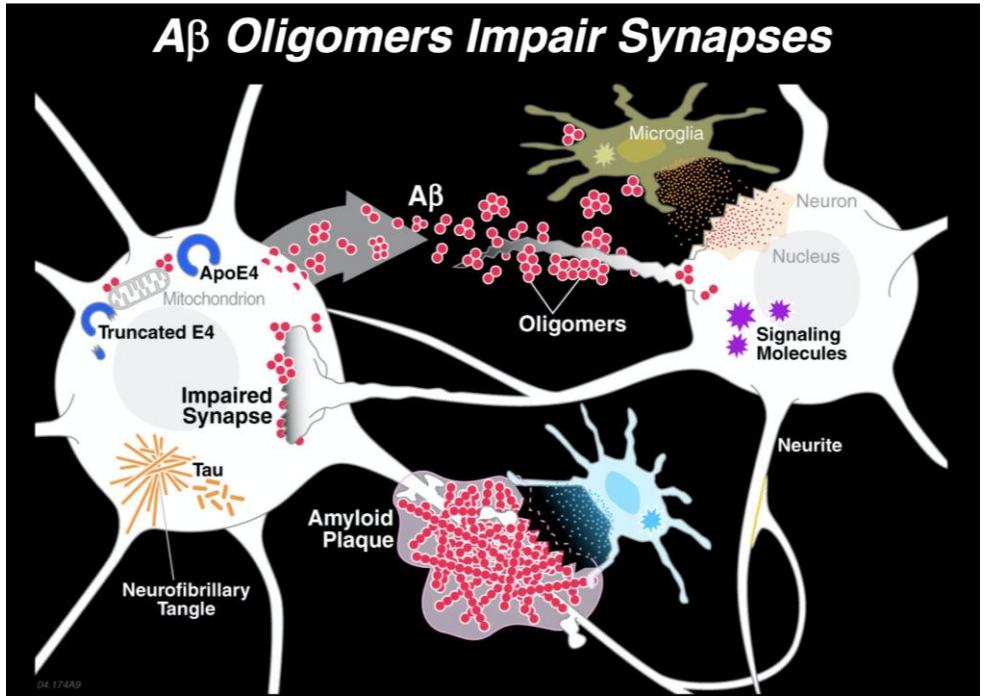
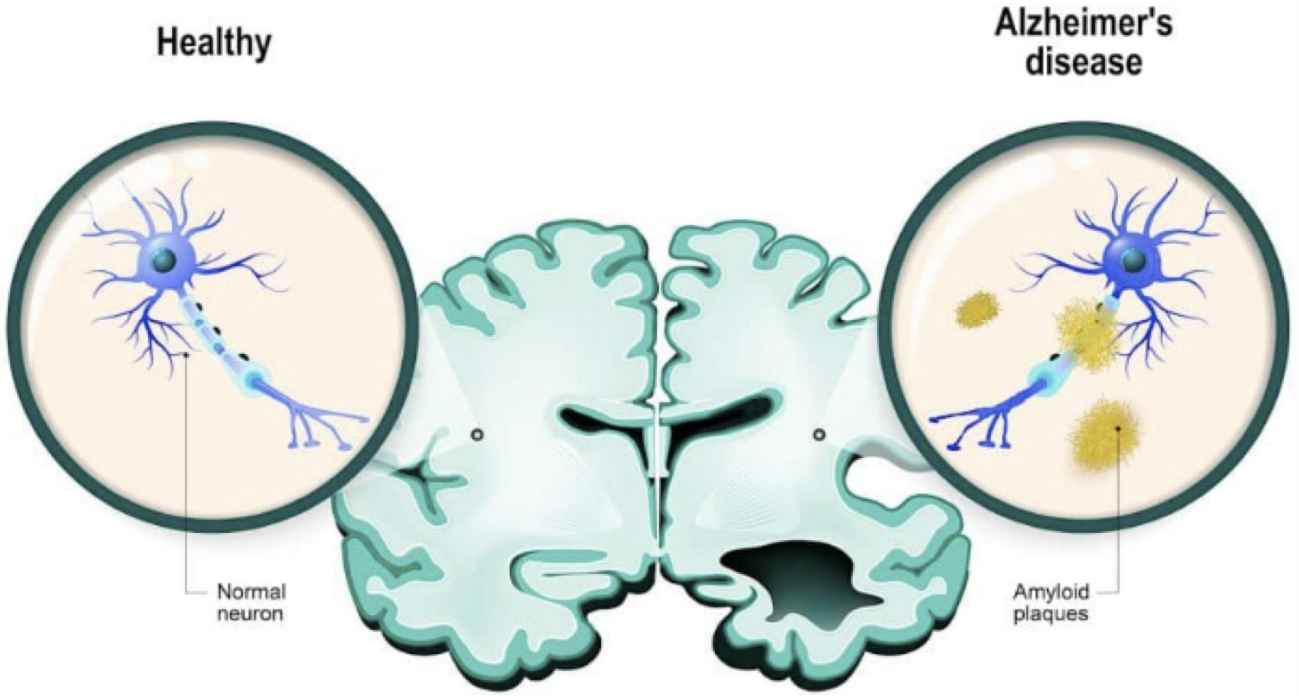
Alzheimer’s disease : epidemiology and symptoms





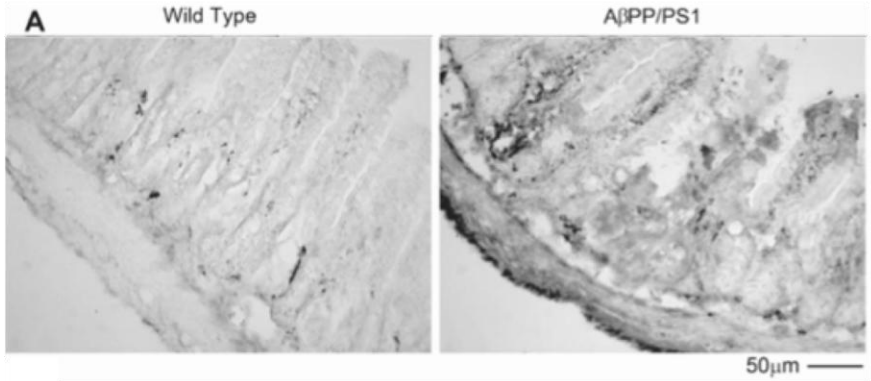
Alzheimer’s disease : Amyloïd-ß toxicity



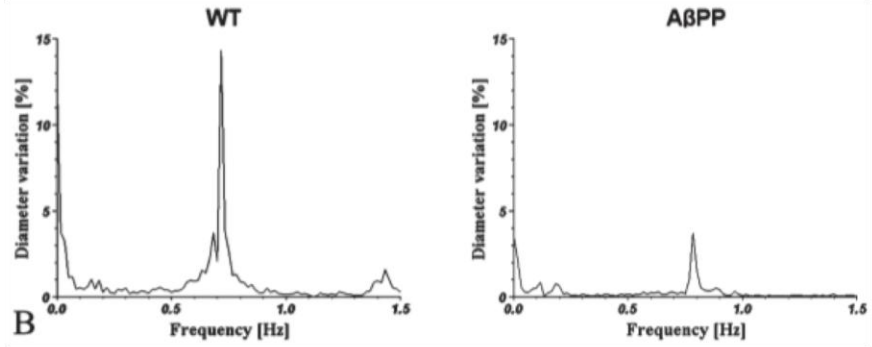


Alzheimer's disease : A gut-brain disease ?

Animal models

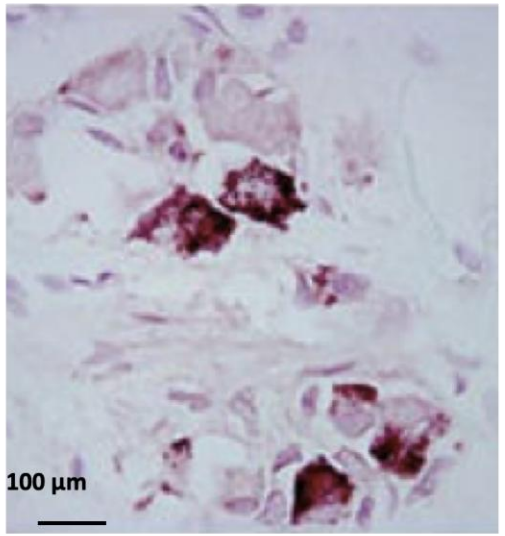


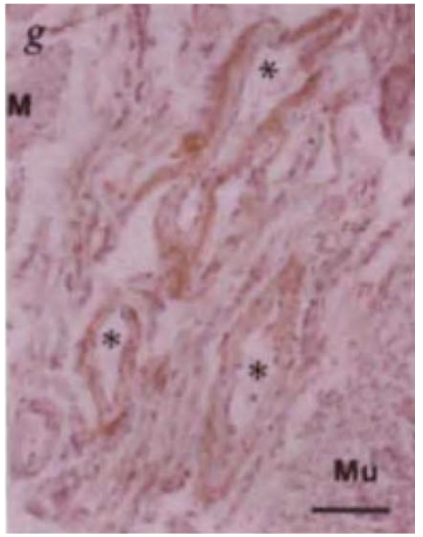
- Intestinal amyloïd aggregates
(Puig et al., 2015)



- Impairments of digestive motricity
(Semar et al., 2013)

Humans





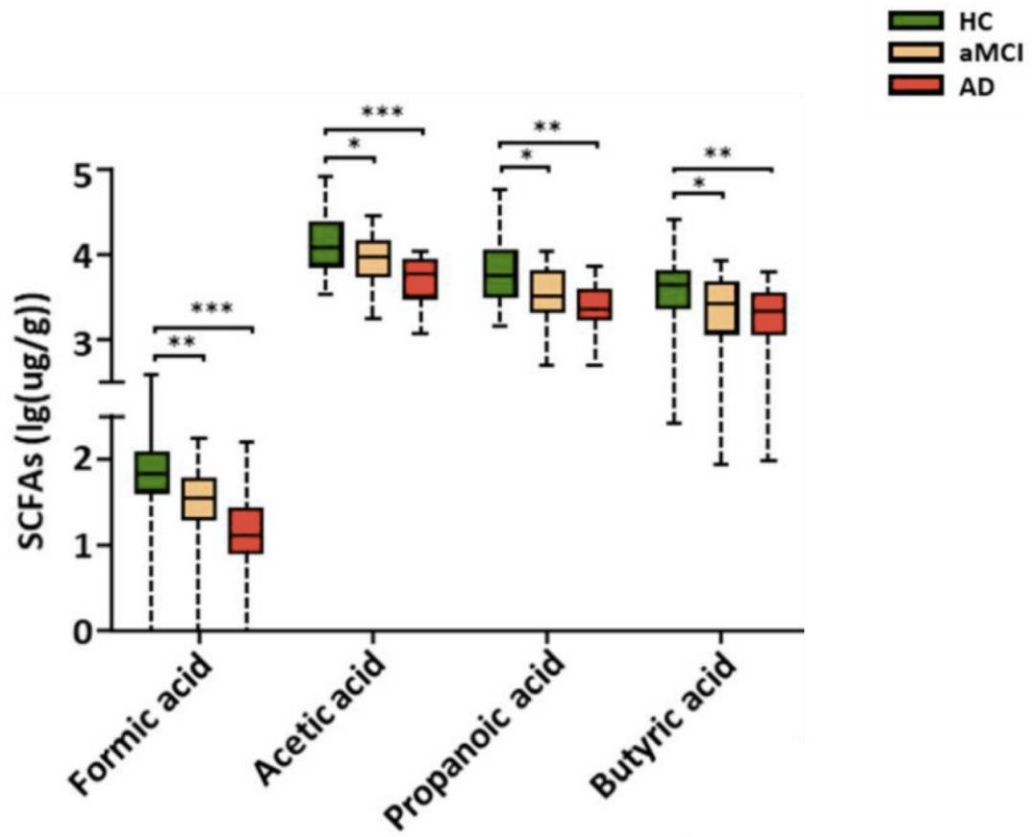
- Intestinal amyloid aggregates
(Joachim et al., 1989 ; Puig et al., 2015)

Alzheimer's disease : A gut-brain disease ?

	Diagnosis within 0–2 years				Diagnosis within 2–10 years			
	UK		France		UK		France	
	OR (corrected 95% CI)	Corrected p value	OR (corrected 95% CI)	Corrected p value	OR (corrected 95% CI)	Corrected p value	OR (corrected 95% CI)	Corrected p value
Major depressive disorder, F32	2·14 (1·77–2·59)	<0·0001	3·41 (3·04–3·84)	<0·0001	1·34 (1·15–1·56)	<0·0001	1·73 (1·45–2·07)	<0·0001
Anxiety, F41	2·02 (1·69–2·41)	<0·0001	1·93 (1·71–2·17)	<0·0001	1·36 (1·18–1·56)	<0·0001	1·5 (1·26–1·78)	<0·0001
Reaction to severe stress and adjustment disorders, F43	2·34 (1·76–3·11)	<0·0001	2·1 (1·61–2·75)	<0·0001	1·4 (1·12–1·77)	<0·0001	1·83 (1·36–2·46)	<0·0001
Hearing loss, H91	1·28 (1·07–1·52)	0·0001	1·95 (1·35–2·82)	<0·0001	1·19 (1·04–1·36)	0·0006	1·51 (1·01–2·26)	0·033
Constipation, K59	1·41 (1·23–1·63)	<0·0001	1·66 (1·47–1·87)	<0·0001	1·31 (1·16–1·49)	<0·0001	1·59 (1·33–1·89)	<0·0001
Spondylosis, M47	1·25 (0·93–1·69)	0·933	1·45 (1·22–1·72)	<0·0001	1·26 (1·05–1·5)	0·0005	1·62 (1·32–1·98)	<0·0001
Memory loss symptom, R41	31·5 (24·18–41·05)	<0·0001	16·5 (10·39–26·19)	<0·0001	7·63 (5·95–9·79)	<0·0001	4·41 (2·3–8·48)	<0·0001
Malaise and fatigue, R53	1·36 (1·17–1·58)	<0·0001	1·78 (1·59–2·0)	<0·0001	1·23 (1·08–1·39)	<0·0001	1·59 (1·36–1·86)	<0·0001
Syncope and collapse, R55	1·95 (1·53–2·48)	<0·0001	2·49 (1·68–3·69)	<0·0001	1·23 (1·01–1·5)	0·034	1·57 (1·06–2·34)	0·007
Abnormal weight loss, R63	2·1 (1·68–2·62)	<0·0001	3·12 (2·41–4·02)	<0·0001	1·47 (1·22–1·77)	<0·0001	1·88 (1·35–2·62)	<0·0001

