The liver-brain-gut neural arc

2020/10/24 Ayumu Watanabe

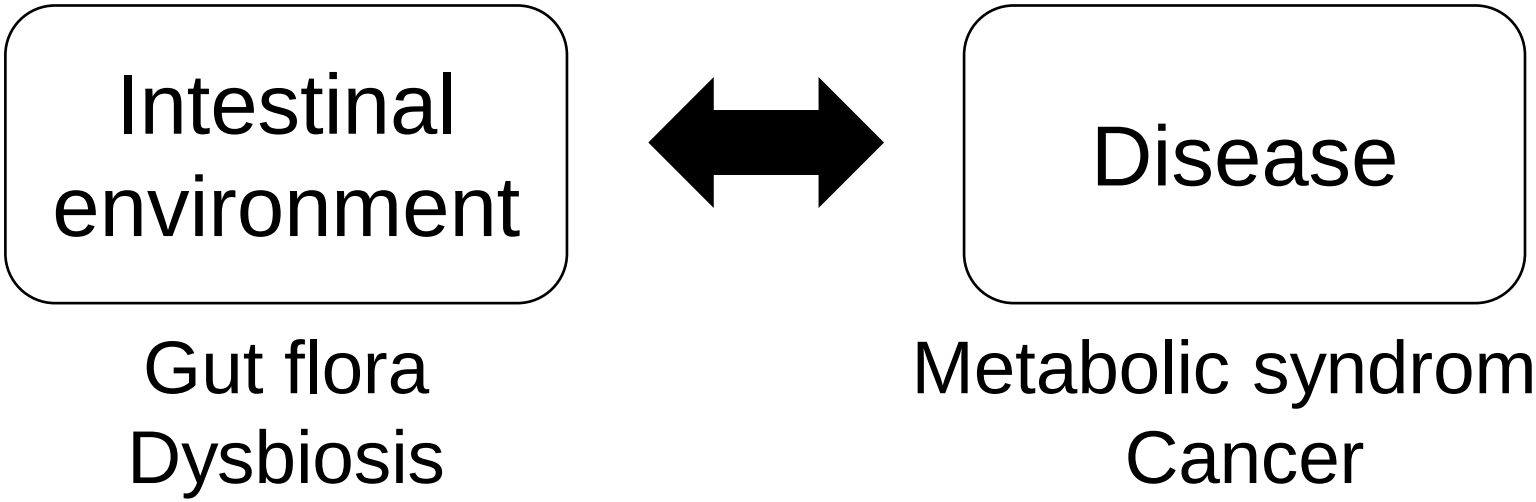
Contents

1. Introduction

2. The liver-brain-gut neural arc maintains the Treg cell niche in the gut
   (T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.)

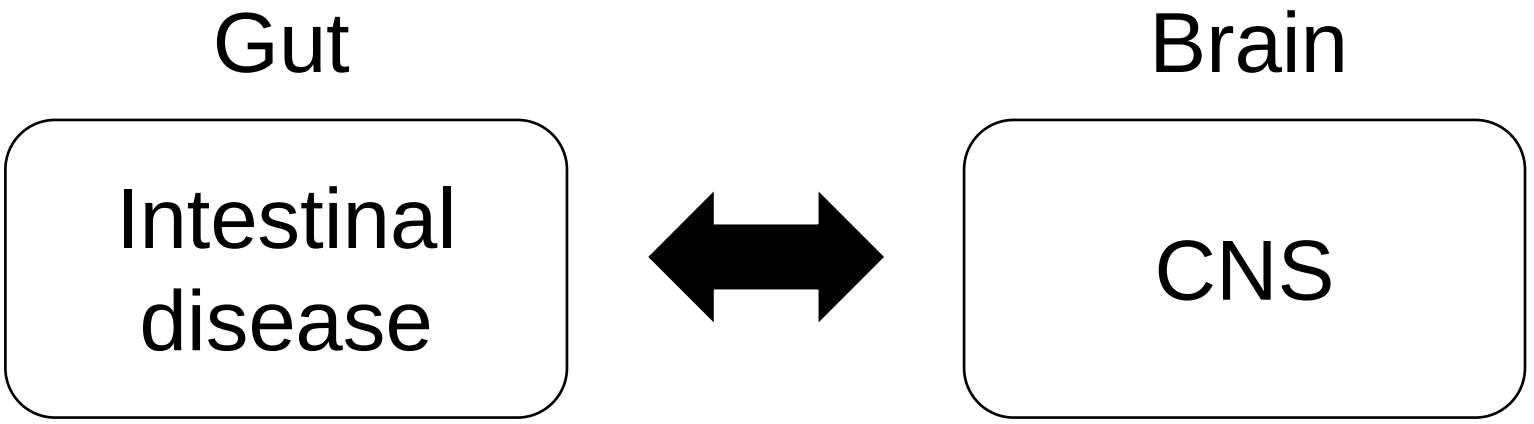
Contents

The gut-brain axis



- **A balanced diet leads to a healthy body.**

The gut-brain axis



Ex.)

