Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease

R. Sampson et al. Cell 167, 1469–1480, December 1, 2016

GROUP E

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Neo Itilo, Mani

Introduction - PD Pathogenesis

- Neurodegenerative **Synucleinopathy** Disorder
- Hallmark Neuropathology : **aberrant aggregation** of specific neuronal protein (α-synuclein) that disrupts many cellular functions
- Symptoms : **motor deficits** including tremors, muscle rigidity, bradykinesia, and impaired gait.

PD in Numbers

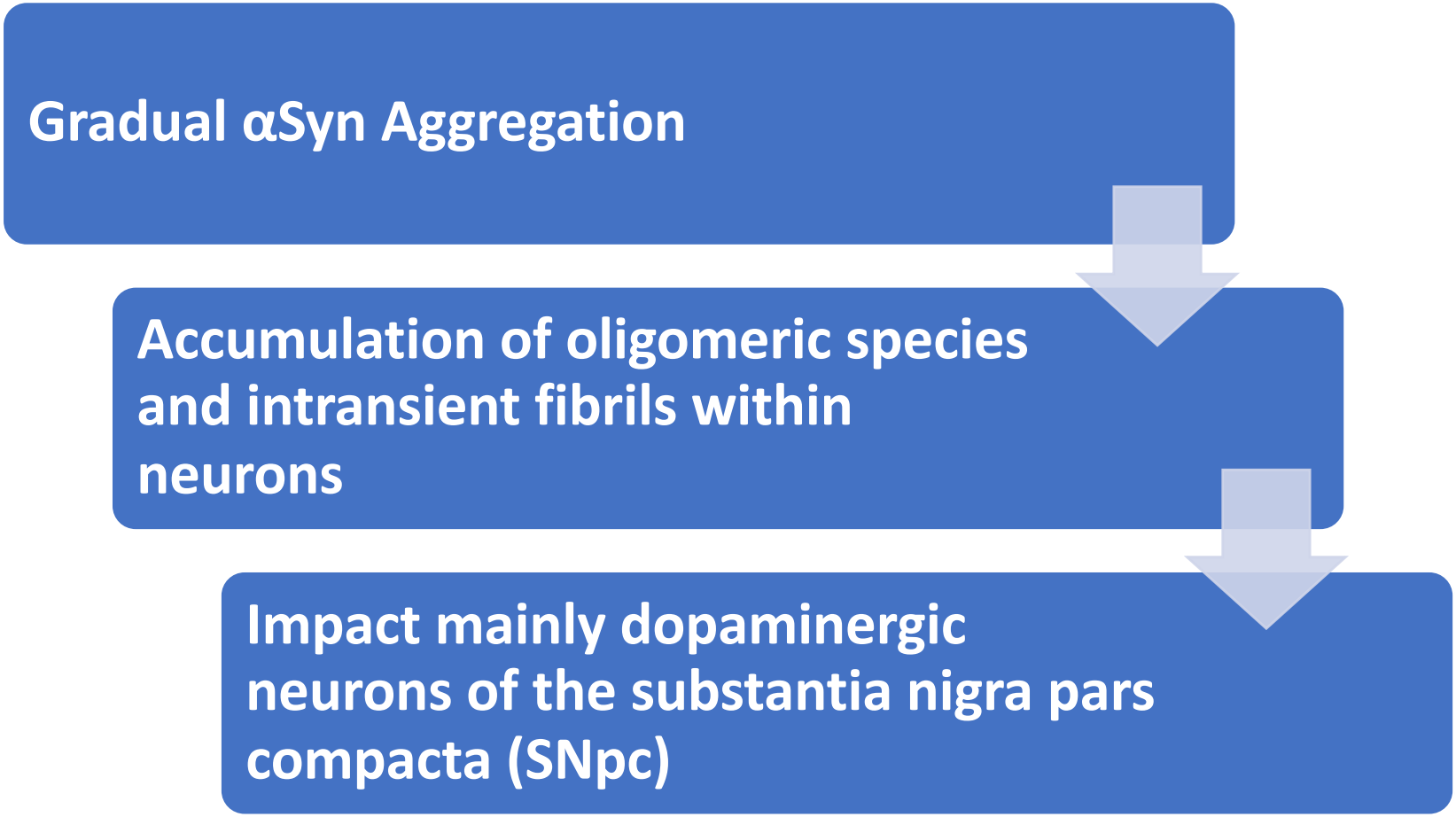
- **Second** most common neurodegenerative disease in the US
- **1% of the US population** over 60 years of age
- **3 million** patients and caregivers suffer from the symptoms worldwide

(Nalls et al., 2014)

Genetic Background in PD

- **Multifactorial** Disorder
- Strong **environmental** component
- **Less than 10%** of cases are hereditary

Steps in Pathogenetic Mechanism



Current Therapeutic Approach

- **Dopamine modulators** are a first-line therapeutic in PD
- Serious **Side effects**
- Can **lose effectiveness**
- NEED FOR **SAFE** AND **EFFECTIVE** DRUGS

Peripheral Influences in Disease

- Data suggests bidirectional communication between the **gut and the brain** in :
  - ✓Anxiety
  - ✓Depression
  - ✓Nociception
  - ✓Autism Spectrum Disorder

(Mayer et al., 2014)

- CNS signals impact gastrointestinal (GI) physiology. Gut signals may in turn affect brain activity

(Selkrig et al., 2014; Wall et al., 2014)

The Role of Microbiota in Models

Research in mouse models reveals the **effect of microbiota** on neurodevelopment and the CNS:

- Germ-free (GF) mice and antibiotic-treated specific-pathogen free (SPF) mice are **altered in hippocampal neurogenesis** (impaired spatial and object recognition)
- GF mice have **altered cortical myelination** and impaired blood-brain barrier function
- Microbiota promotes **enteric and circulating serotonin** and affects anxiety, hyperactivity, and cognition

(Moehle et al., 2016; Braniste et al., 2014; Hoban et al., 2016)

The Role of Microbiota in Humans

- Dysbiosis of the human microbiome reported in subjects diagnosed with neurological diseases

- Fecal and mucosa-associated gut microbes were reported to be different between individuals with PD and healthy controls

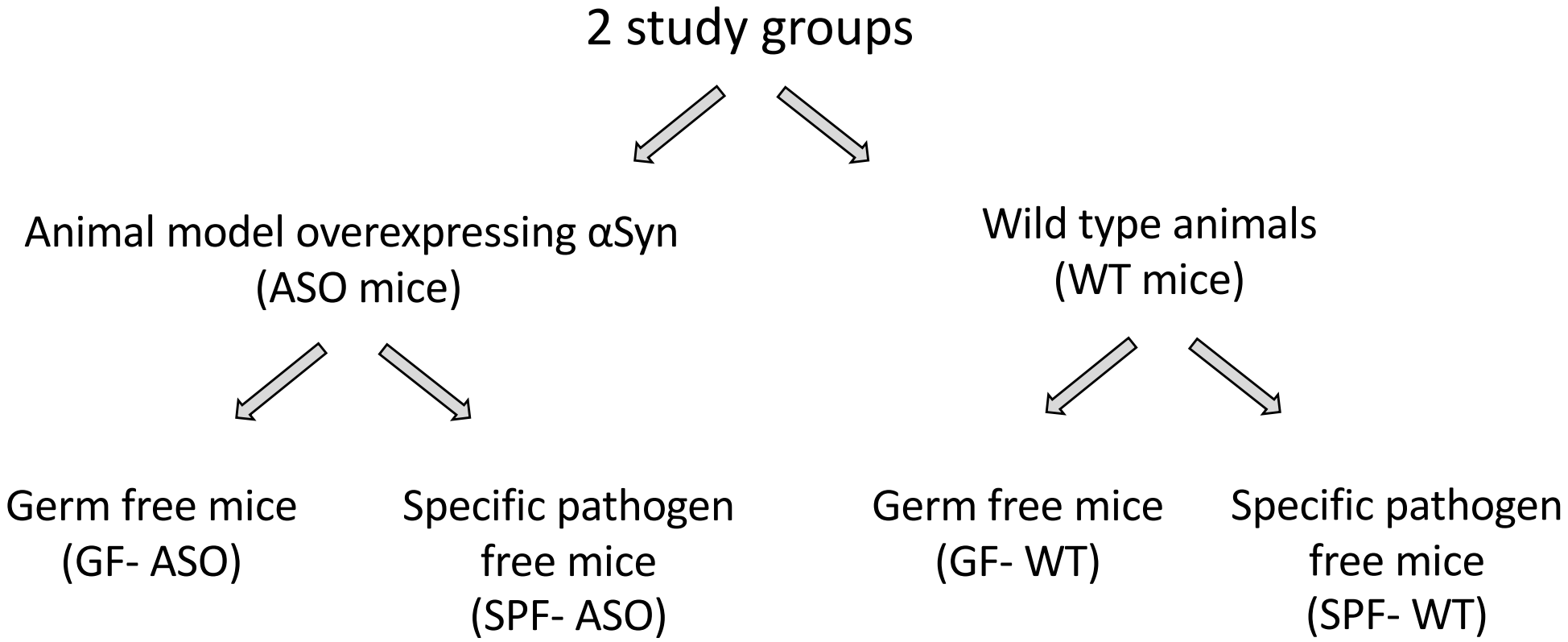
(Schroeder and Baeckhed, 2016; Hasegawa et al., 2015)