Both CIs and p values were corrected for multiple comparisons. OR=odds ratio. *Cannot be calculated because an insufficient number of presentations w

Table 2: **ORs for all variables individually associated with a future diagnosis of Alzheimer's disease in the 2–10 years before diagnosis**



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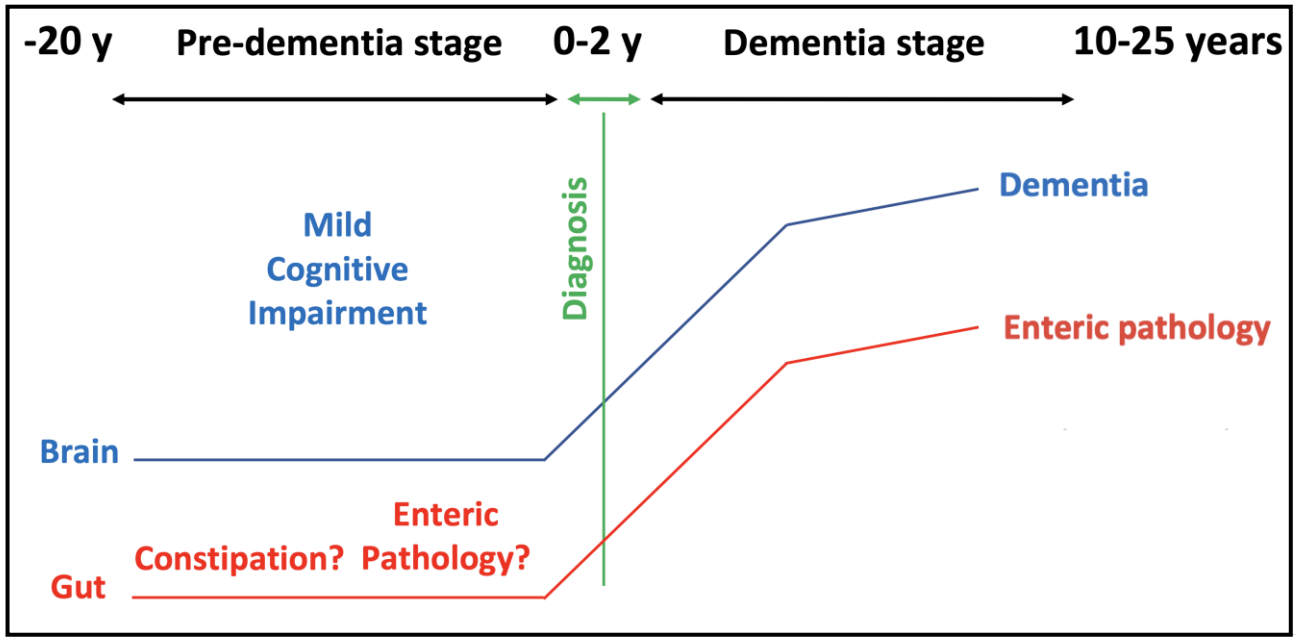

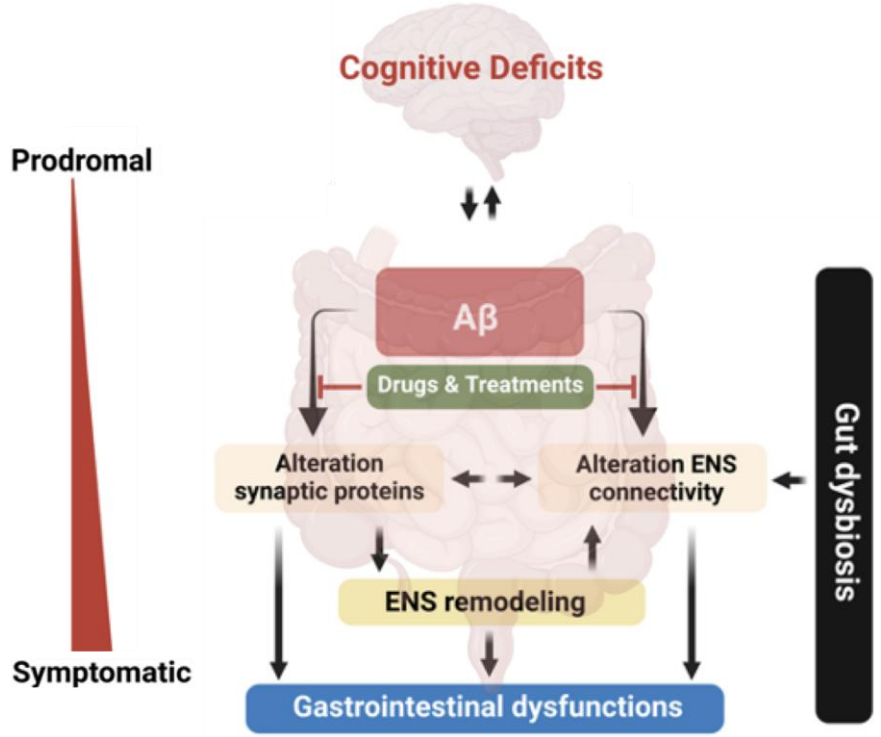

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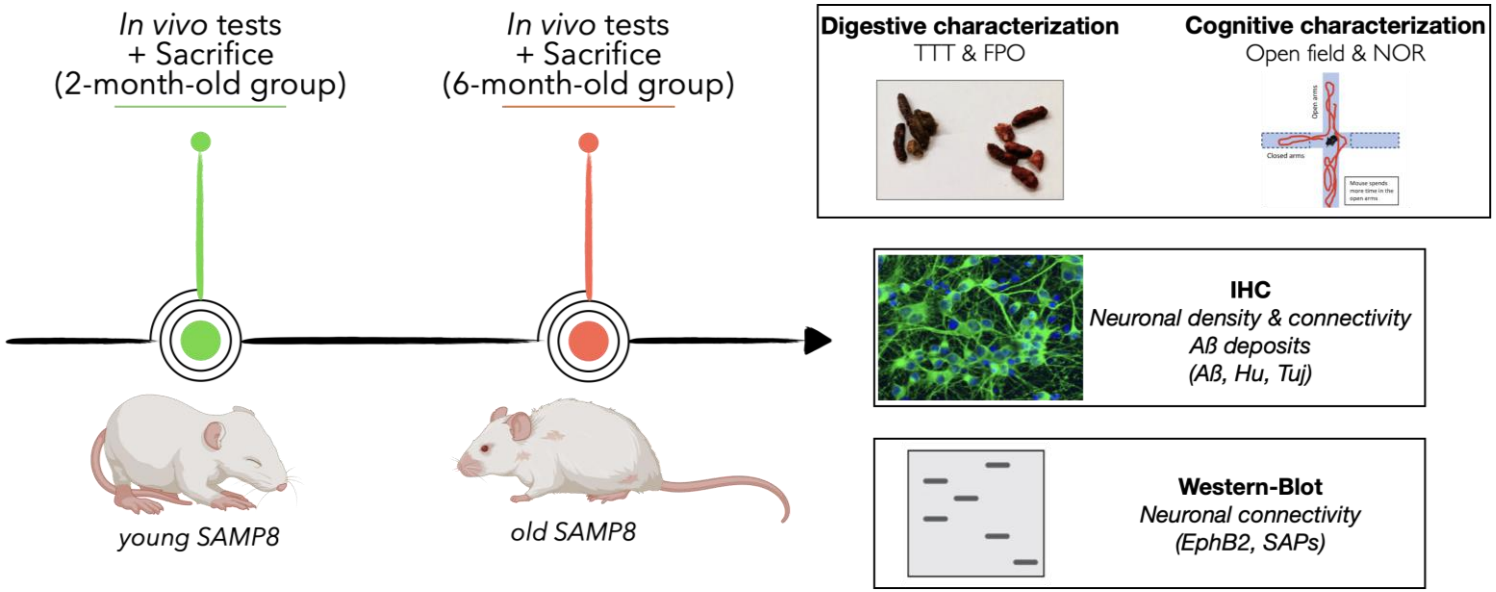

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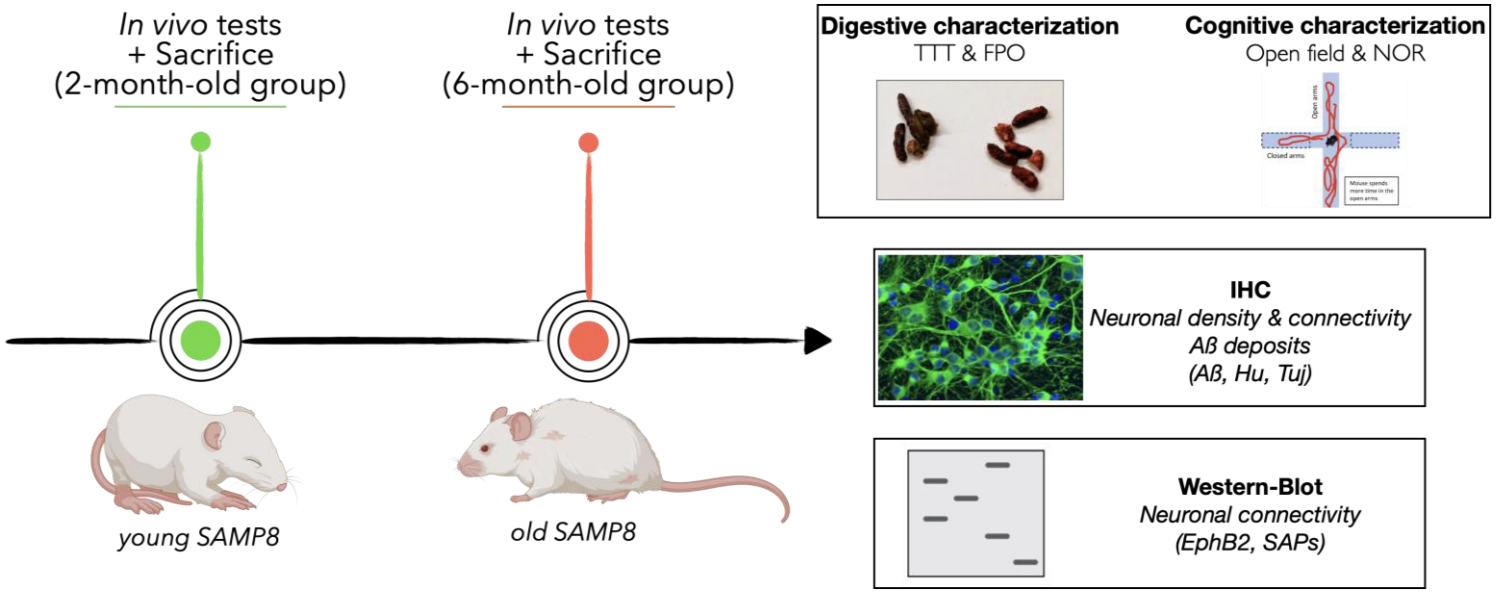

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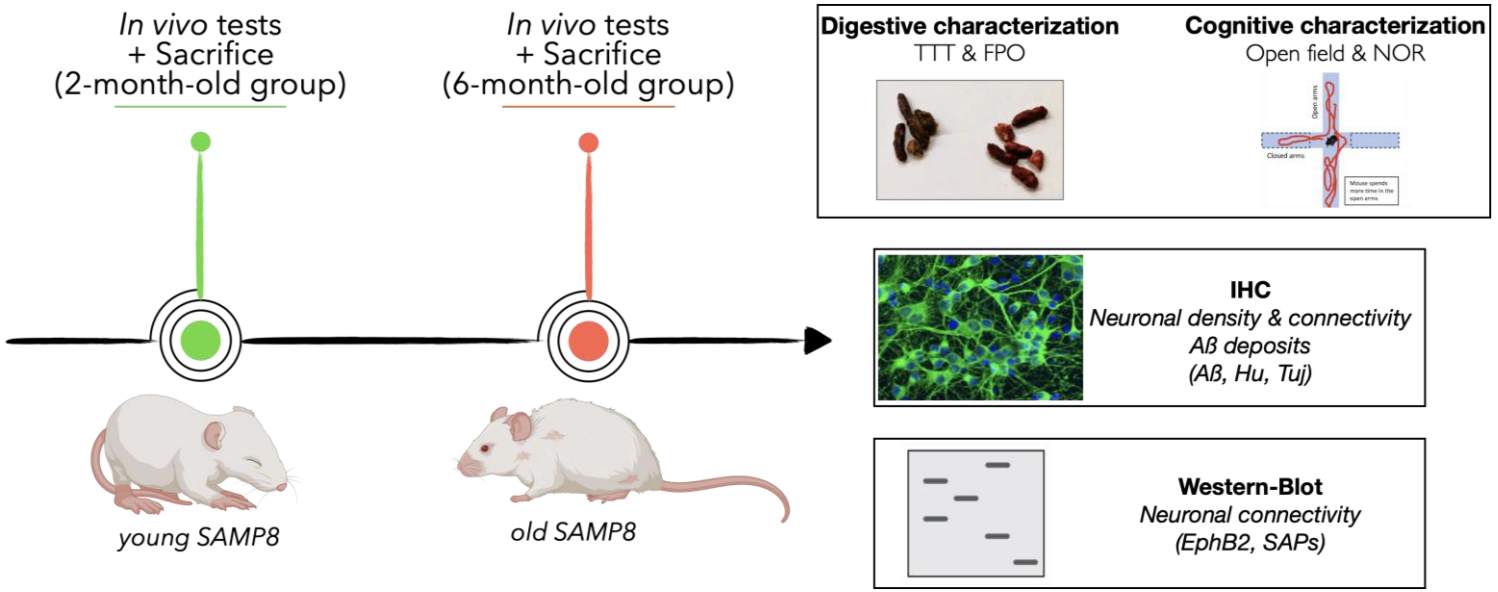