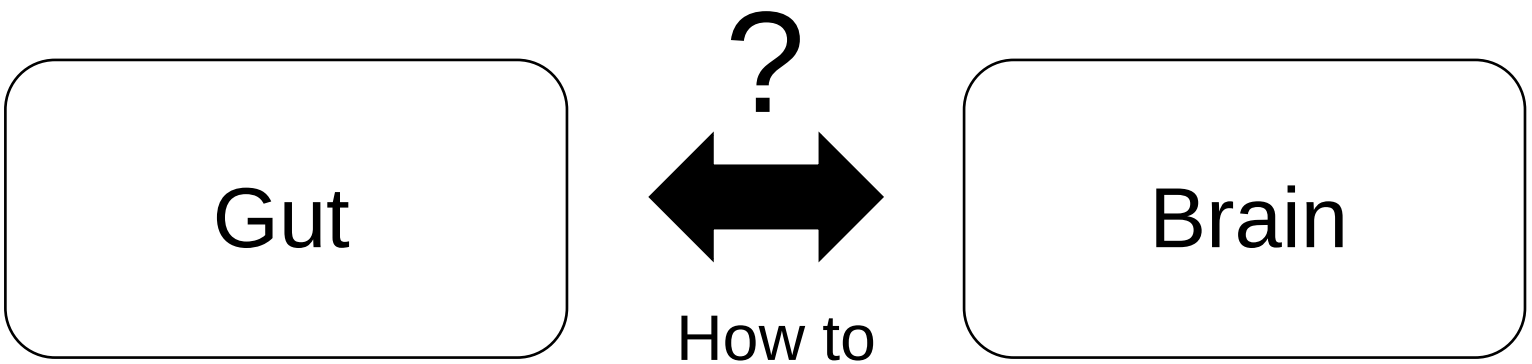
- IBDs (Inflammatory bowel diseases)

- Depression
- IBS

The gut-brain axis is conceptually feasible

1) G. Sharon; T. R. Sampson; D. H. Geschwind; S. K. Mazmanian, *Cell*, **2016**, *167*, 915

The gut-brain axis



How to
communicate
for the immune homeostasis

If unraveled in detail

New targets in drug discovery

1) G. Sharon; T. R. Sampson; D. H. Geschwind; S. K. Mazmanian, *Cell*, **2016**, *167*, 915

Tregs

About Tregs (regulatory T cells)

- Suppressor T cells ($CD4^+$ T cells)
  Essential for immunological self-tolerance and immune homeostasis
  Related to the inflammatory and allergic disease

- Foxp3 is the master regulatory gene
  Mutation of the Foxp3 leads to the autoimmune disease known as IPEX syndrome

1) K. Otsubo; H. Kanegane; I. Kobayashi; T. Miyawaki, *Jpn. J. Clin. Immunol*, **2010**, *33*, 196-206.
2) S. Hori; T. Nomura; S. Sakaguchi, *Science*, **2003**, *299*, 1057-1061.

Tregs

About Tregs (regulatory T cells)

- Target of the immune checkpoint inhibitor
  PD-1 and CTLA-4 is expressed in Tregs
  Related to the immune tolerance of the cancer

- tTreg and pTreg
  tTreg: thymus-derived Treg
  pTreg: peripherally-derived Treg
  Most of pTreg exist in the intestine

1) K. Wing; Y. Onishi; P. P. Martin; T. Yamaguchi; M. Miyara; Z. Fehervari; T. Nomura; S. Sakaguchi, *Science*, **2008**, *322*, 271-275.

APC

About APC (antigen presenting cell)

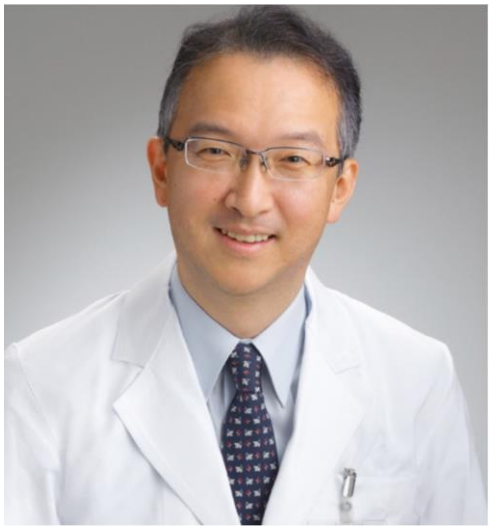
- Immune cells (MHC-II$^+$ cells)
  Process antigens and present them to T-cells (antigen presentation)
  Proliferation, Differentiation and Activation of helper T-cells are induced
  Ex.) Dendritic cells, Macrophages and B cells

In the main paper, regarded as an important factor for Differentiation and Maintenance of pTregs

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Contents

Prof. Takanori Kanai



Career:

1988	B.S. @Keio University School of Medcine
1992	Research Associate @Keio University School of Medcine
1995	M.D. @Shizuoka City Shimizu Hospital
2001	Research Associate @Tokyo Medical and Dental University
2003-2007	Senior Lecturer @Tokyo Medical and Dental University
2008-2012	Associate professor @Keio University School of Medcine
2013-	Professor @Keio University School of Medcine
2016-	Director of the Center for IBD, Keio University Hospital
2020-	General manager of Joint Research Coronavirus Task Force

Research topics:
IBD (Inflammatory bowel disease),
Intestinal flora, Probiotics, Dysbiosis