Hypothesis

"Gut bacteria regulate the hallmark motor deficits and pathophysiology of synucleinopathies"

METHODS

Animal models



GF mice were generated via caesarian section → fostered by GF dams (female parent) in GF environment

SPF mice were treated with an antibiotic cocktail
(neomycin, gentamycin, ampicillin, erythromycin, vancomycin)

Motor Function Tests

- ASO mice develop Parkinson symptoms
  - Fine and gross motor function deficits
  - Gut mobility deficits

Defects in coordinated motor tasks become evident at 12 weeks of age

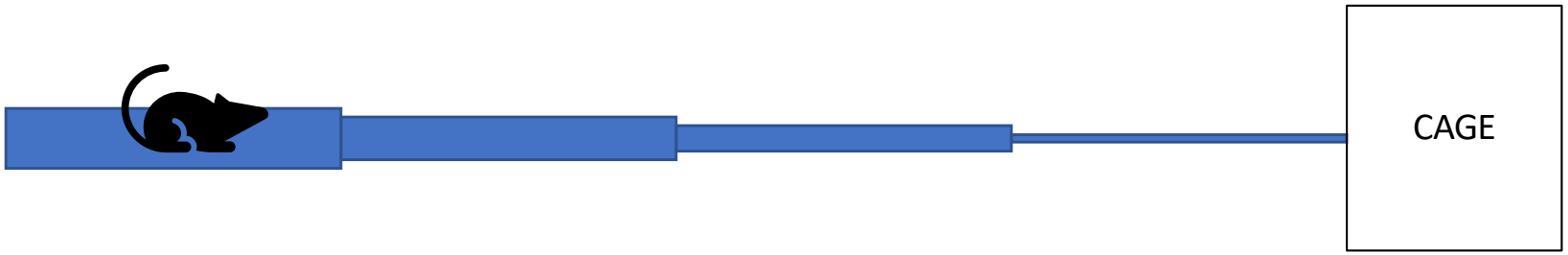
- Motor function tests
  - Beam traversal
  - Pole descent
  - Inverted grid

} Gross motor function

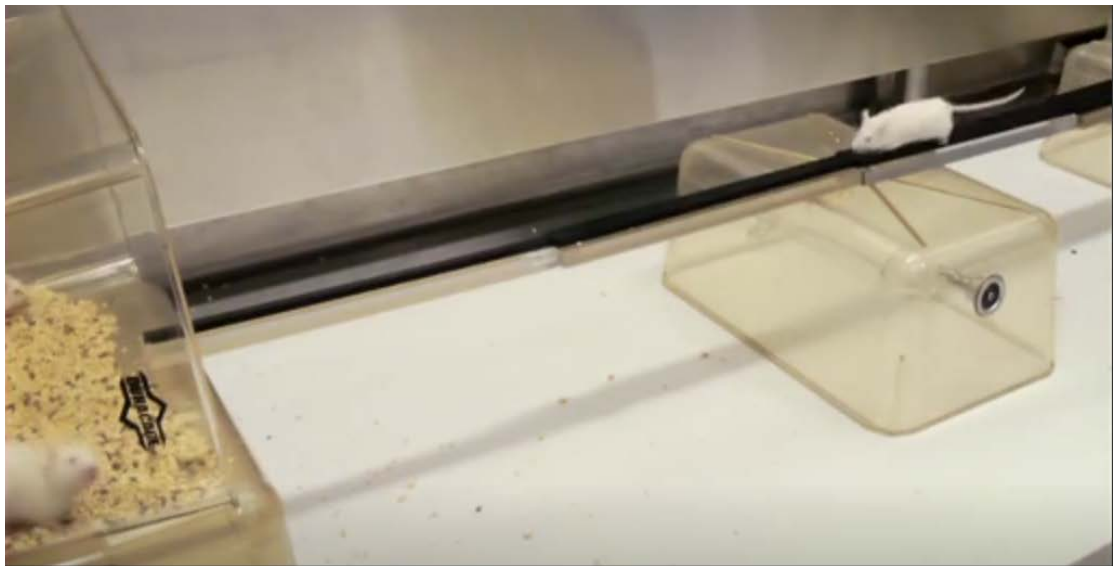
  - Nasal adhesive removal
  - Hindlimb clasping reflexes

} Fine motor function

Beam Traversal



Animals had two days of training before testing



Pole Descent

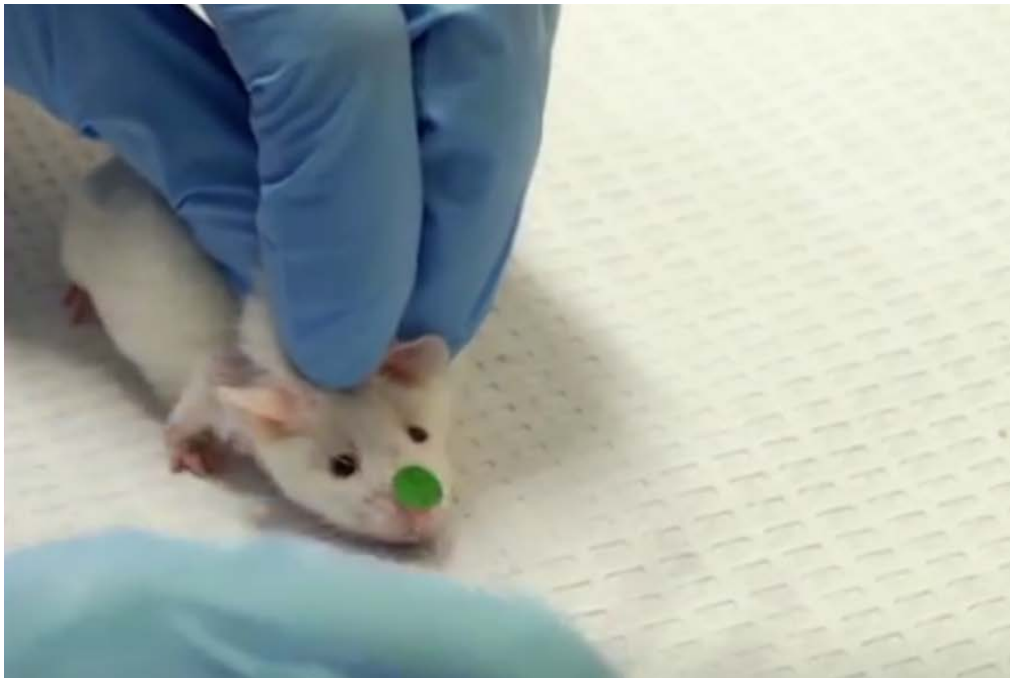
Pole wrapped with non-adhesive shelf liner

Animals had two days of training before testing

Adhesive removal

Adhesive stickers between the nostrils and forehead

Time to remove the sticker



Hindlimb Clasping Reflex

Marker of disease progression

<u>Scoring</u>

0 - hindlimbs are consistently splayed outward, away from the abdomen

1 - one hindlimb is retracted toward the abdomen

2 - both hindlimbs are partially retracted toward the abdomen

3 - hindlimbs are entirely retracted and touching the abdomen

Inverted Grid

It is a test of muscle strength using all four limbs

<u>Scoring</u>

<60 sec → **FAIL**

>60 sec → **PASS**

Gastrointestinal (GI) Dysfunction Test

- Constipation test via fecal output method

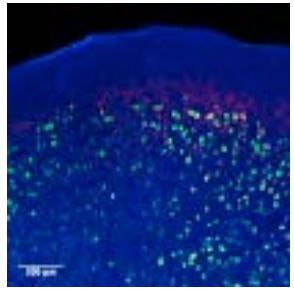
1. Mice removed from cages and placed into a transparent cylinder.