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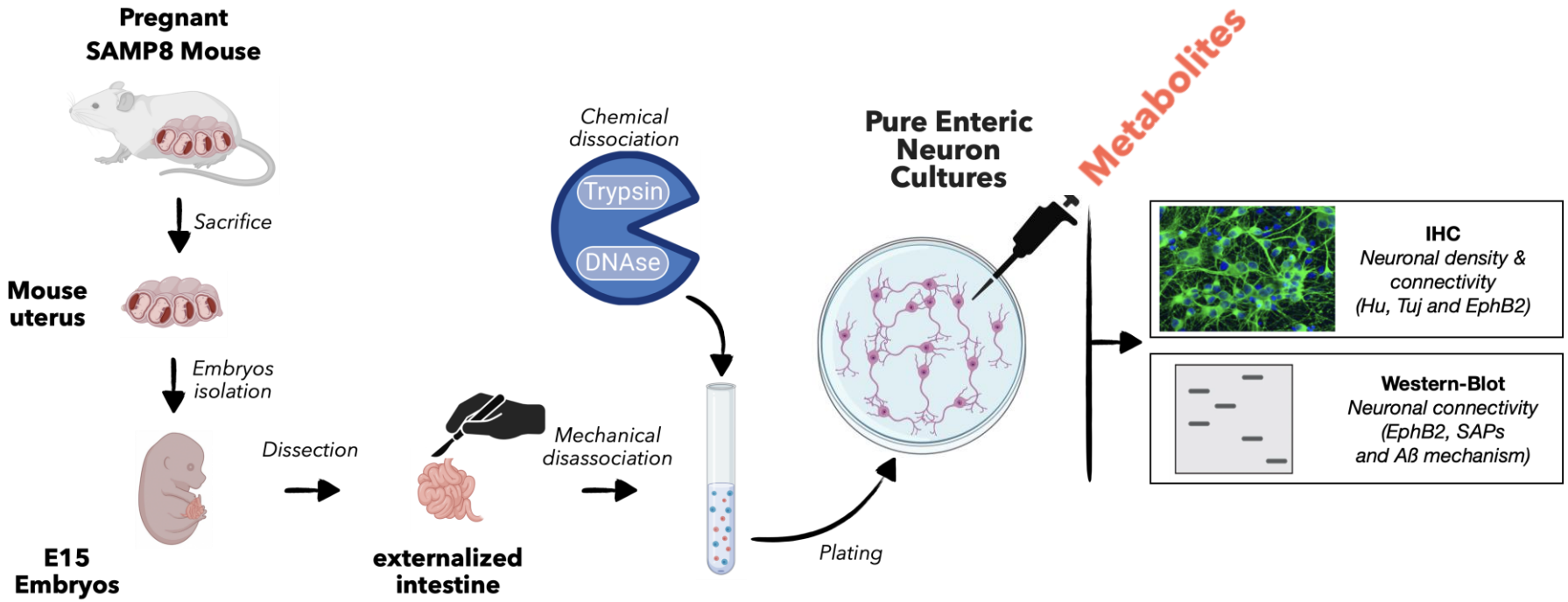

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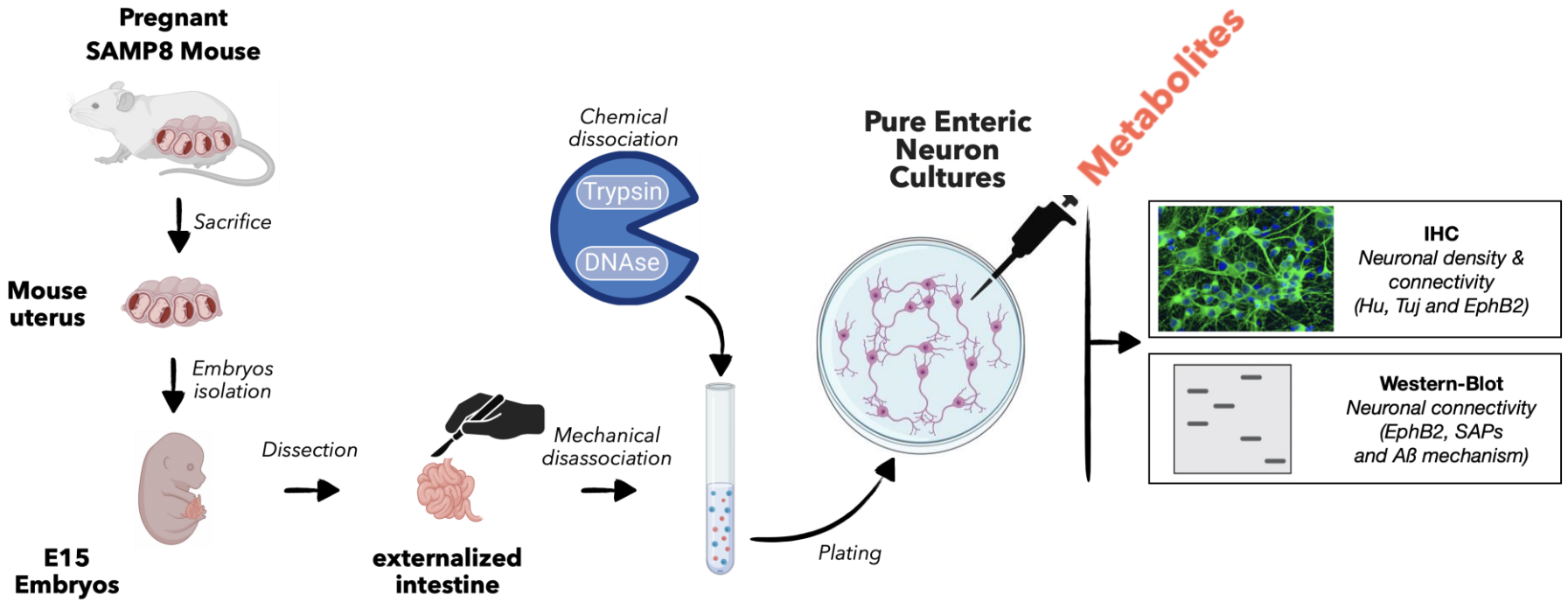

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Butyrate treatment improves the enteric neuronal connectivity *in vitro*

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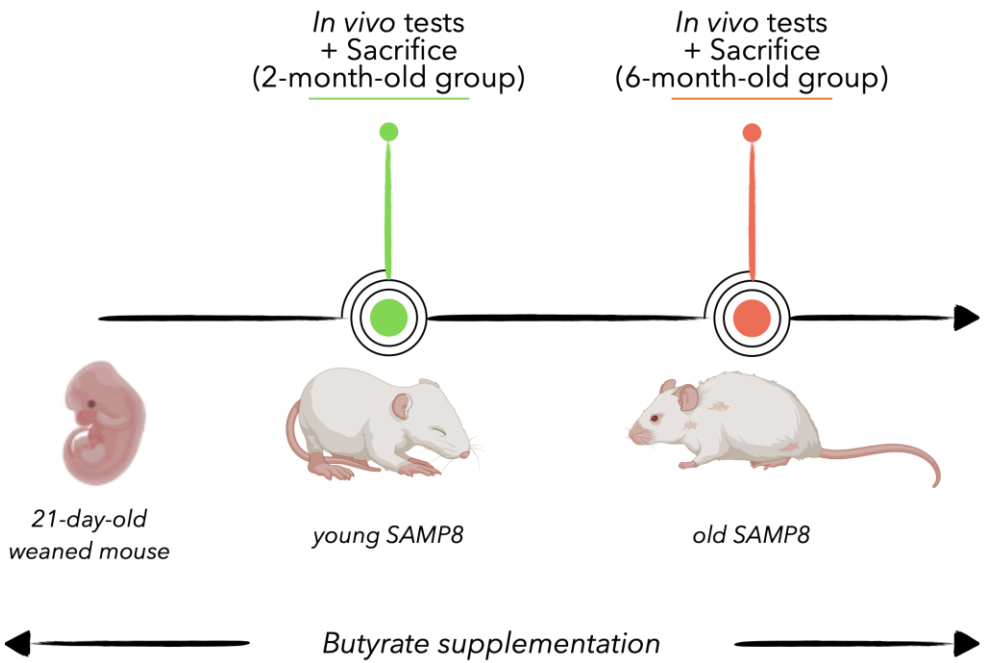

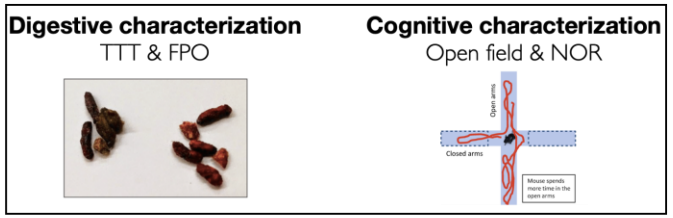

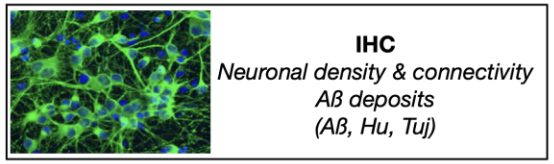

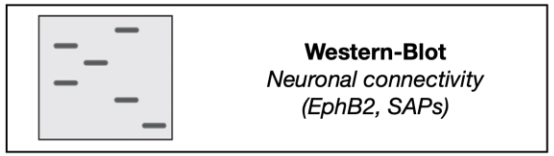

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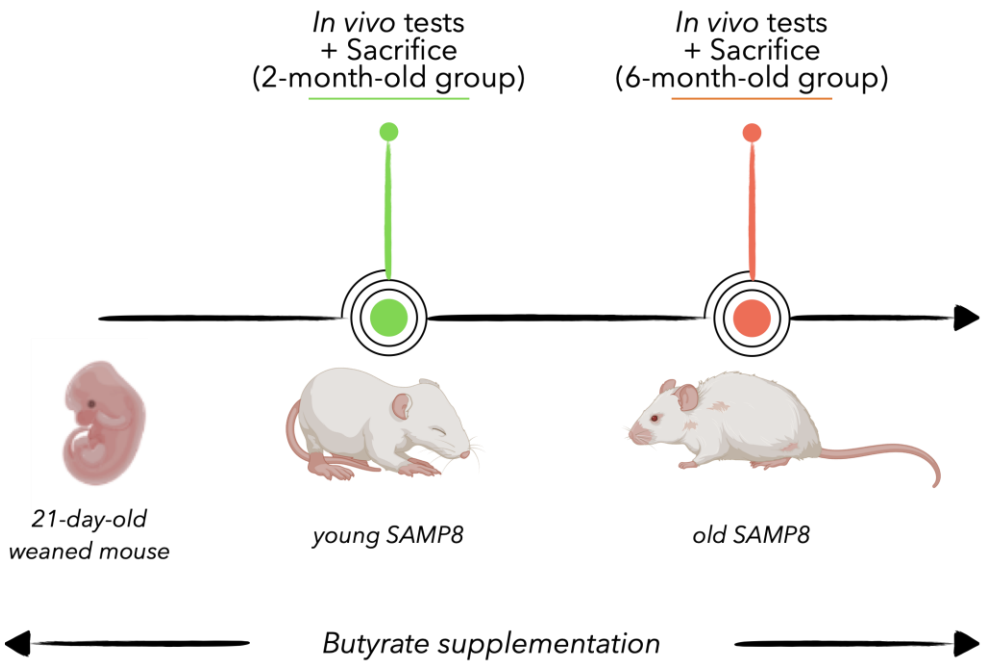

Digestive characterization
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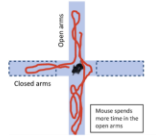

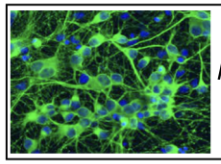

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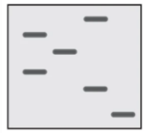
Neuronal density & connectivity
Aß deposits
(Aß, Hu, Tuj)



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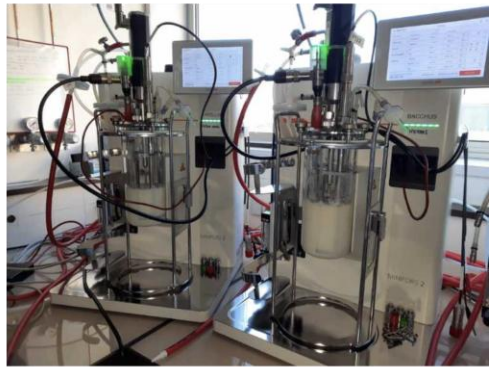

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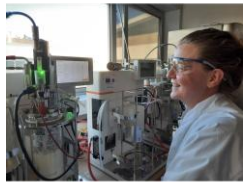

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Butyrate production through milk fermentation
using a bacterial consortium

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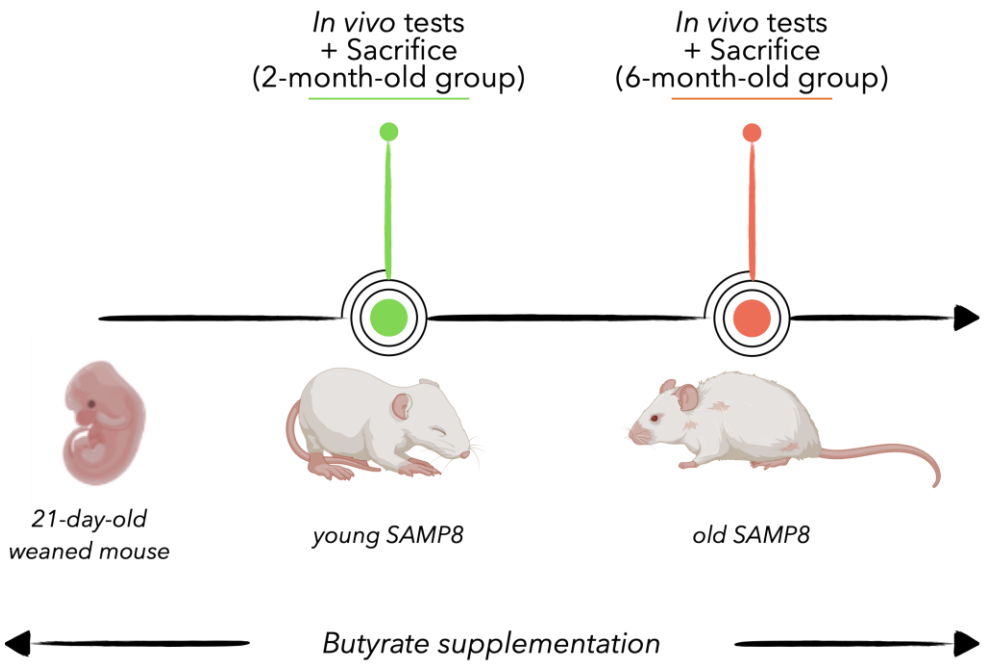

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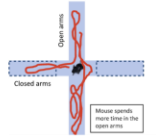

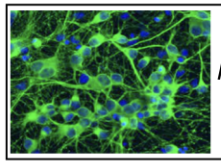

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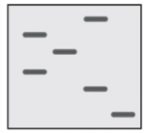