Contents of Main Paper

1. What is the role of colonic APCs in maintaining the pTreg?

2. How the pTreg homeostasis is regulated?

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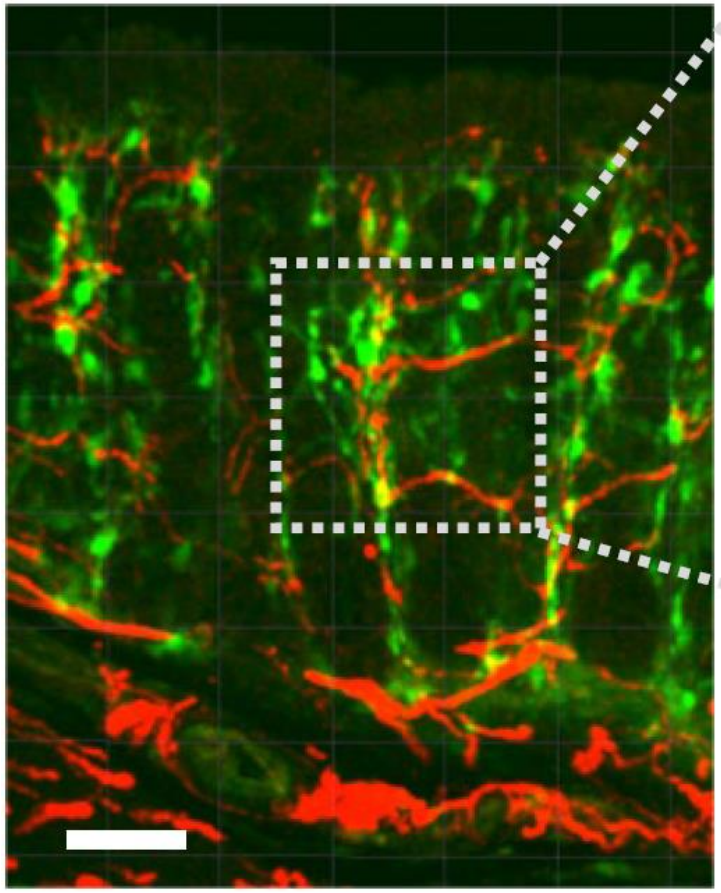
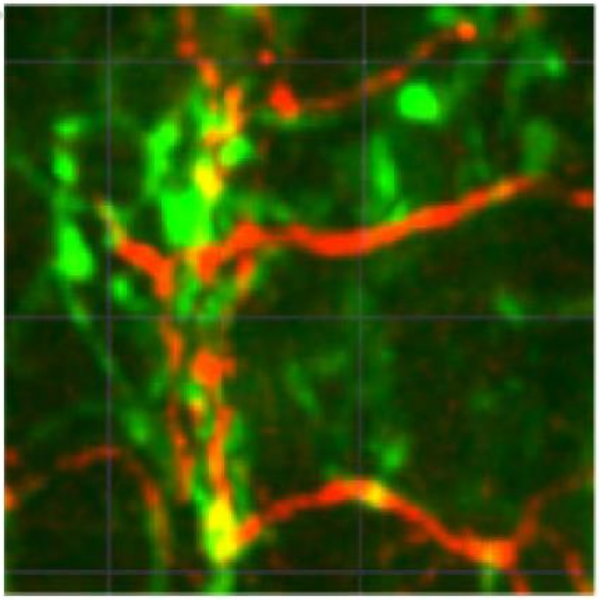
1. What is the role of colonic APCs in maintaining the pTreg?

2. How the pTreg homeostasis is regulated?

The localization of neurons and APCs





CX3CR1 = marker of MNPs (one of the APCs)