2. Fecal pellets collection timeline

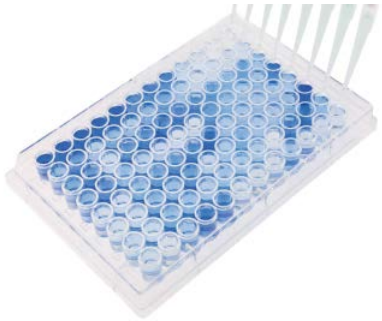
3. Total fecal pellet produced was recorded

αSyn Aggregation

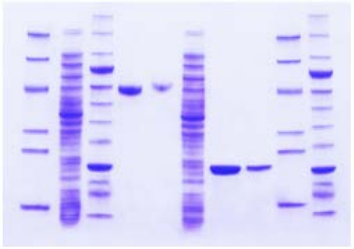
1. Immunostaining (brain sections)



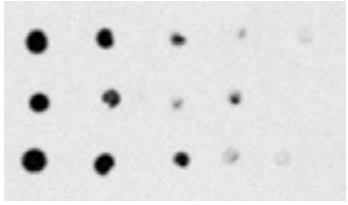
2. ELISA (brain tissue homogenates)



3. Western blot (tissue tissue homogenates)



3. Dot blot (tissue tissue homogenates)

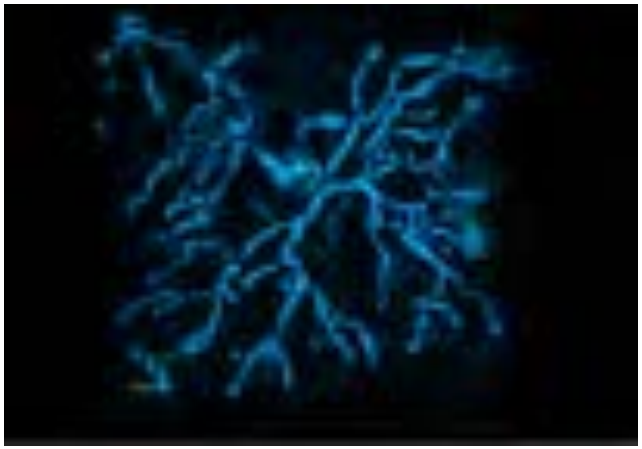


Microglia activation

αSyn aggregates activate microglia in the CNS

morphological reconstructions
(cell size, branch length)

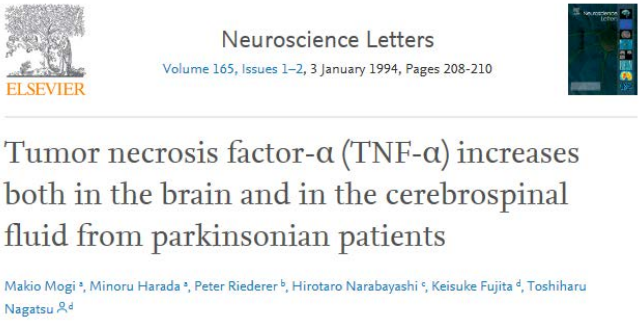
Iba1 (microglia specific protein)

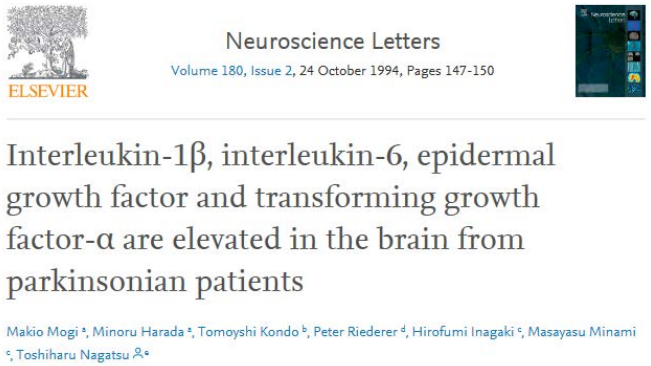


Neuroinflammation

Parkinson's disease

↑TNF-α
↑ IL-6





- ELISA in brain tissue homogenates
- qPCR in microglia RNA (*Tnfa, Il6 genes*)

Microbiome Profiling

"Humanized mice" (ex-GF)

Fecal samples (6 PD patients and 6 controls) ⇨ Fecal transplantation via oral gavage

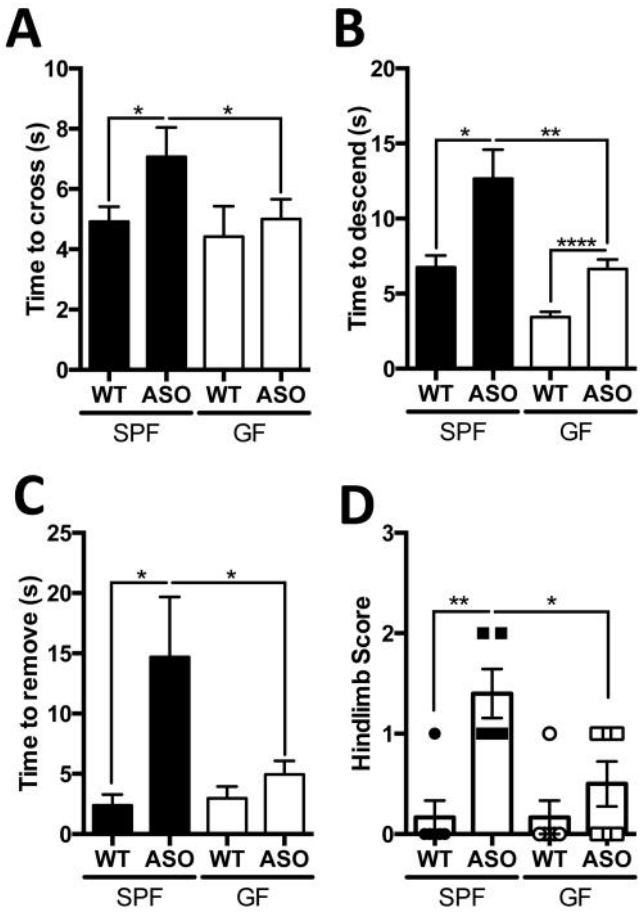
Repetitive fecal sampling after transplantation (Days 7, 14, 21, 49)

DNA extraction from each sample

The microbiota were identified based on sequencing of the V4 hypervariable region of the 16S rRNA gene

RESULTS

Results- SPF-ASO mice depict reduced motor function



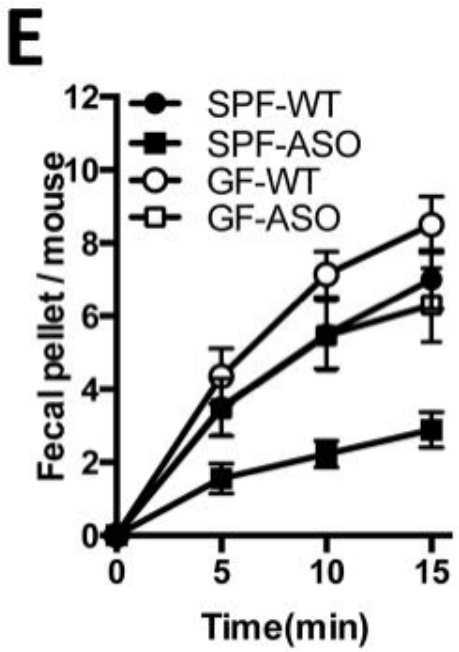
(A) Time to traverse beam apparatus.

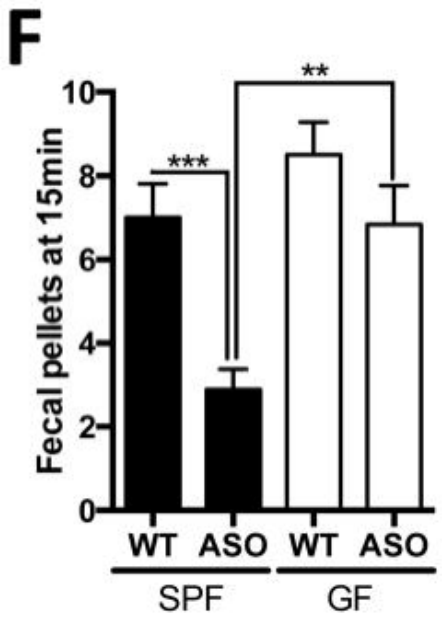
(B) Time to descend pole.