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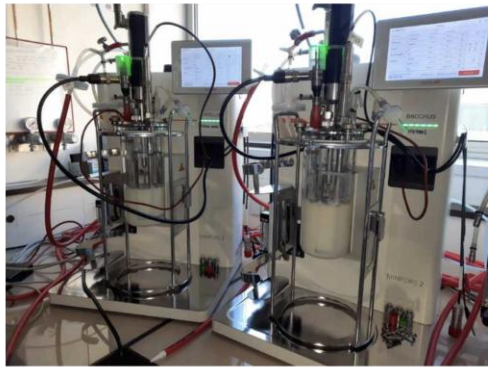

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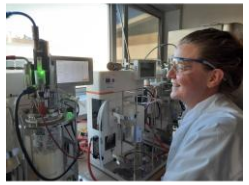

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Work in progress …

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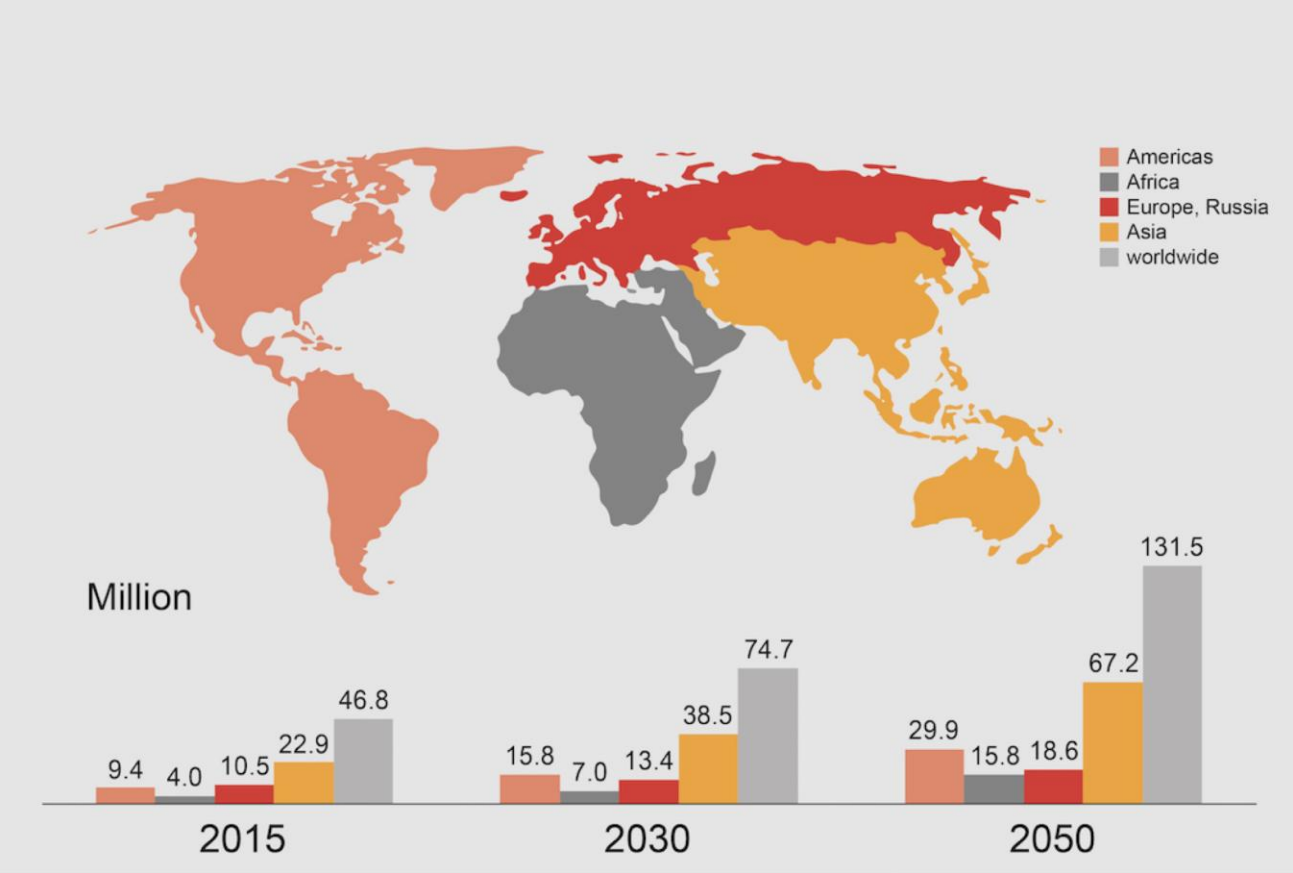
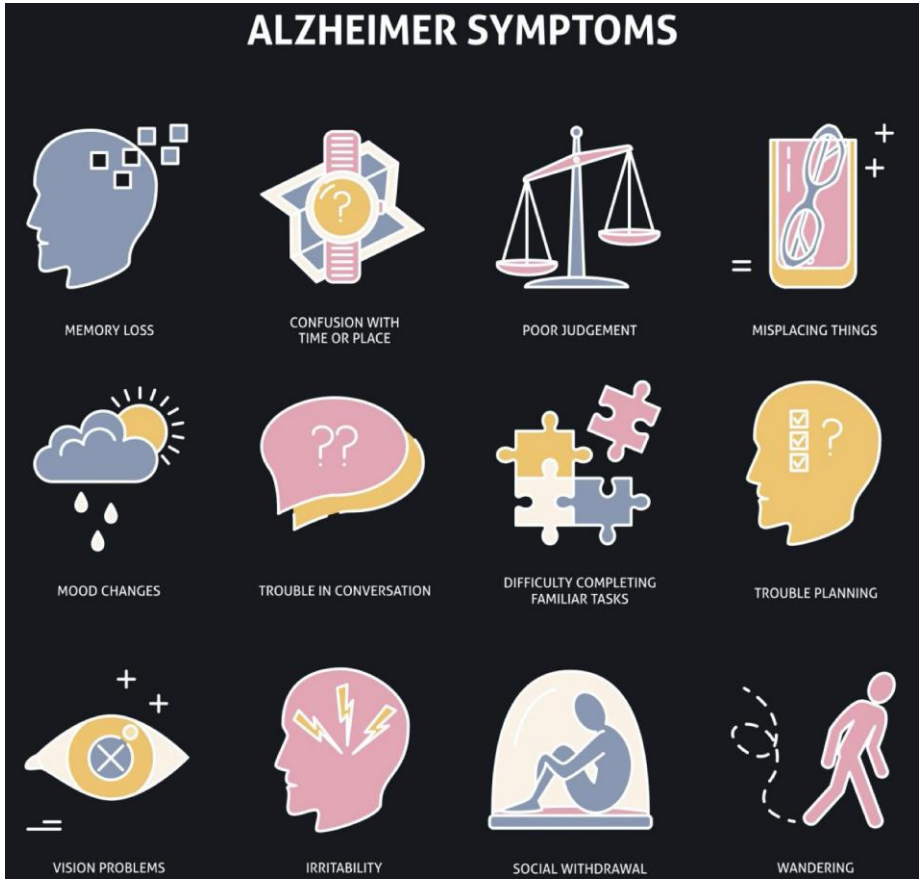
TENS & STLO laboratories

CRNH
CENTRE DE RECHERCHE EN NUTRITION HUMAINE OUEST

OUEST VALORISATION
Ressources d'innovation

Alzheimer’s disease : epidemiology and symptoms





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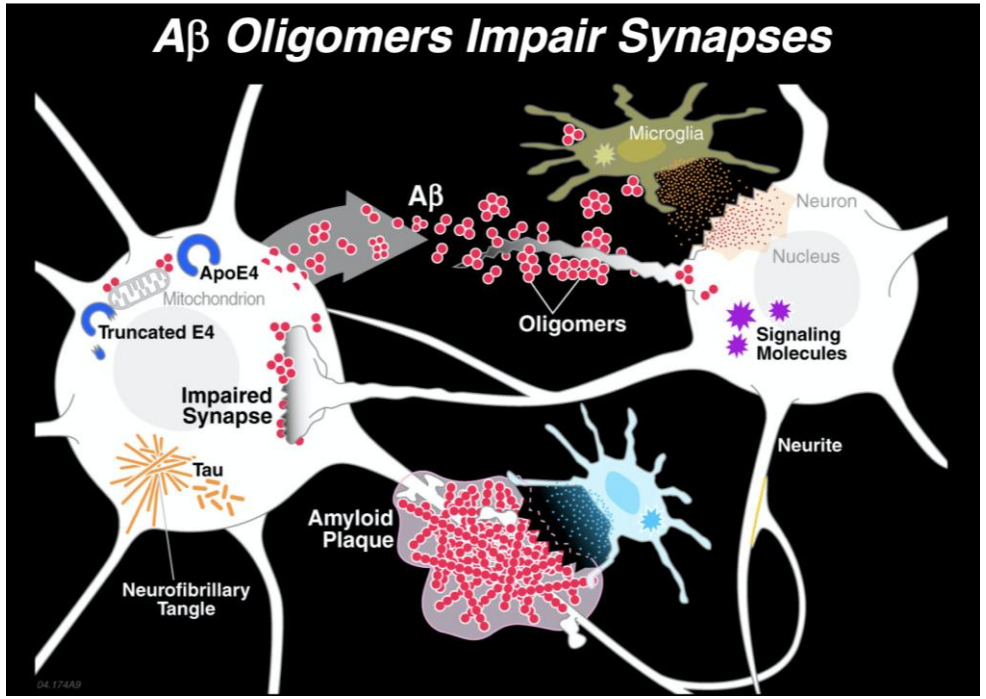
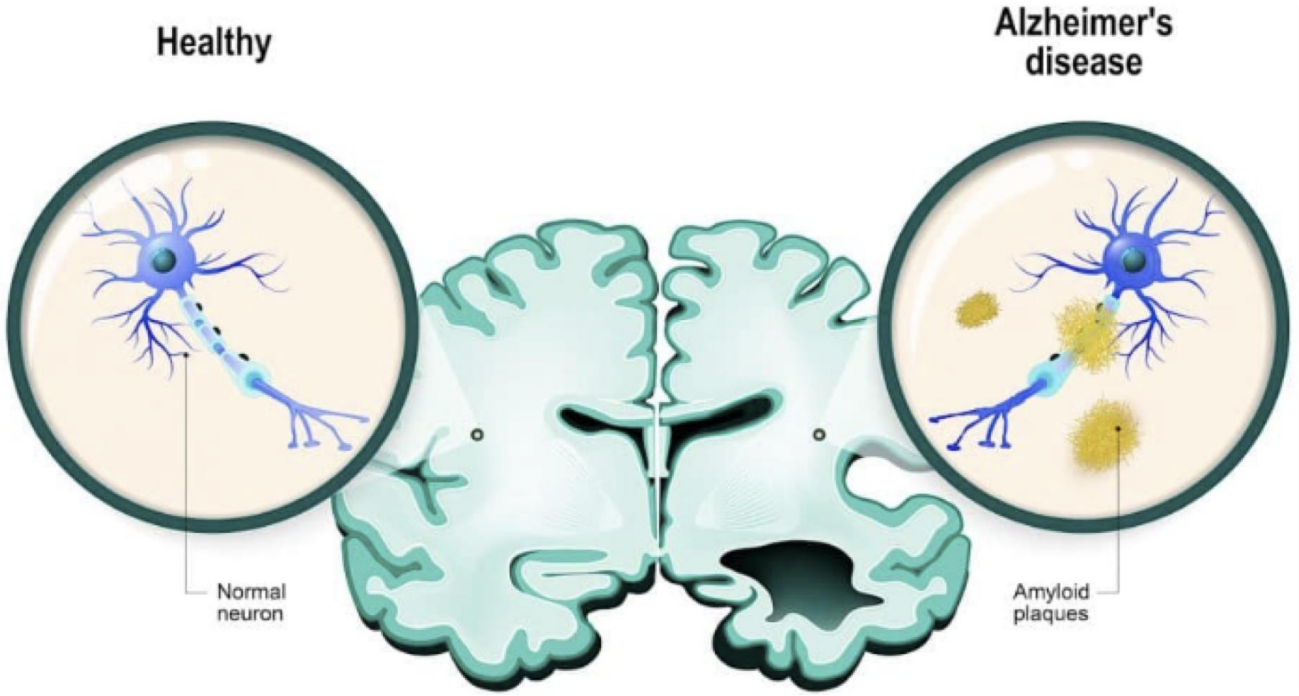
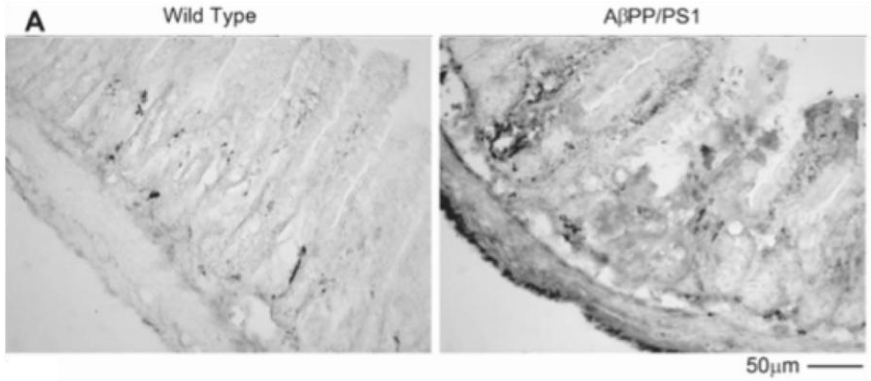


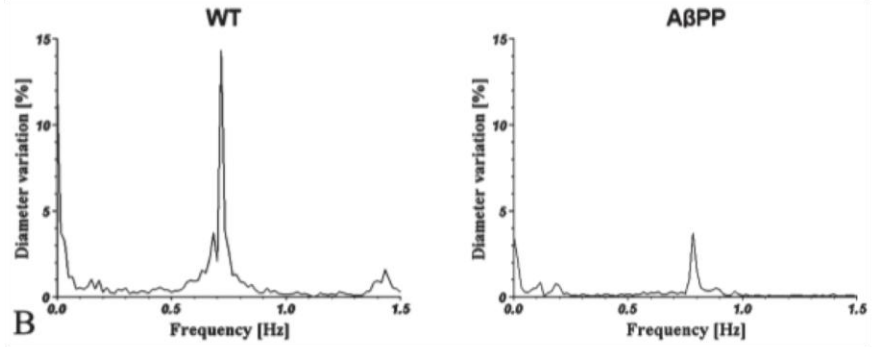


Alzheimer's disease : A gut-brain disease ?