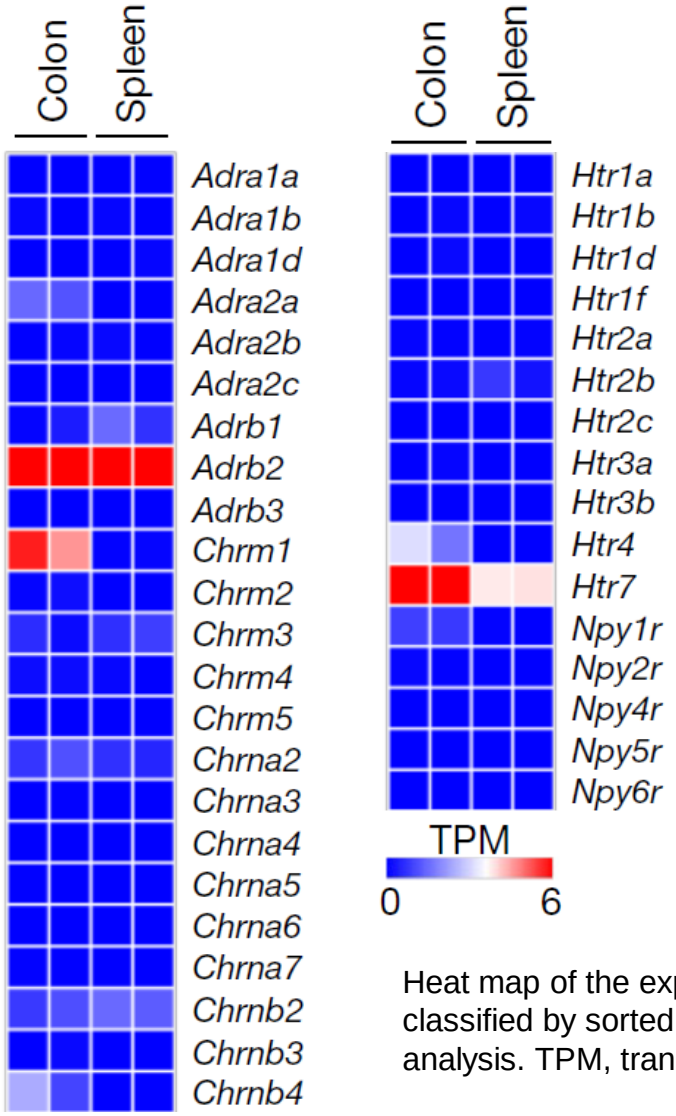
β-tubulin III = marker of neurons

Representative immunofluorescence of CX3CR1–GFP (green) and β-tubulin III (red) in the mouse colon. Scale bar, 50 μm.

→ the proximity of **neurons** and **APCs** in the colon

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Difference of the expression of the gene between colonic and splenic APCs



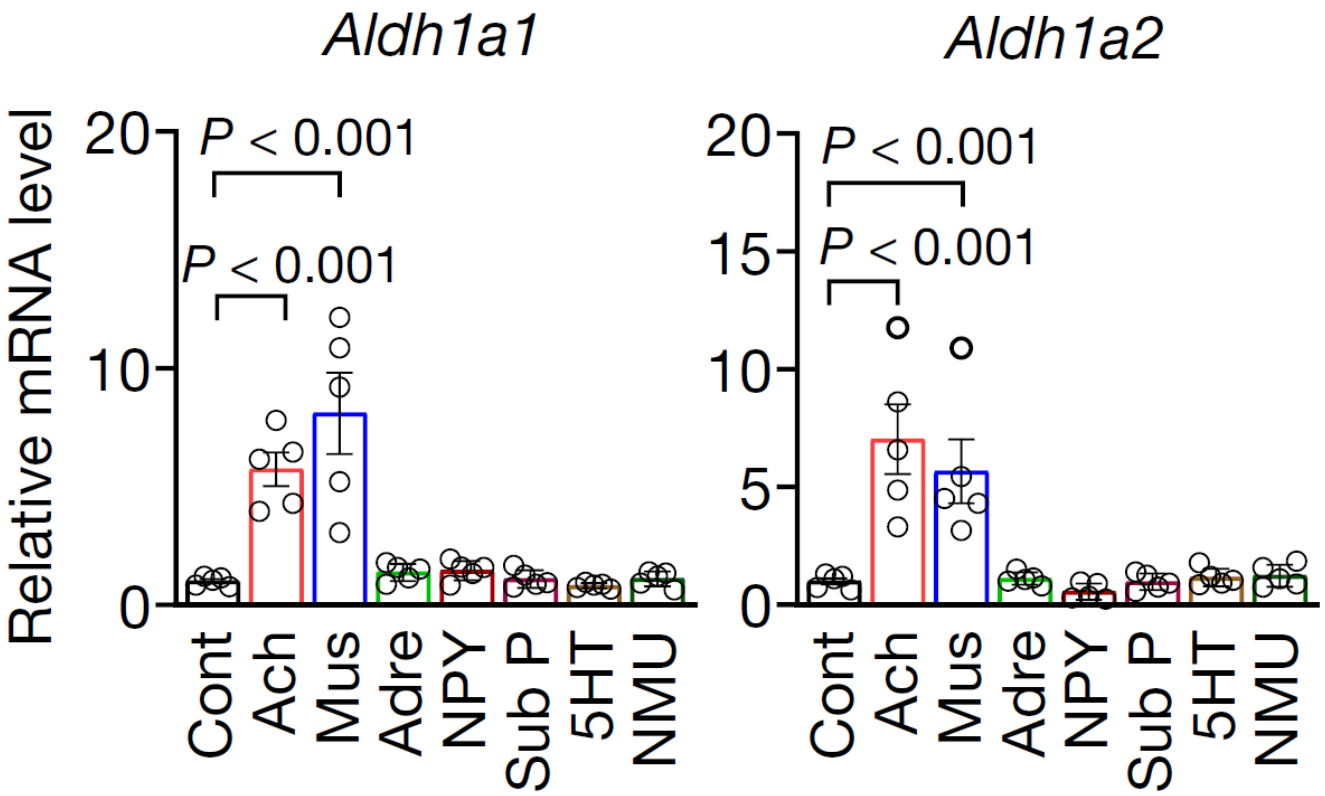
→ In the colon, the expression of the ***Chrm1*** is higher level than in the spleen.

Chrm1: the gene encoding the muscarinic Ach receptor

Heat map of the expression of genes encoding neurotransmitter receptors, classified by sorted colonic and splenic APCs, as determined by RNA-sequencing analysis. TPM, transcripts per million.

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Relative mRNA levels with neurotransmitters



Aldh1a1 and Aldh1a2 mRNA expression in colonic APCs treated with PBS (control), 10 μM acetylcholine (Ach), 10 μM muscarine (Mus), 100 nM adrenaline (Adre), 100 μM neuropeptide Y (NPY), 100 nM substance P (Sub P), 10 μM serotonin (5-HT) or 100 ng ml−1 neuromedin U (NMU) for 12 h (n = 5 per group).