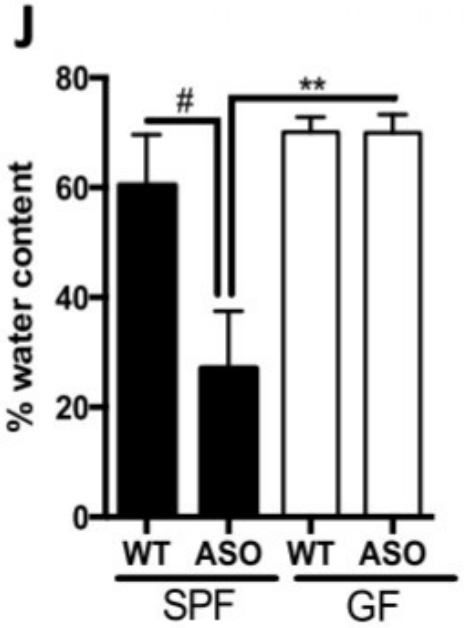
(C) Time to remove adhesive from nasal bridge.

(D) Hind-limb clasping reflex score

SPF-ASO mice depict increased GI Dysfunction



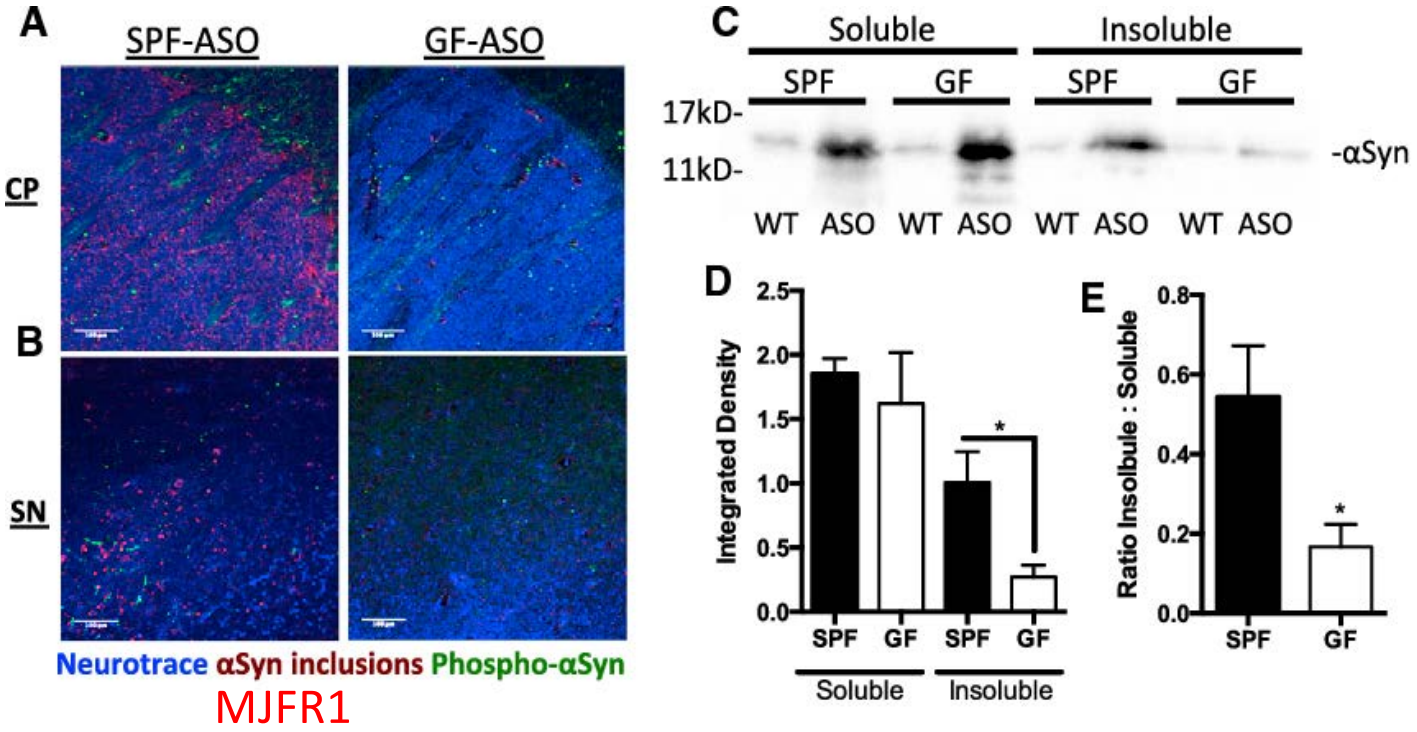




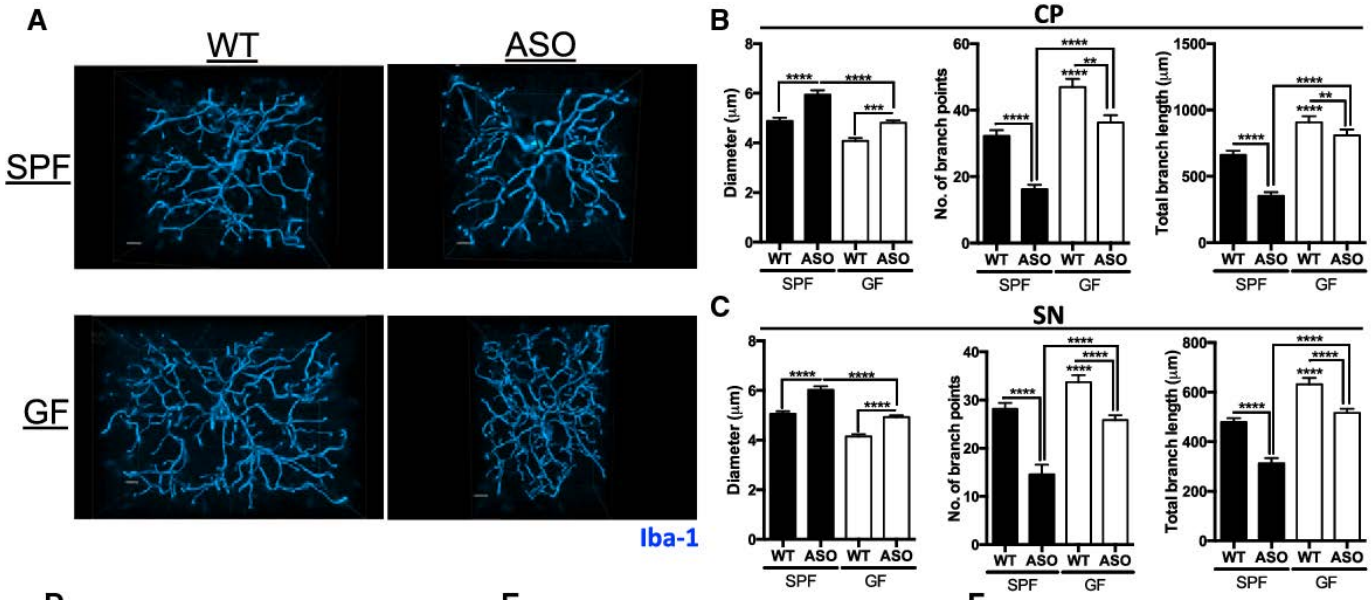
Traits of constipation:

- lower number of faecal pellets over 15 min
- lower number of total fecal pellets in 15 min
- reduced water content