Animal models

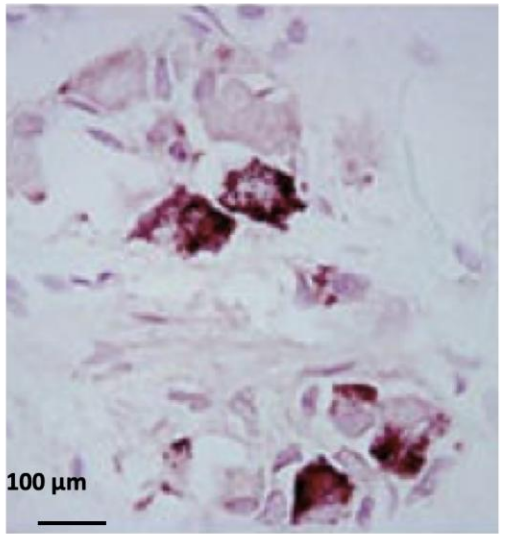


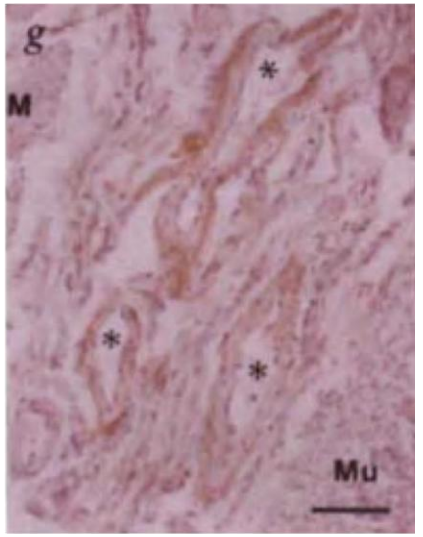
- Intestinal amyloïd aggregates
(Puig et al., 2015)



- Impairments of digestive motricity
(Semar et al., 2013)

Humans





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	Diagnosis within 0–2 years				Diagnosis within 2–10 years			
	UK		France		UK		France	
	OR (corrected 95% CI)	Corrected p value	OR (corrected 95% CI)	Corrected p value	OR (corrected 95% CI)	Corrected p value	OR (corrected 95% CI)	Corrected p value
Major depressive disorder, F32	2·14 (1·77–2·59)	<0·0001	3·41 (3·04–3·84)	<0·0001	1·34 (1·15–1·56)	<0·0001	1·73 (1·45–2·07)	<0·0001
Anxiety, F41	2·02 (1·69–2·41)	<0·0001	1·93 (1·71–2·17)	<0·0001	1·36 (1·18–1·56)	<0·0001	1·5 (1·26–1·78)	<0·0001
Reaction to severe stress and adjustment disorders, F43	2·34 (1·76–3·11)	<0·0001	2·1 (1·61–2·75)	<0·0001	1·4 (1·12–1·77)	<0·0001	1·83 (1·36–2·46)	<0·0001
Hearing loss, H91	1·28 (1·07–1·52)	0·0001	1·95 (1·35–2·82)	<0·0001	1·19 (1·04–1·36)	0·0006	1·51 (1·01–2·26)	0·033
Constipation, K59	1·41 (1·23–1·63)	<0·0001	1·66 (1·47–1·87)	<0·0001	1·31 (1·16–1·49)	<0·0001	1·59 (1·33–1·89)	<0·0001
Spondylosis, M47	1·25 (0·93–1·69)	0·933	1·45 (1·22–1·72)	<0·0001	1·26 (1·05–1·5)	0·0005	1·62 (1·32–1·98)	<0·0001
Memory loss symptom, R41	31·5 (24·18–41·05)	<0·0001	16·5 (10·39–26·19)	<0·0001	7·63 (5·95–9·79)	<0·0001	4·41 (2·3–8·48)	<0·0001
Malaise and fatigue, R53	1·36 (1·17–1·58)	<0·0001	1·78 (1·59–2·0)	<0·0001	1·23 (1·08–1·39)	<0·0001	1·59 (1·36–1·86)	<0·0001
Syncope and collapse, R55	1·95 (1·53–2·48)	<0·0001	2·49 (1·68–3·69)	<0·0001	1·23 (1·01–1·5)	0·034	1·57 (1·06–2·34)	0·007
Abnormal weight loss, R63	2·1 (1·68–2·62)	<0·0001	3·12 (2·41–4·02)	<0·0001	1·47 (1·22–1·77)	<0·0001	1·88 (1·35–2·62)	<0·0001

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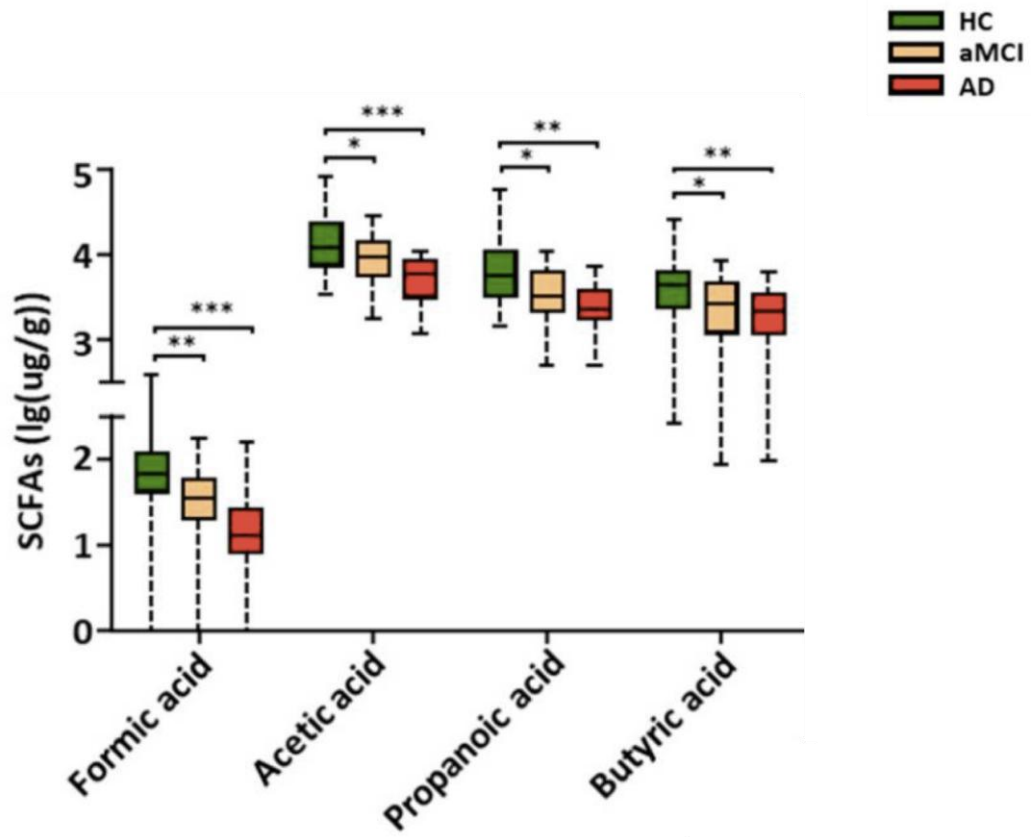

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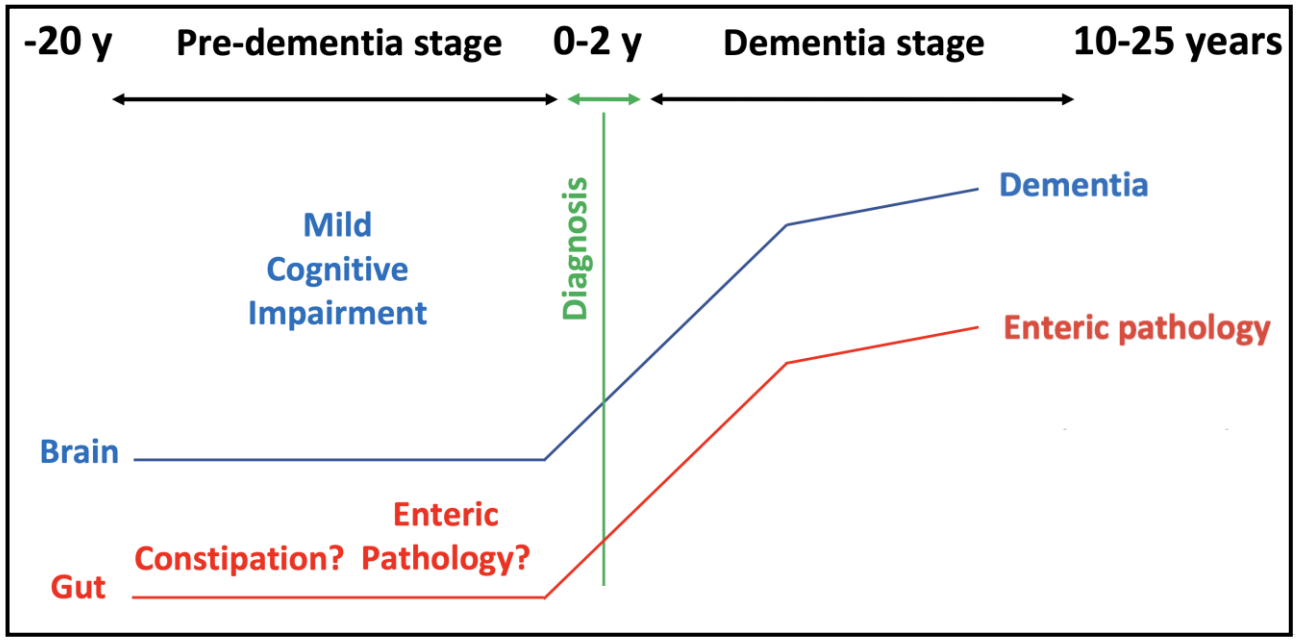

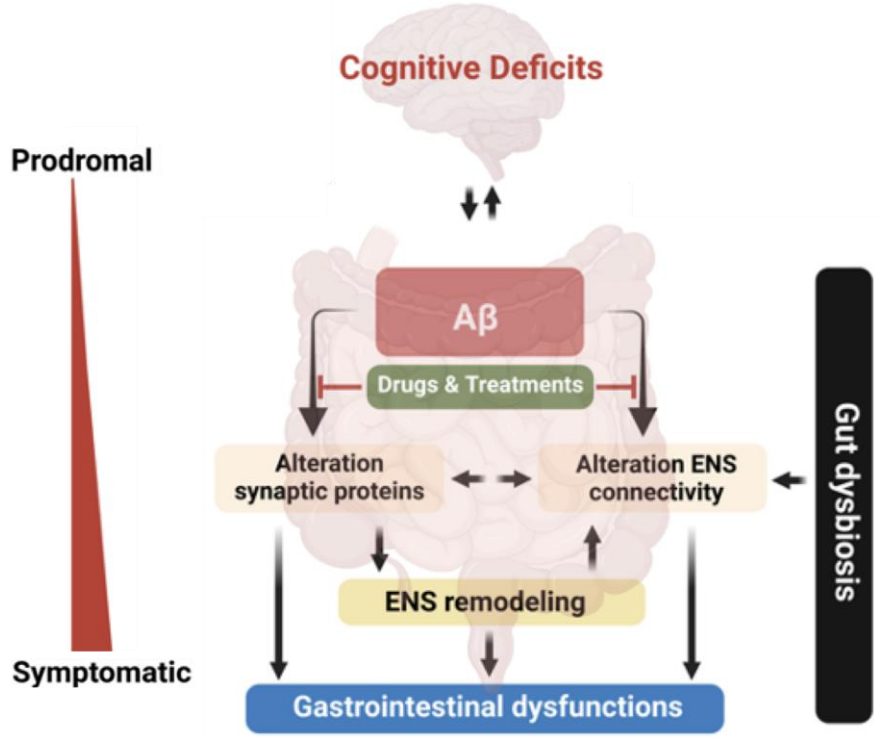

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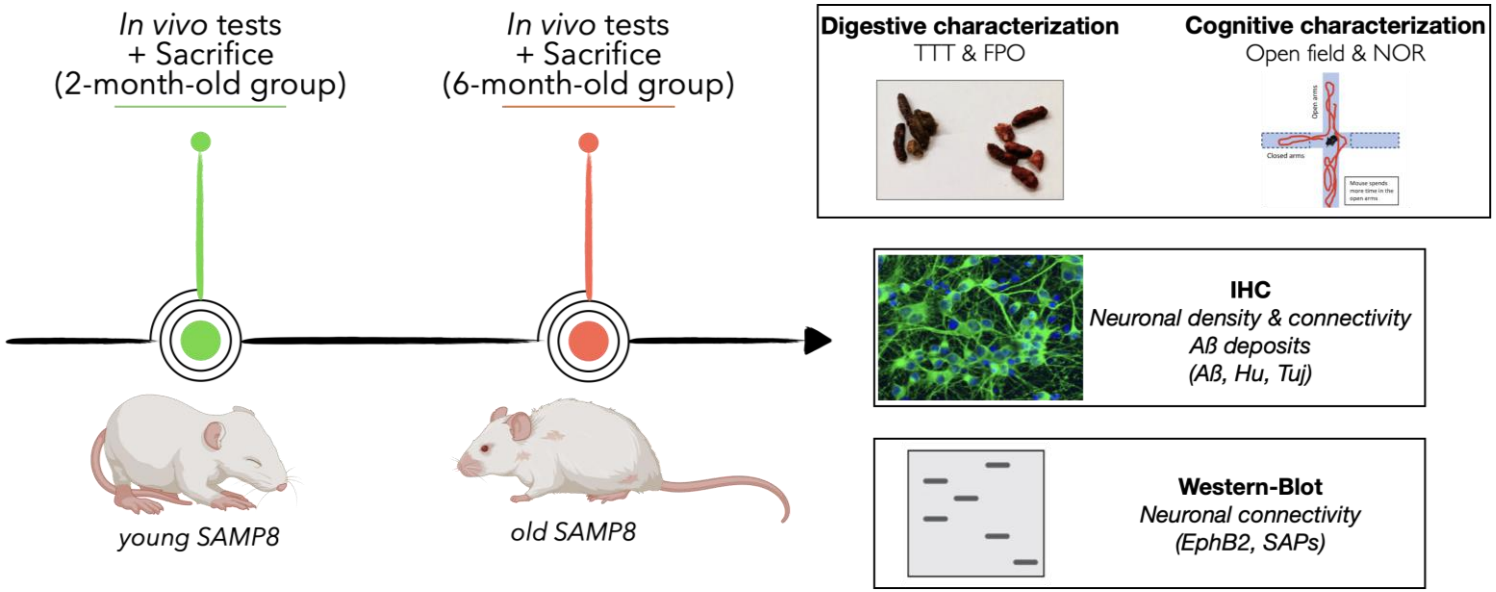