Gated on APCs:
$CD45^{+}CD3^{-}B220^{-}NK1.1^{-}MHC\text{-}II^{+}$

P < 0.05 was considered significant

→ ***Aldh1a1 , 2*** *was* increased by **Ach** and **Mus**

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Aldh1a1, 2

Aldh1a1 , 2 ↑ (gene)

⬇

ALDH ↑ (enzyme)

⬇

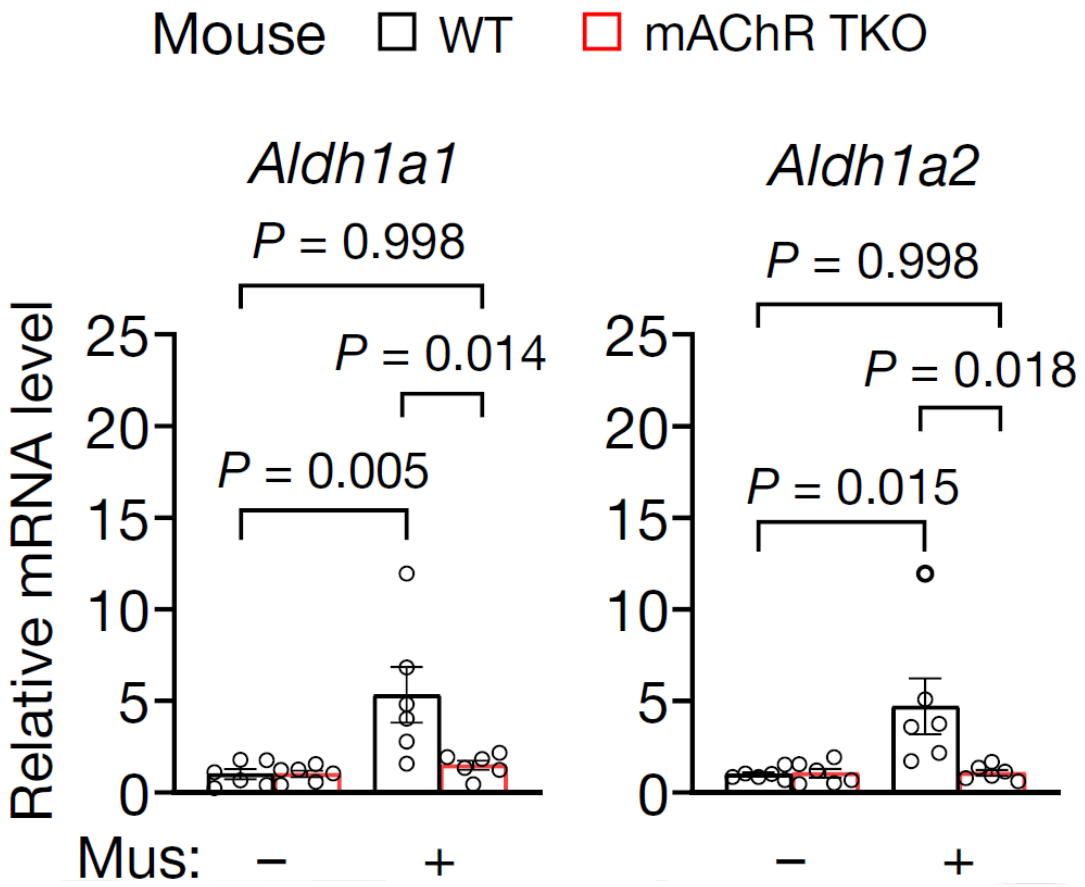
Retinoic acid ↑

⬇

The generation of pTreg is promoted

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

The role of the mAchR

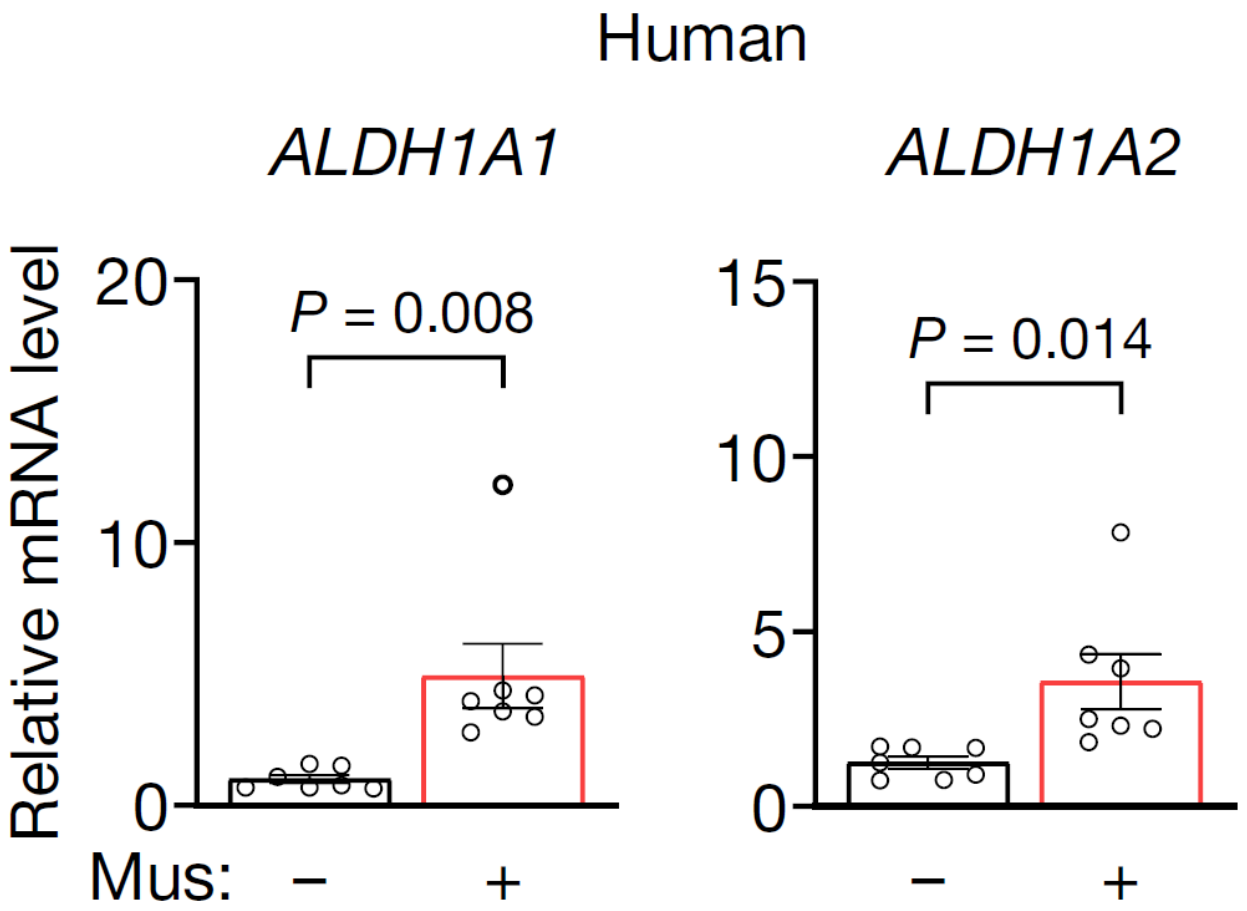


Aldh1a1 and Aldh1a2 expression in wild-type (WT) and mAChR TKO colonic APCs.
Colonic APCs were isolated from wild-type or mAChR TKO mice and treated with 10 μM muscarine or untreated for 12 h (n = 6 per group).