The Gut Microbiota Is Required for aSyn Pathology



aSyn-Dependent Microglia Activation by the Microbiota

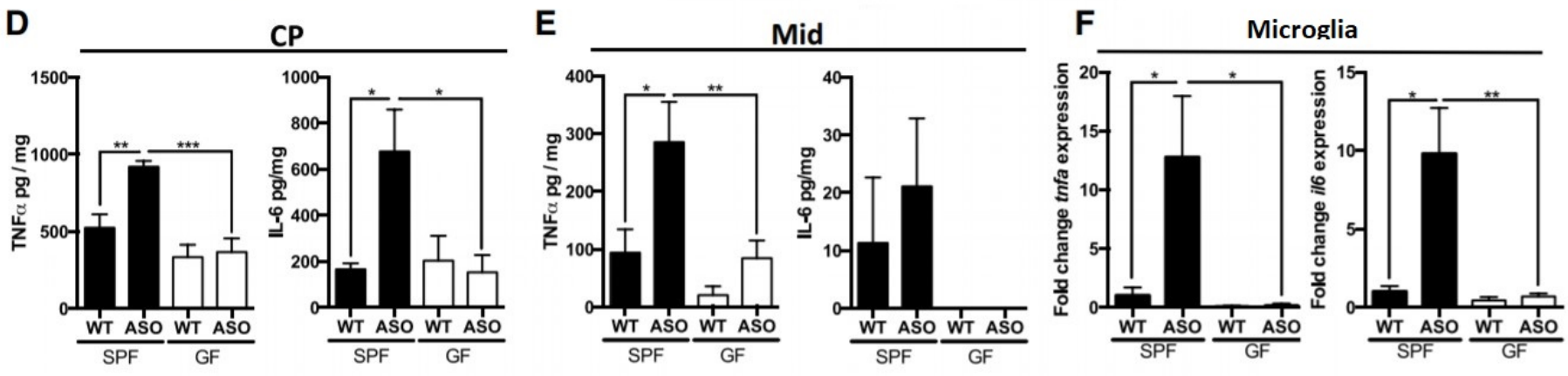


In CP

Micriglia activation:

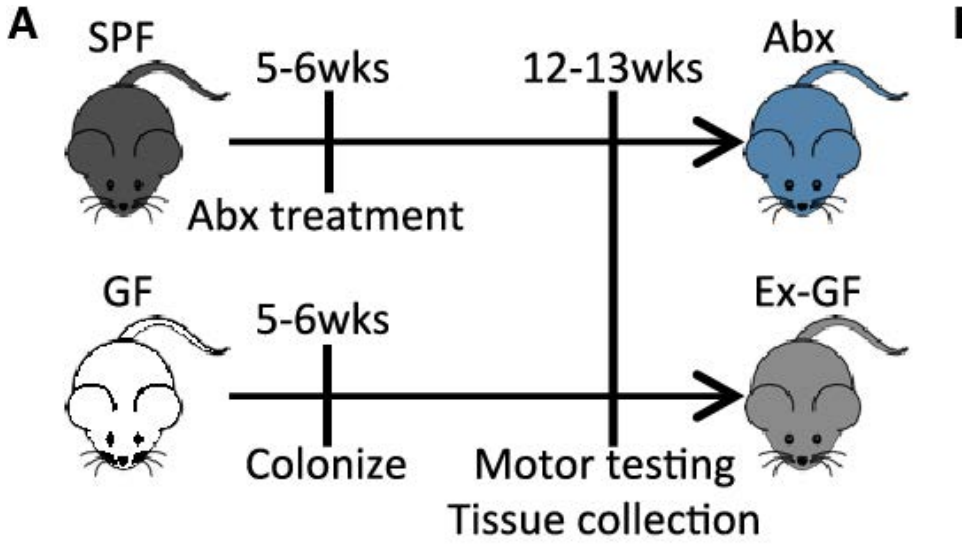
- Increase in diameter
- Reduction in number of branches

Microglia activation leads to neuroinflammation



(D) ELISA analysis for TNF-a and IL-6 present in homogenates from the CP.
(E) ELISA analysis for TNF-a and IL-6 present in homogenates from the inferior midbrain (Mid).
(F) qPCR analysis of microglia cells derived from brain homogenate for tnfa and il6.

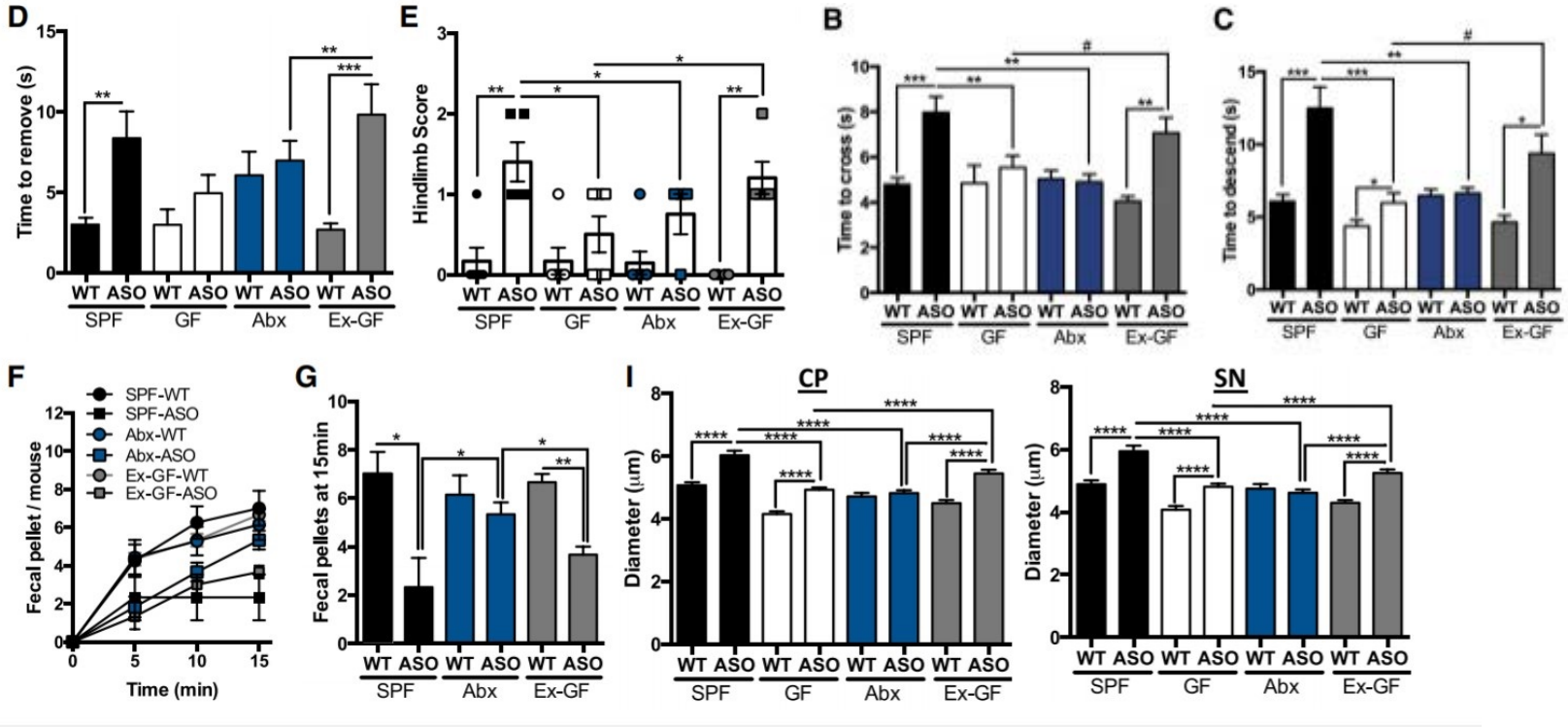
Prenatal Postnatal Microbial Differentiation



Microbiota:

- Neurological output during gestation
- Via active gut-to-brain signalling in adulthood

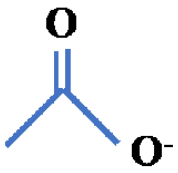
Postnatal Microbial Signals Modulate aSyn-Dependent Pathophysiology



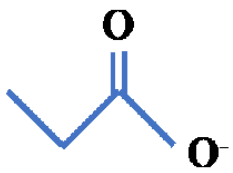
The role of Short Chain Fatty Acids (SCFAS)

- Microbial metabolites

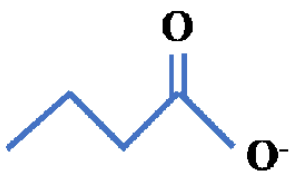
- Are SCFAs sufficient to promote αSyn-mediated neuroinflammation?