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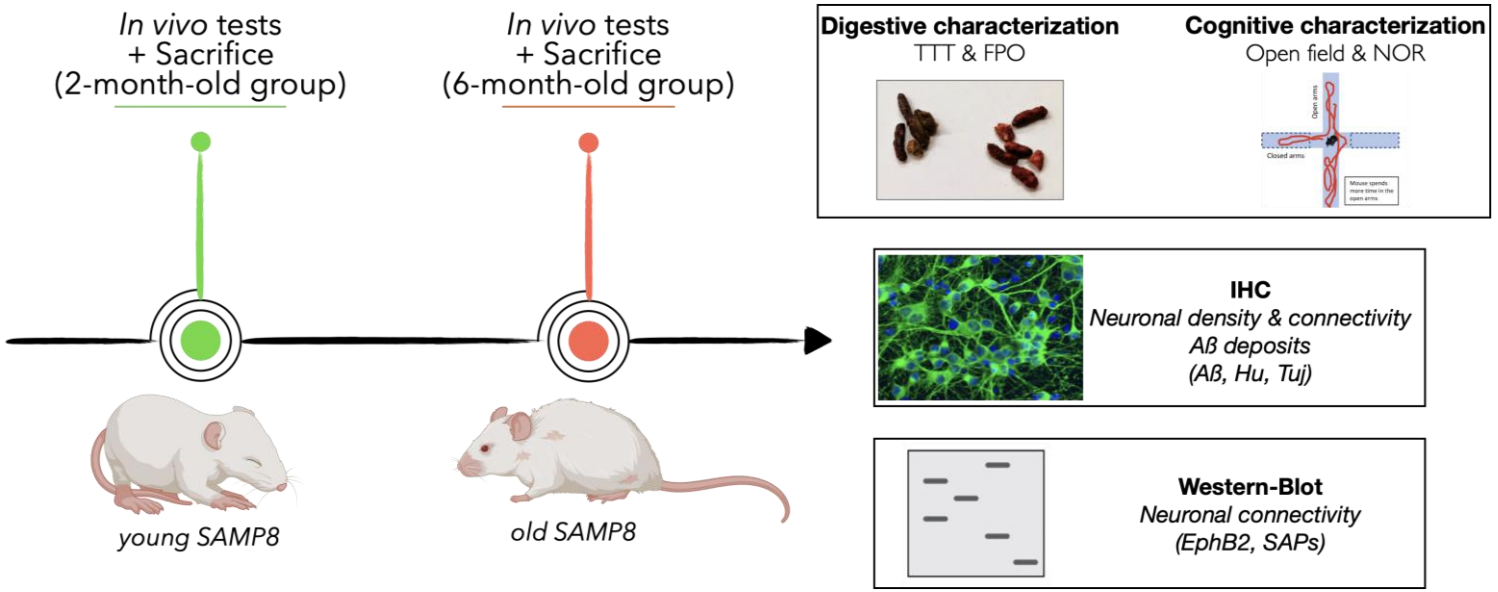

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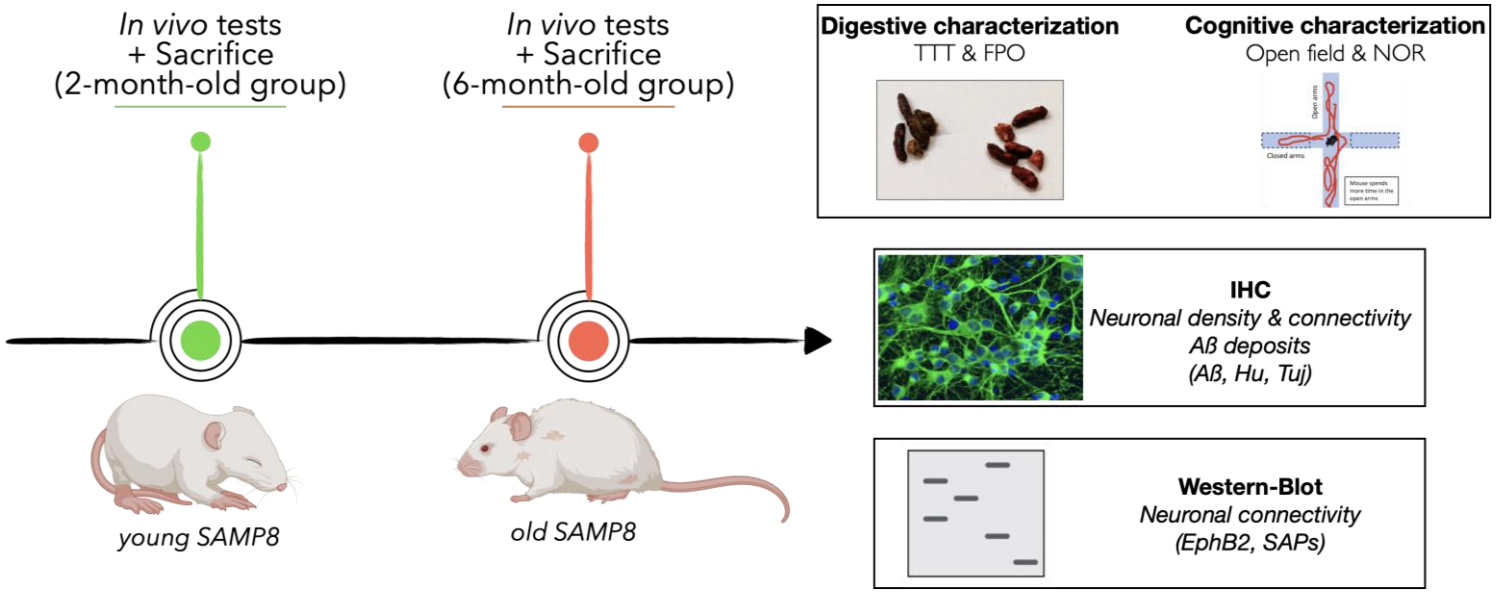

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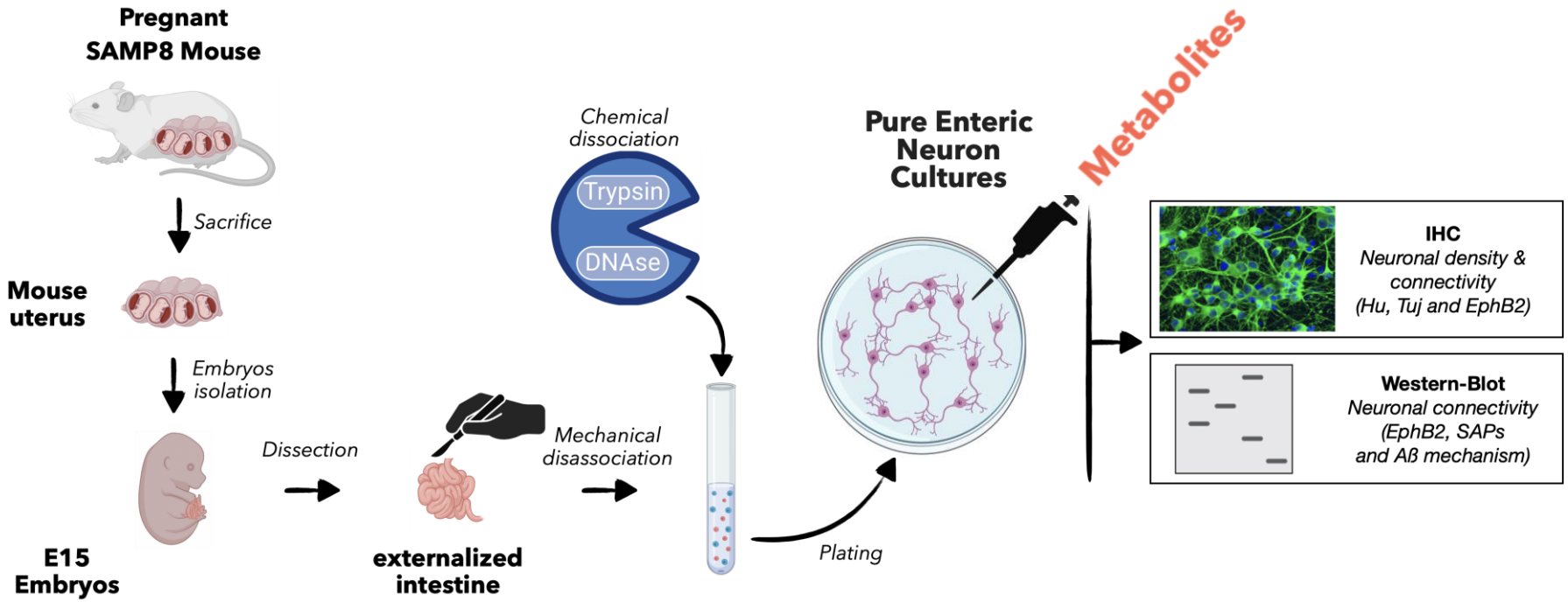

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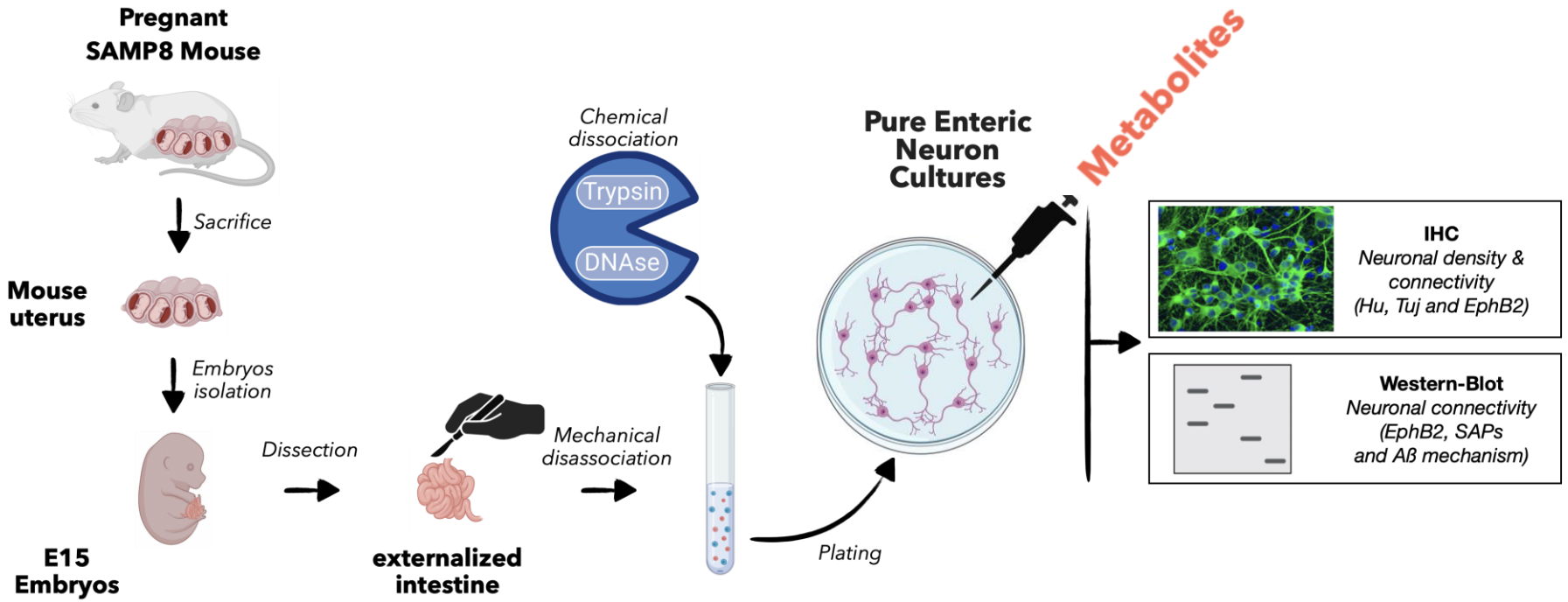