→ **mAChR TKO** (mAChR-deficient) mice:
Aldh1a1 and 2 ***not*** *increased*

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

In human colonic APCs

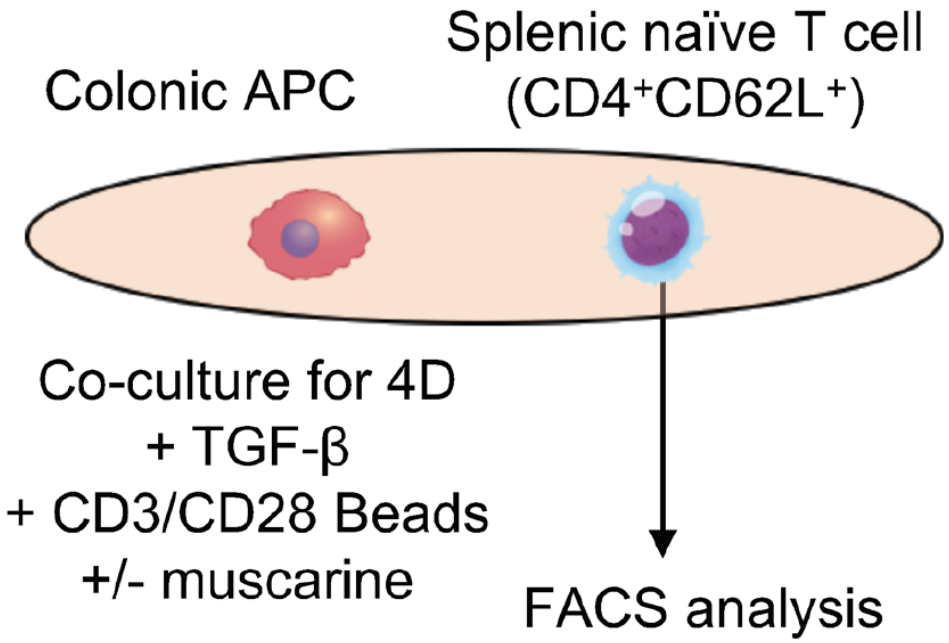


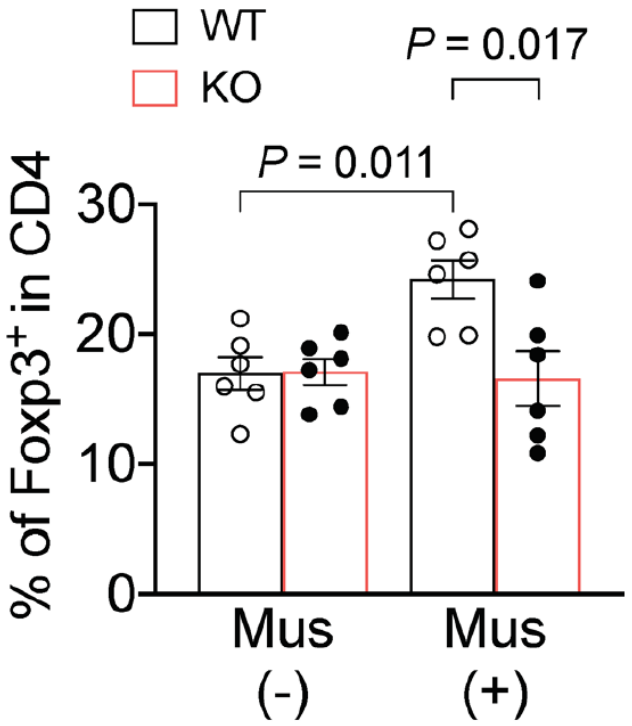
ALDH1A1 and ALDH1A2 mRNA levels in human colonic APCs. Colonic APCs were treated with 10 μM Mus or untreated for 12 h (n = 7 per group).

→ In **human** colonic APCs,
ALDH1A1 and 2 increased by Mus

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

The effect of APC and Mus to the amount of pTreg