Acetate



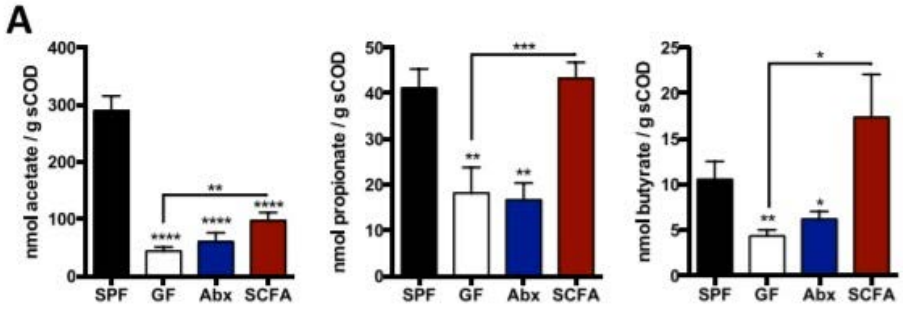
Propionate



Butyrate

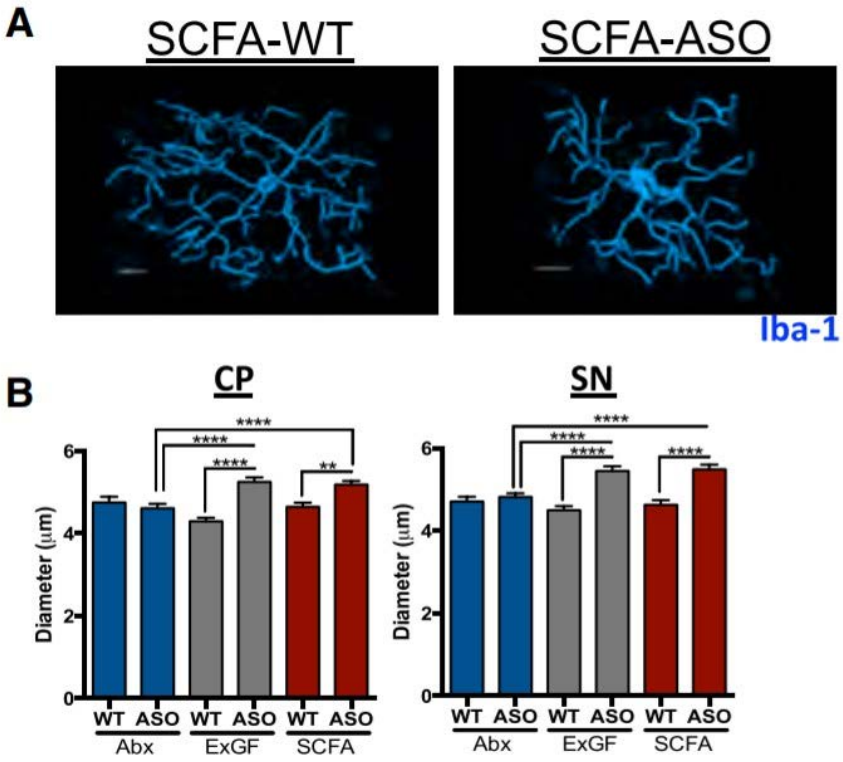
Short Chain Fatty Acids treatment

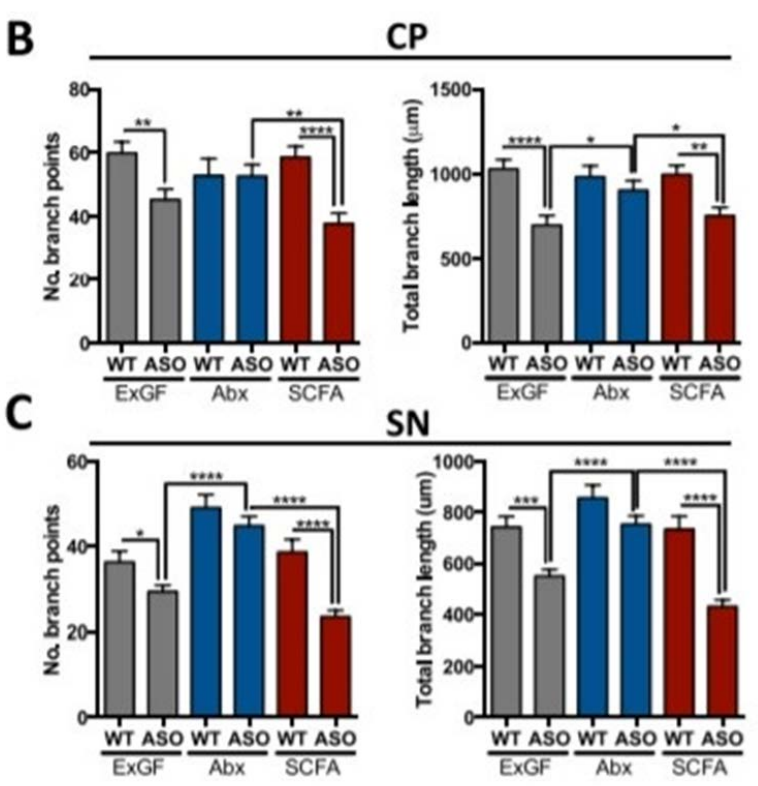
- GF-ASO GF-WT were treated with SCFA to restore fecal concentration



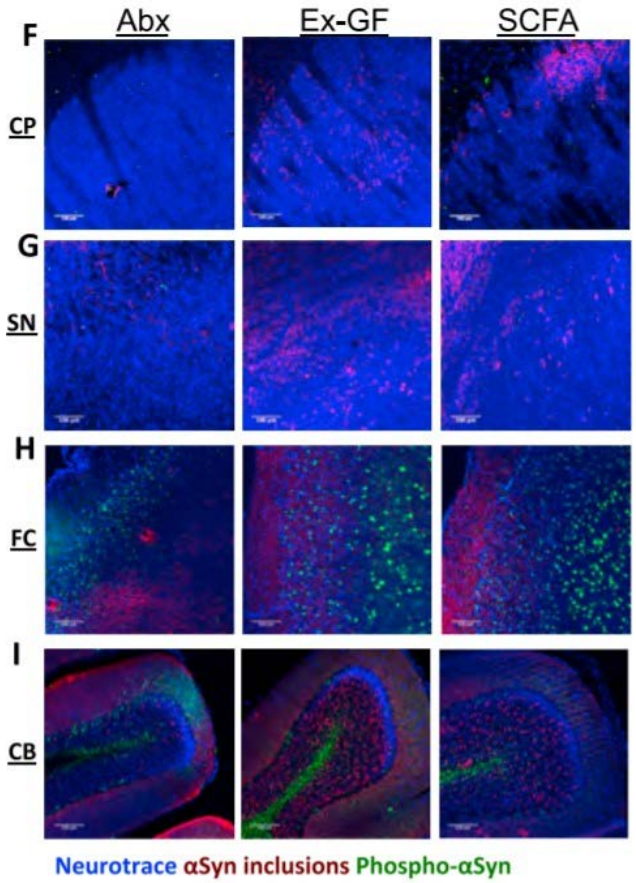
- Increased microglia activation
- Increased αSyn aggregation

Increased microglia activation

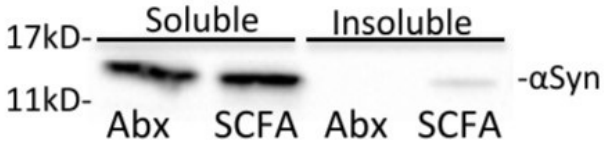




Increased αSyn Pathology



- Increased αSyn aggregation in the CP and SN confirmed by quantification of dot blots and western blot

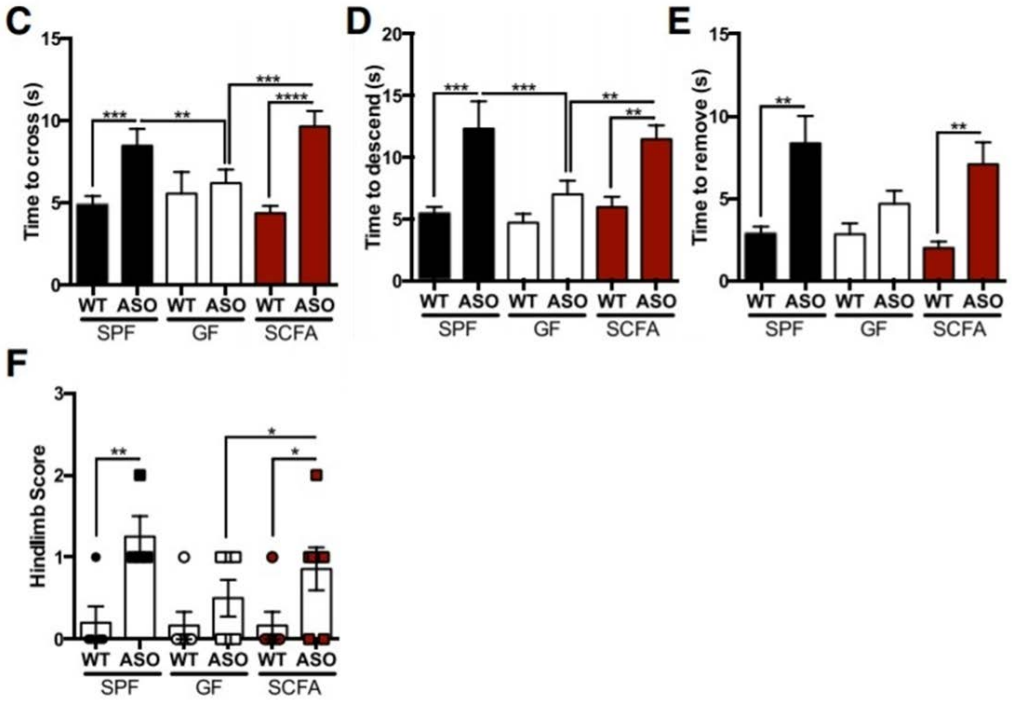


- Region specific
- SCFAs accelerate in vivo αSyn aggregation but they do not accelerate in vitro αSyn aggregation