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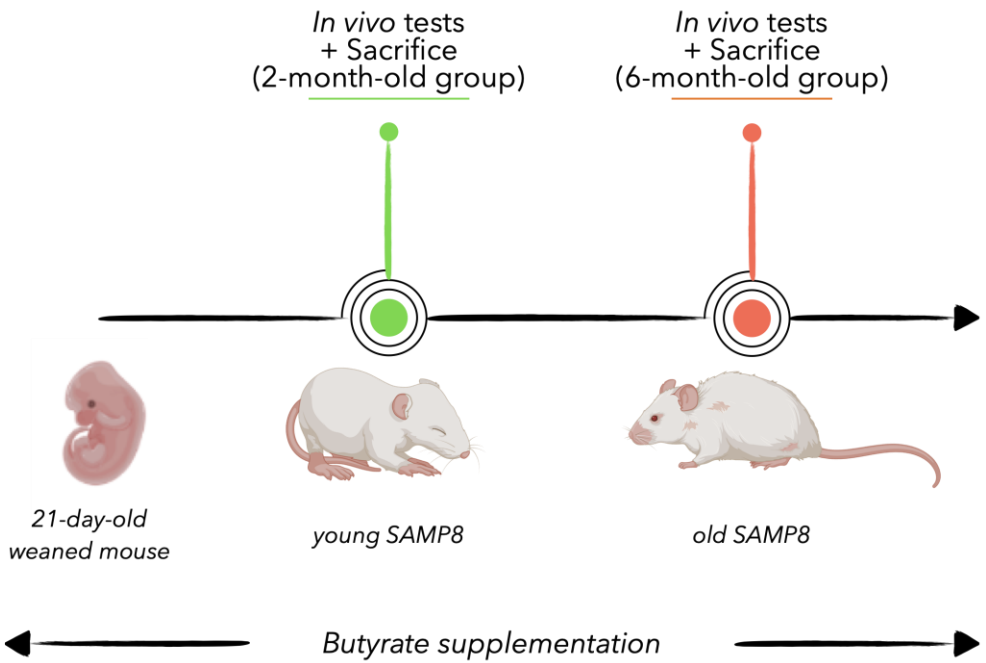
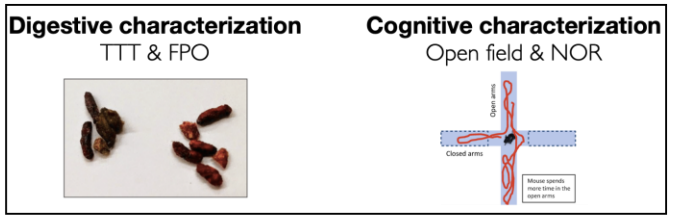
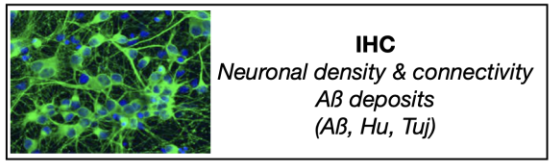
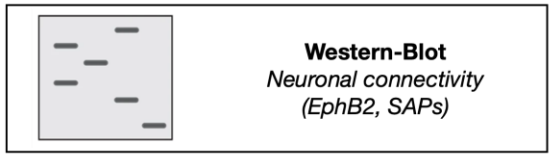
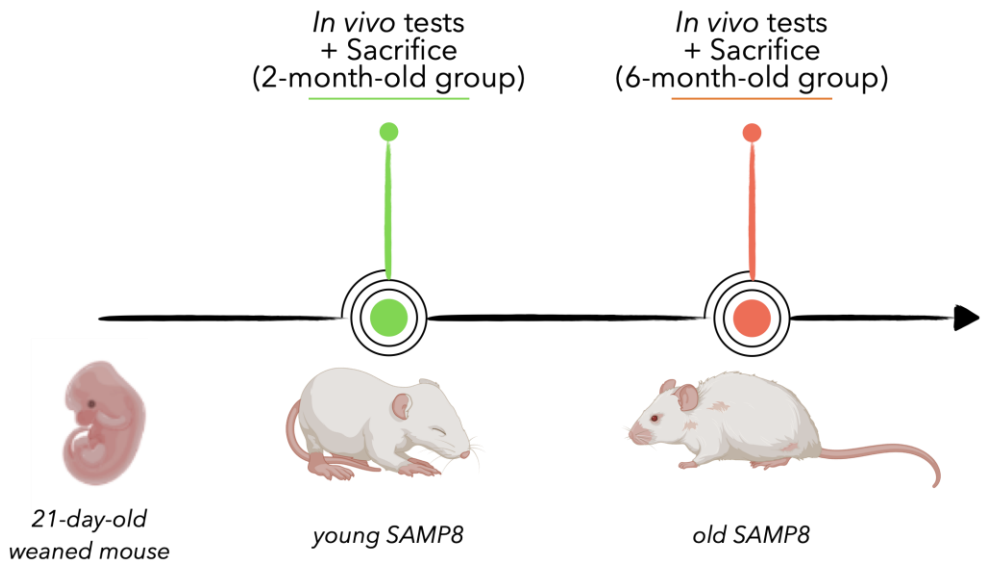






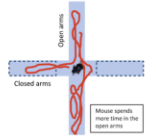


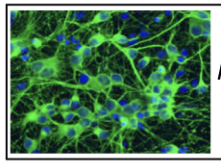
Could butyrate enhance cognitive and digestive functions in Alzheimer's mouse model ?



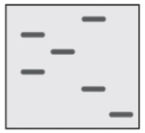
Digestive characterization
TTT & FPO

Cognitive characterization
Open field & NOR

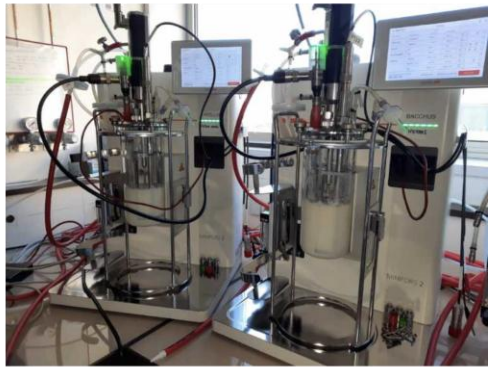




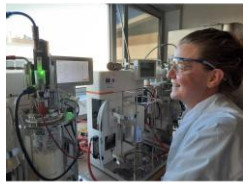
IHC
Neuronal density & connectivity
Aß deposits
(Aß, Hu, Tuj)



Western-Blot
Neuronal connectivity
(EphB2, SAPs)



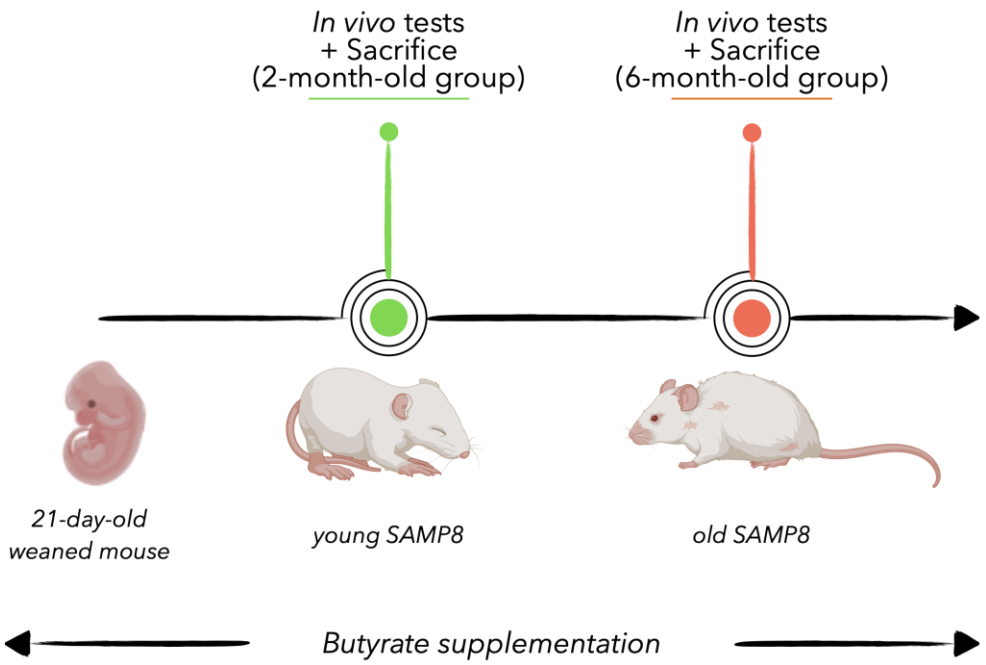
Dr. Hélène Falentin



Marina Giblaine

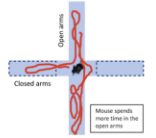
Dairy product enriched with butyrate

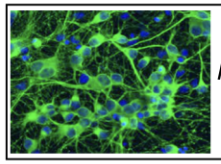
Could butyrate enhance cognitive and digestive functions in Alzheimer's mouse model ?



Digestive characterization
TTT & FPO

Cognitive characterization
Open field & NOR





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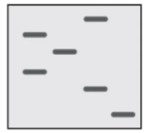

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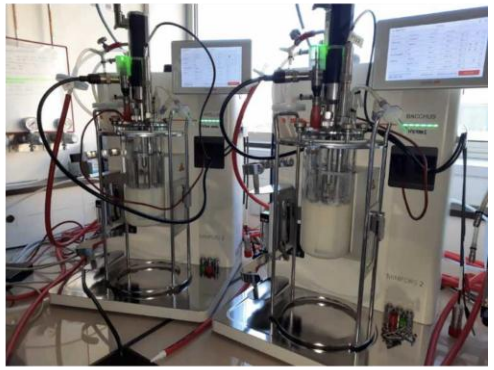
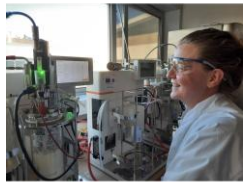


Dr. Hélène Falentin



Marina Giblaine

Butyrate production through milk fermentation
using a bacterial consortium

Work in progress …

Questions
& Answers

Fermentation as a lever for improving Infant Formulas : Design of a fermented formula improving brain development in newborns

Sarah BLANCHET
Directed by Sophie BLAT and by Sergine EVEN

Context

Human milk

→ Optimal post-natal nutrition for the newborn.

Context

Human milk

Infant Formula

Context

Human milk

Infant Formula

Meet the nutritional needs of the newborn by:

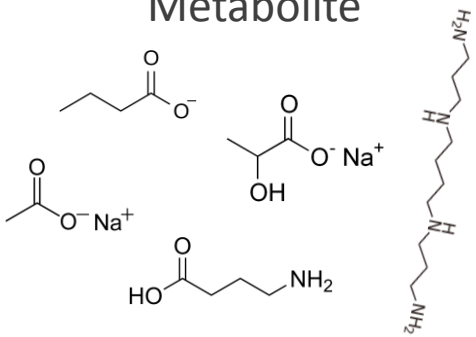
Macronutrients : Proteins, Carbohydrates, Lipids

Micronutrients : Minerals, vitamins

Context

Human milk

Human Milk Metabolite



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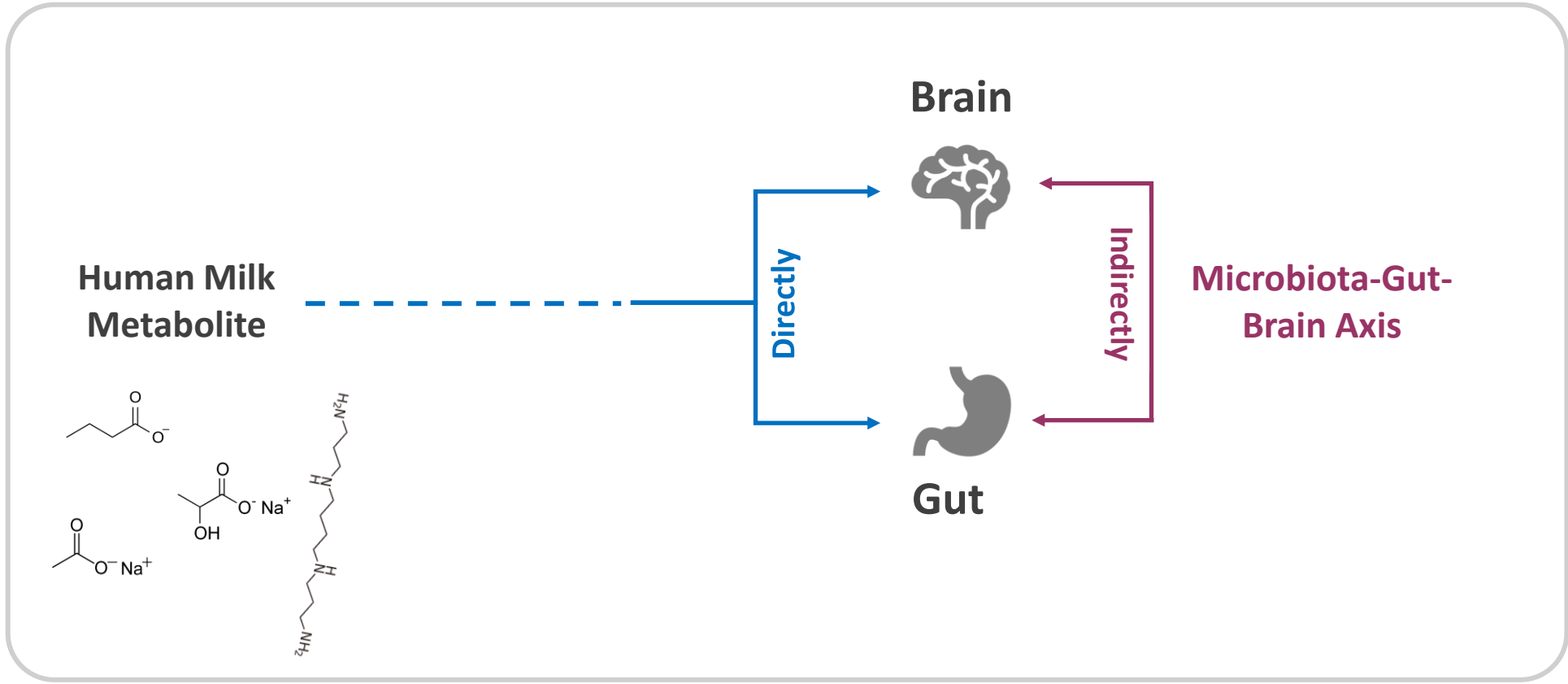
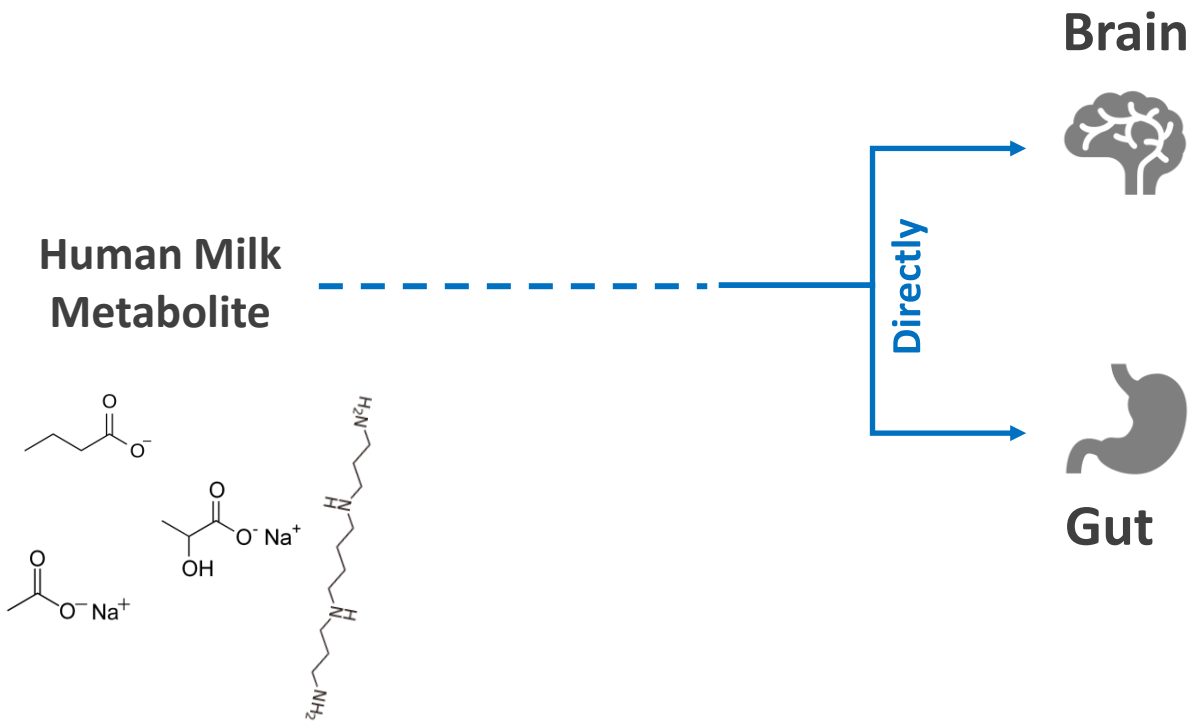
Infant Formula