Muscarinic signalling in colonic APCs promoted induction of Treg. left, Schema of experience. right, Frequency of Foxp3+ Treg among CD4+ T cells. (left panel) Representative contour plots. (right panel) Quantification (n = 6/group).

→ pTreg level was **enhanced** by **APC** and **Mus** from **WT mice**, but not from **KO mice**

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Vagus nerve

Stimulation of the mAchR on the APCs

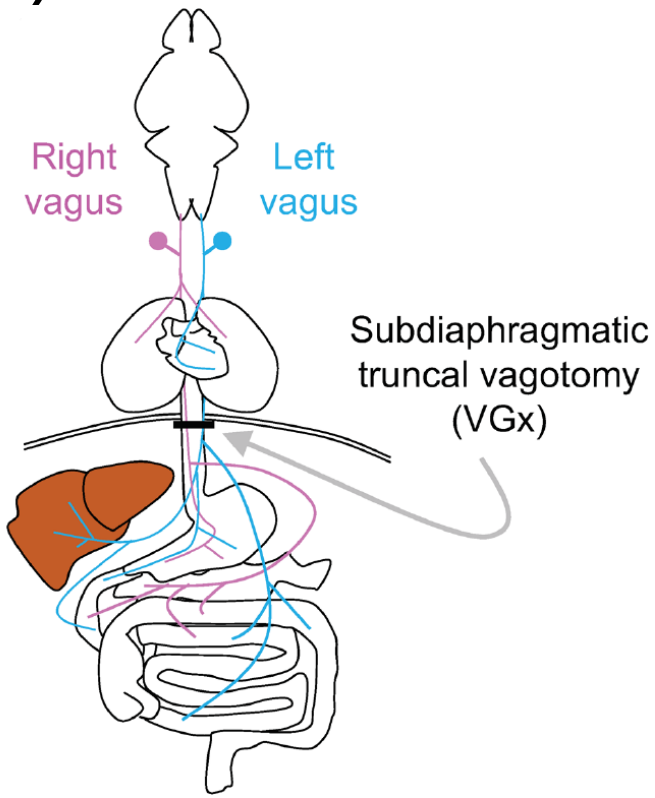
⬇

pTreg ↑ (*in vitro*)

mAchR:
parasympathetic innervation

⬇

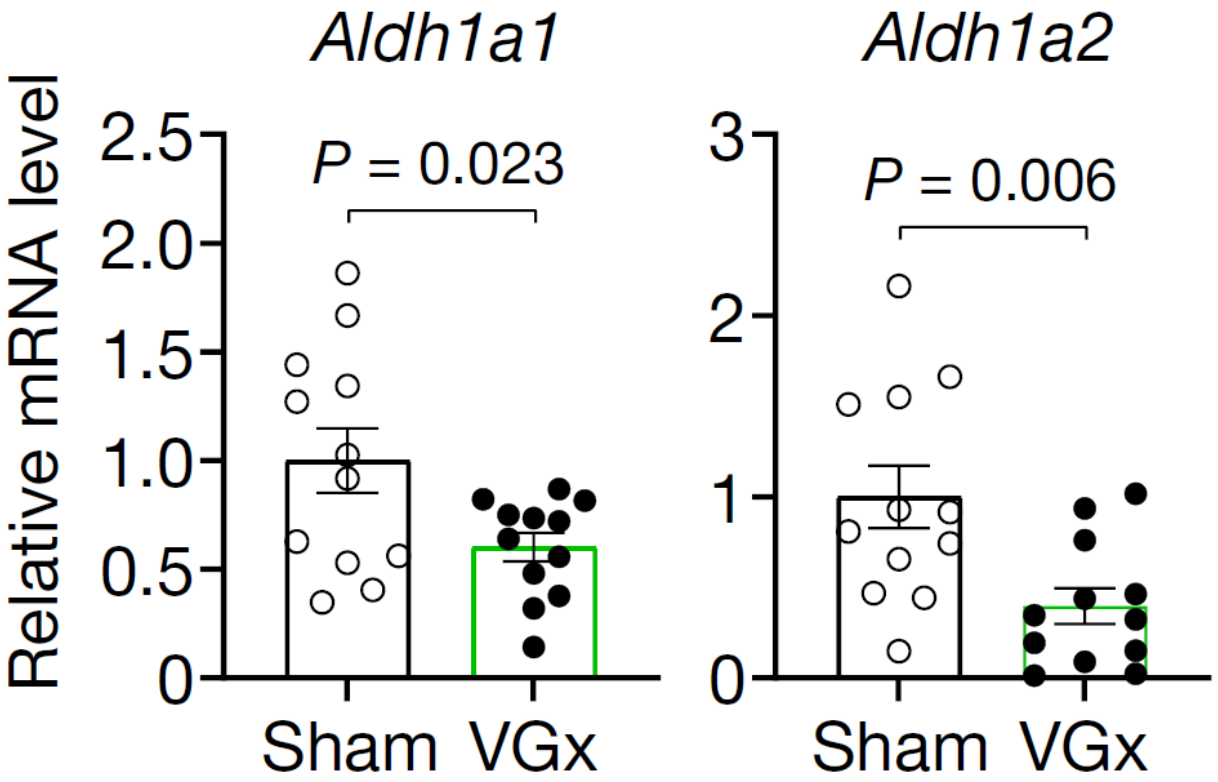
Surgical perturbation of
the Vagus nerve
= **VGx**