-> there is no direct link

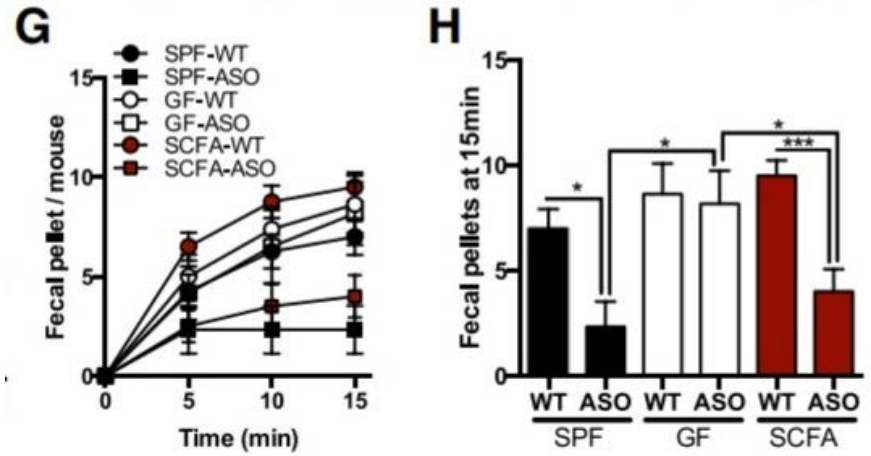
- Possible indirect link via microglia activation

Are SCFAs sufficient to promote motor deficits?



- SCFA treated-ASO mice display significantly impaired performance in several motor tasks compared to untreated GF-ASO mice

GI deficits



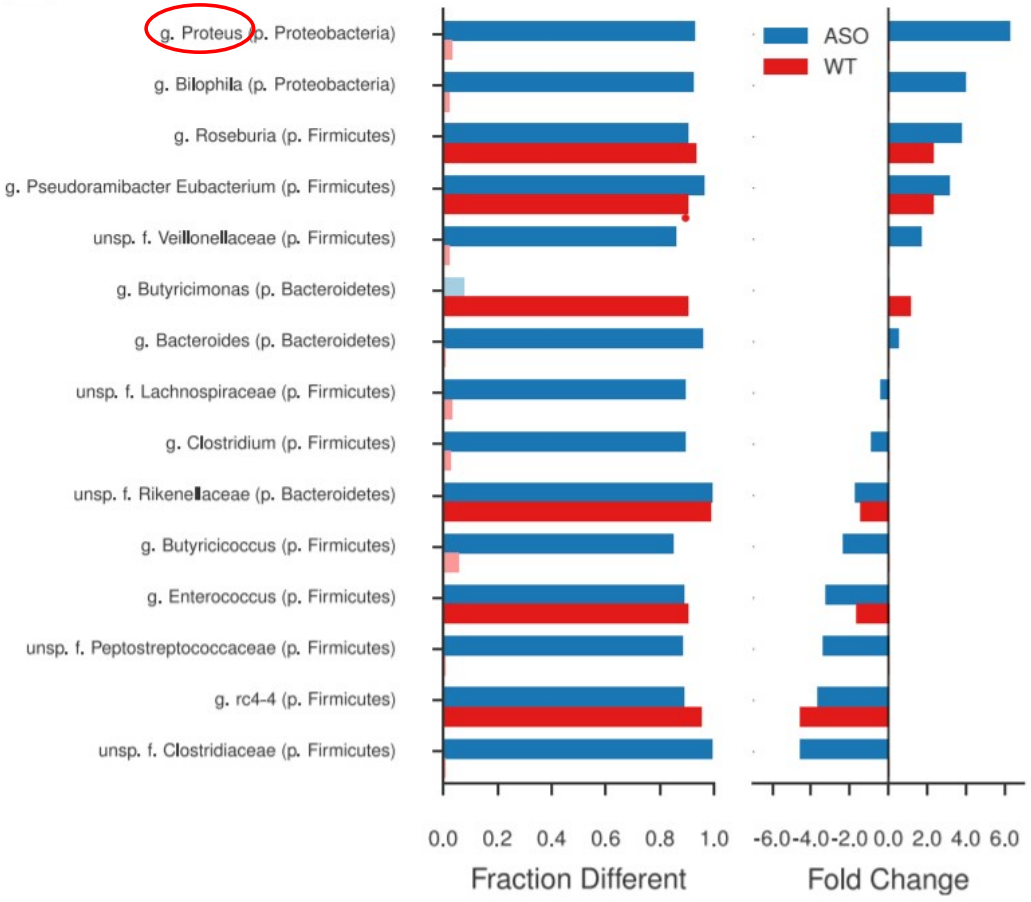
- (G) Time course of fecal output in a novel environment over 15 min.
- (H) Total fecal pellets produced in 15 min.

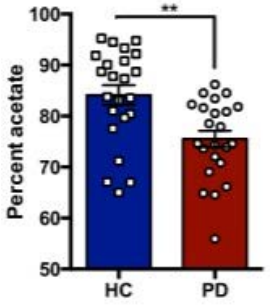
Dysbiosis of the PD microbiome

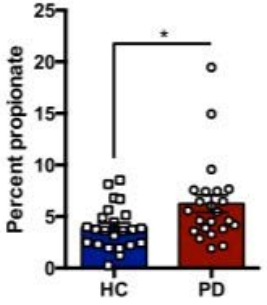
- PD patients display alte$red microbiomes
- Microbiome profiling - fecal samples from six PD patients and six healthy controls

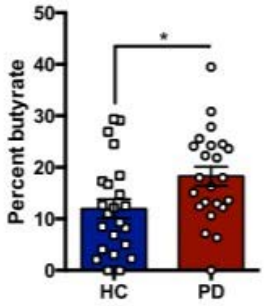
- Genera is altered in animals colonized with PD microbiota compared to Healthy controls
- Genotype effects microbial community configuration
- Significant difference PD/H in ASO background compared to WT
- Altered KEGG pathways
- Significantly altered SCFA profile

E


g. Proteus (p. Proteobacteria)
g. Bilophila (p. Proteobacteria)
g. Roseburia (p. Firmicutes)
g. Pseudoramibacter Eubacterium (p. Firmicutes)
unsp. f. Veillonellaceae (p. Firmicutes)
g. Butyricimonas (p. Bacteroidetes)
g. Bacteroides (p. Bacteroidetes)
unsp. f. Lachnospiraceae (p. Firmicutes)
g. Clostridium (p. Firmicutes)
unsp. f. Rikenellaceae (p. Bacteroidetes)
g. Butyricicoccus (p. Firmicutes)
g. Enterococcus (p. Firmicutes)
unsp. f. Peptostreptococcaceae (p. Firmicutes)
g. rc4-4 (p. Firmicutes)
unsp. f. Clostridiaceae (p. Firmicutes)
ASO
WT
0.0 0.2 0.4 0.6 0.8 1.0
Fraction Different
-6.0 -4.0 -2.0 0.0 2.0 4.0 6.0
Fold Change