Research questions

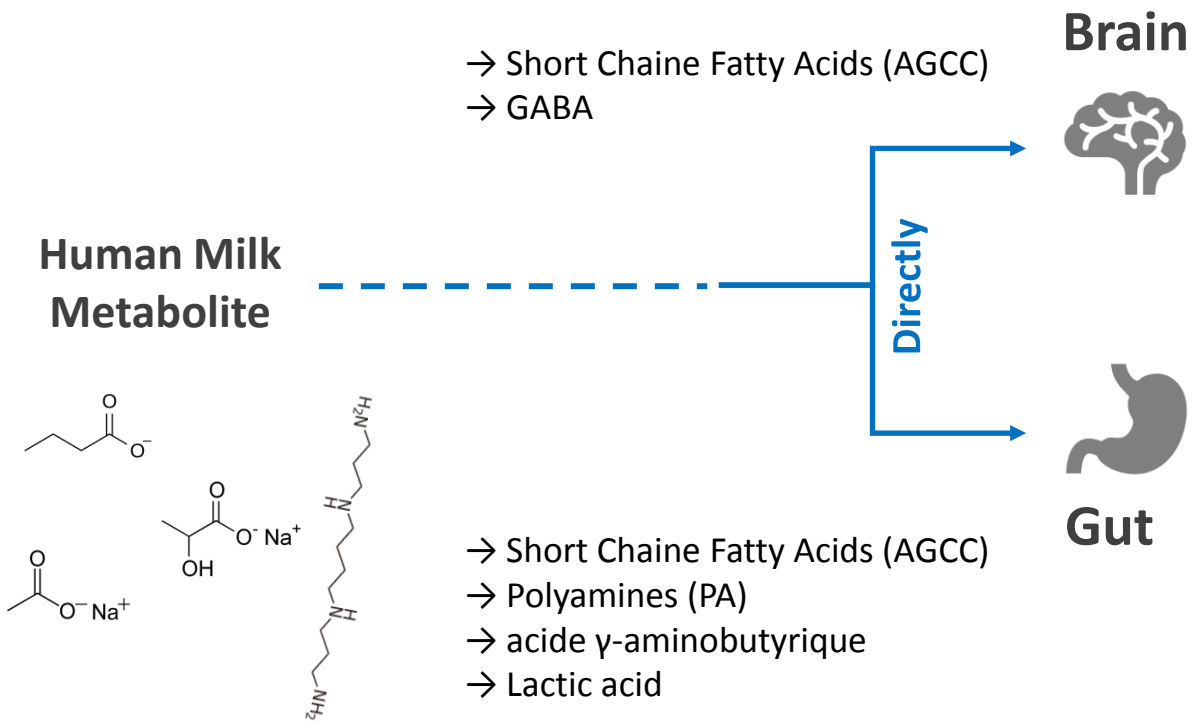


In vitro study of the metabolites



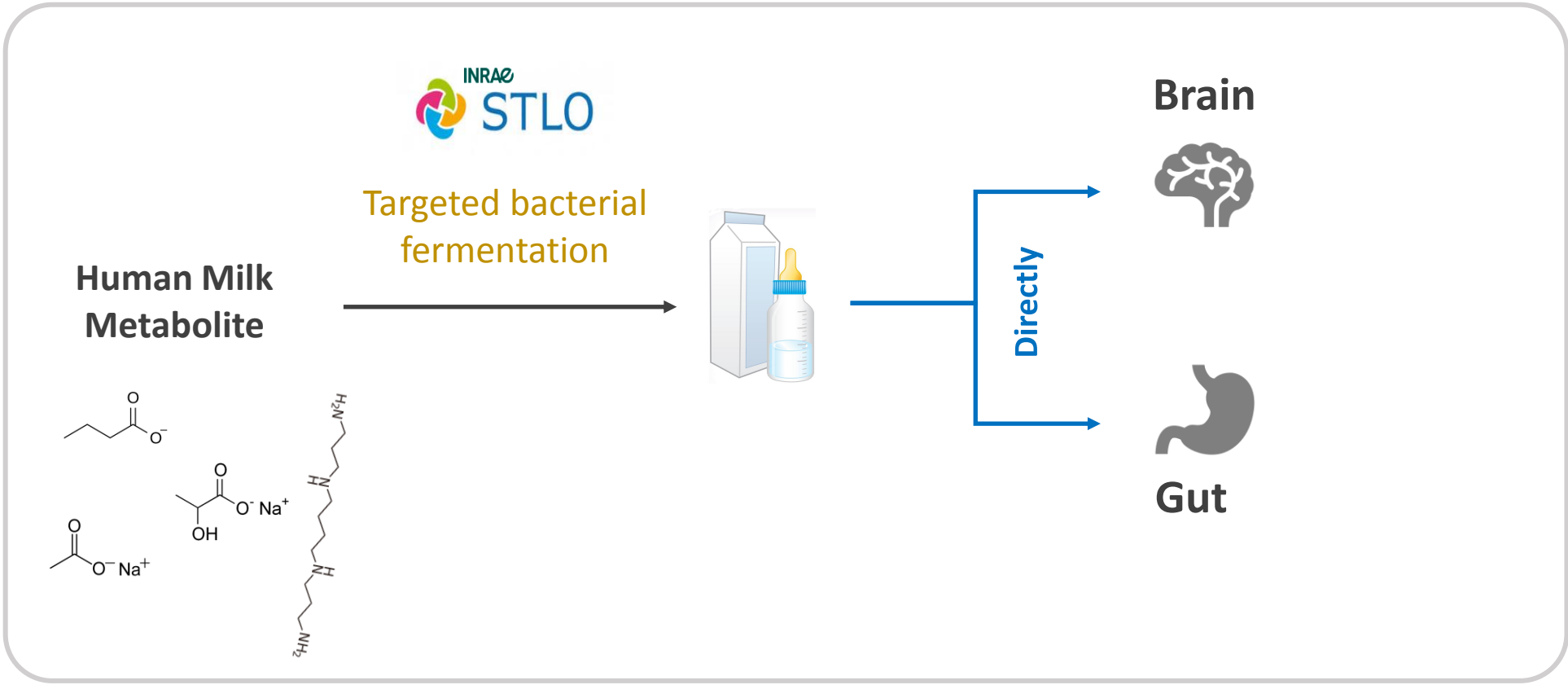
in vitro : **cellular models of intestinal epithelium** and **central neurons** (screening of relevant metabolites)

In vitro study of the metabolites

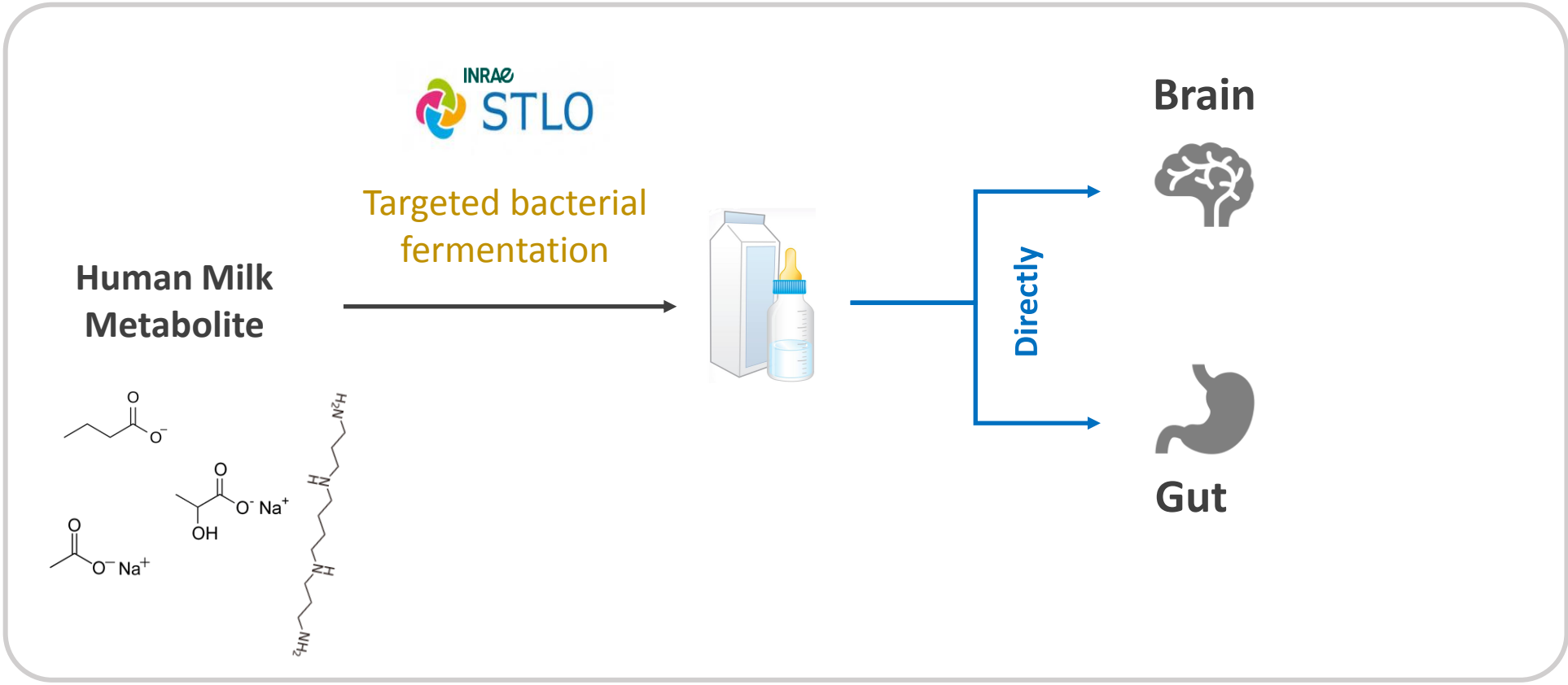


in vitro : **cellular models of intestinal epithelium** and **central neurons** (screening of relevant metabolites)

In vivo study of metabolites produced by targeted bacterial fermentation

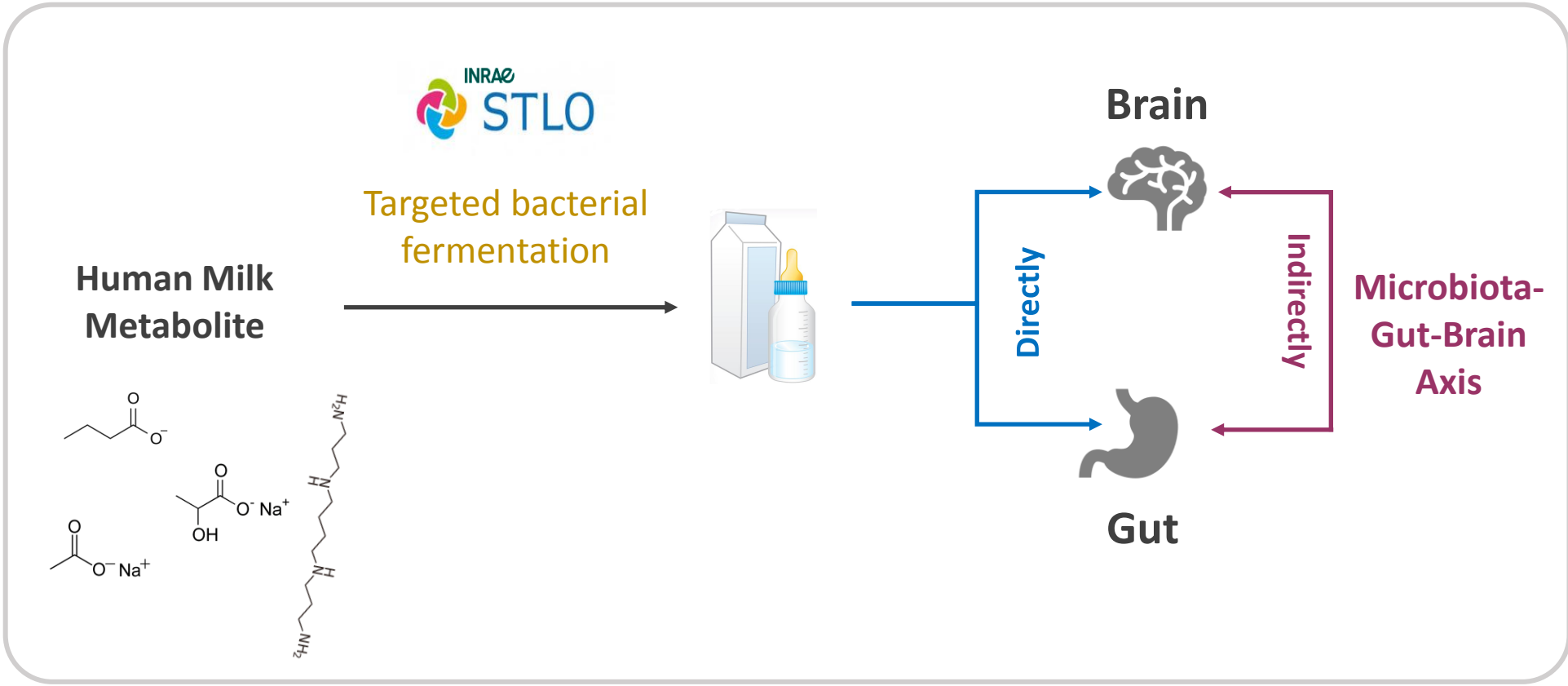


In vivo study of metabolites produced by targeted bacterial fermentation



in vivo : piglet ; **the Yucatan mini-pig** (preclinical model)

In vivo study of metabolites produced by targeted bacterial fermentation



in vivo : piglet ; **the Yucatan mini-pig** (preclinical model)

Thank you for your attention

Questions
& Answers

CRNH
CENTRE DE RECHERCHE EN NUTRITION HUMAINE OUEST

OUEST VALORISATION
Ressources d'innovation

Thank you for attending our session

Need more information? Contact:

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- Clair-Yves BOQUIEN : clair-yves.boquien@univ-nantes.fr
- Candice LAMOUREUX : candice.lamoureux@ouest-valorisation.fr
- Pierre-Etienne SADO : pierre-etienne.sado@univ-nantes.fr