Percent acetate
100
90
80
70
60
50
**
HC
PD


Percent propionate
25
20
15
10
5
0
*
HC
PD

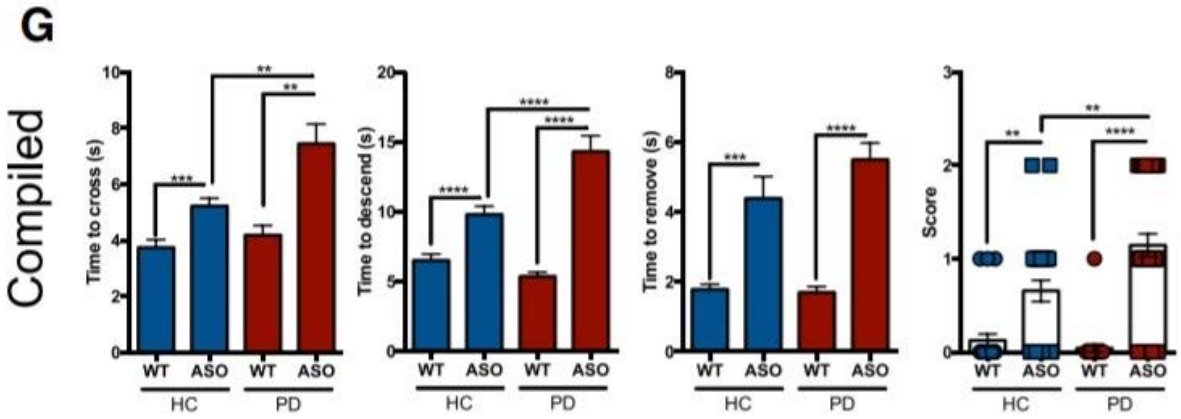

Percent butyrate
50
40
30
20
10
0
*
HC
PD

Dysbiosis of the PD microbiome

- Fecal microbial communities between PD patients and controls can be maintained after transfer into mice
- αSyn overexpression engenders distinct alterations to the gut microbiome profile after transplantation in ASO animals

PD-derived gut microbiota and motor dysfunction

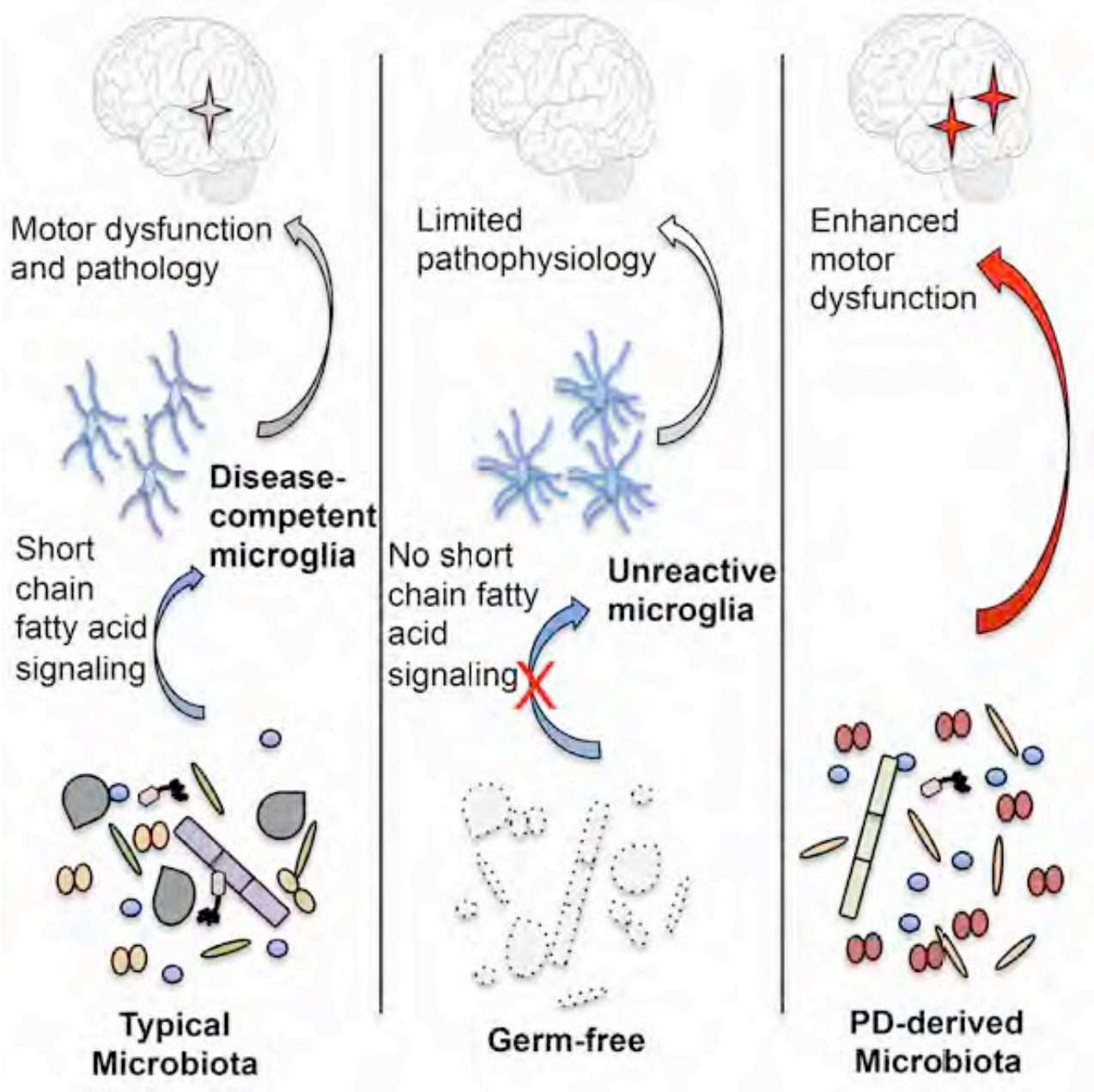
- microbiota from PD patients promoted increased “αSyn-mediated” motor dysfunction
- Results were consistent among 4/6 pairs



PD-derived gut microbiota and motor dysfunction

- The observation that gut bacteria from PD patients compared to healthy controls enhance motor deficits in a mouse model provides evidence for a functional contribution by the microbiota to synucleinopathies

Discussion


Motor dysfunction and pathology
Disease-competent microglia
Short chain fatty acid signaling
Typical Microbiota
Limited pathophysiology
No short chain fatty acid signaling
Unreactive microglia
Germ-free
Enhanced motor dysfunction
PD-derived Microbiota

Discussion-Extrapolation to humans

- Gut Microbiota is **REQUIRED** for postnatal motor deficits (in animal models)
- GF-mice demonstrate **reduced microglia activation**→ reduced motor deficits
- Treatments with microbial metabolites (**SCFAs**) **restored** major features of disease in GF-mice
- αSyn overexpression AND Dysbiosis **COMBINE** influence to disease

Role of Microbiota in Inflammation

1. Gut microbiota **promotes full maturation** and inflammation capabilities of microglia (through SCFAs)

2. Increases in the activation state of microglia may **alter neuronal function** and increase cell death

3. SCFAs may cross the BBB → Impact CNS

4. Inflammatory environment→ aSyn aggregation→ migroglia activation→ Inflammatory response (feed-forward cascade)

(Erny et al., 2015; Mitchell et al., 2011)

Other Unexplored Factors

- Other disease-modifying procedures may remain undiscovered
- Microbial effects on **autophagy** and **proteasome** function (clearance of αSyn)
- Likely that microbial signals have important role during **prenatal** neurodevelopment

(Lin et al., 2014; Beilina and Cookson, 2015; Nalls et al., 2014; Cleynen et al., 2014)

Bacteria from PD Patients

- Transplantation of gut bacteria from PD patients result to **more severe motor impairments** to microbiota than those from healthy controls

- Fecal Transplants: **alter bacteria genera** in ASO mice

- Altered microbial communities seem to **impact immune responses** in the gut and periphery

  - (Hooper et al., 2012)

The Role of Dysbiosis

- Altered intestinal physiological functions in affected individuals may lead to **changes in microbiome**

- **Speculation** : extracellular α-Syn may act as antimicrobial, thus shaping the PD microbiome

- PD Microbiota :
  - ✓ Missing or reduced protective microbes
  - ✓ Enhanced pathogenic resident microbes
  - ✓ **OR BOTH**

  →differential production of microbial molecules in gut

  →differentiated secretion of metabolites in circulation which impact neurological function

The Role of Dysbiosis

- IDENTIFICATION of **altered** bacterial taxa or metabolites
- DISEASE **BIOMARKERS** and DRUG **TARGETS**
- INTERVENTION that aims to **correct dysbiosis** may **slow or halt** disease progression

Conclusions

Microbiota are required for the hallmark motor and GI dysfunction in a mouse model of PD via postnatal gut-brain signaling by microbial molecules that impact neuroinflammation and aSyn aggregation.

- ✓Certain neurologic conditions that have classically been studied as disorders of the brain may also have etiologies in the gut.

Thank You !

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