Vagus nerve: parasympathetic nerve in gastrointestinal tract

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

The effect of VGx (1)

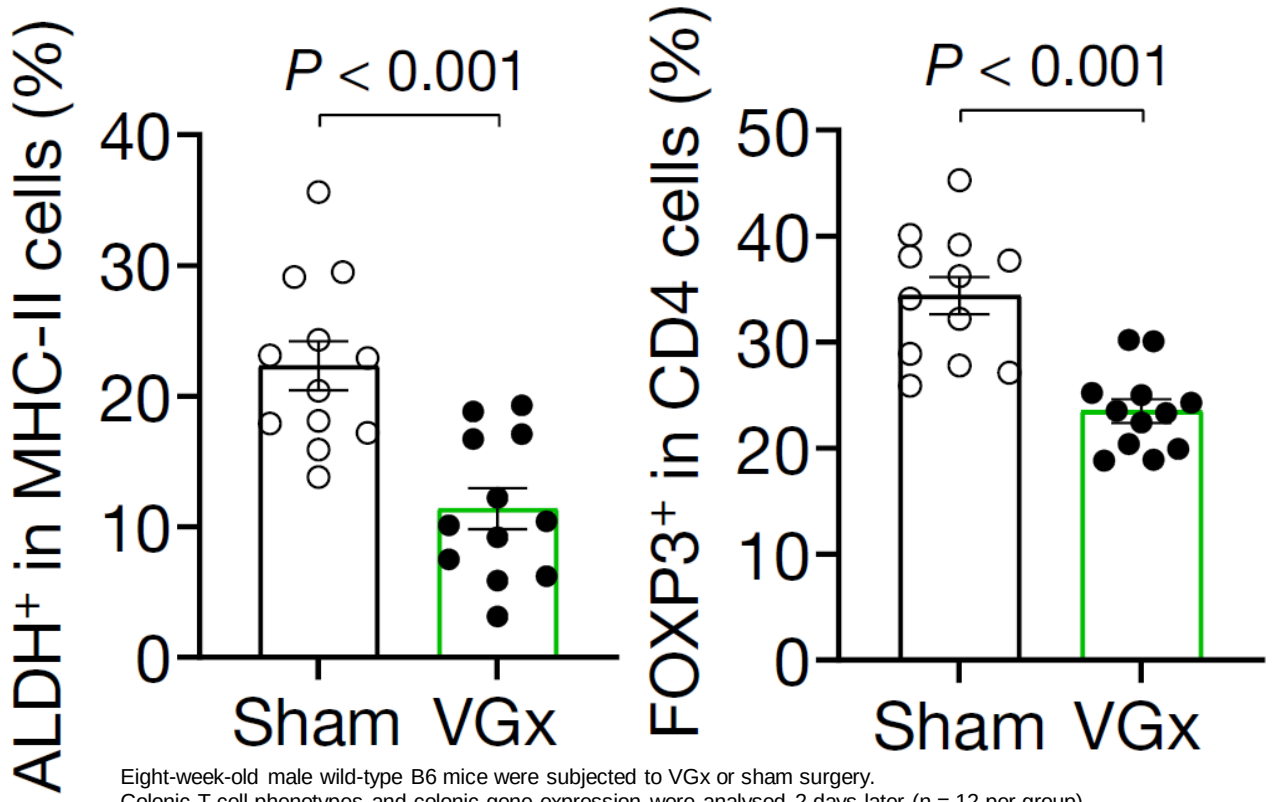


Eight-week-old male wild-type B6 mice were subjected to VGx or sham surgery.
Colonic T cell phenotypes and colonic gene expression were analysed 2 days later (n = 12 per group).
Expression of Aldh1a1 and Aldh1a2 mRNA in colonic APCs.

→ *Aldh1a1, 2* level was **decreased** by **VGx**

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

The effect of VGx (2)

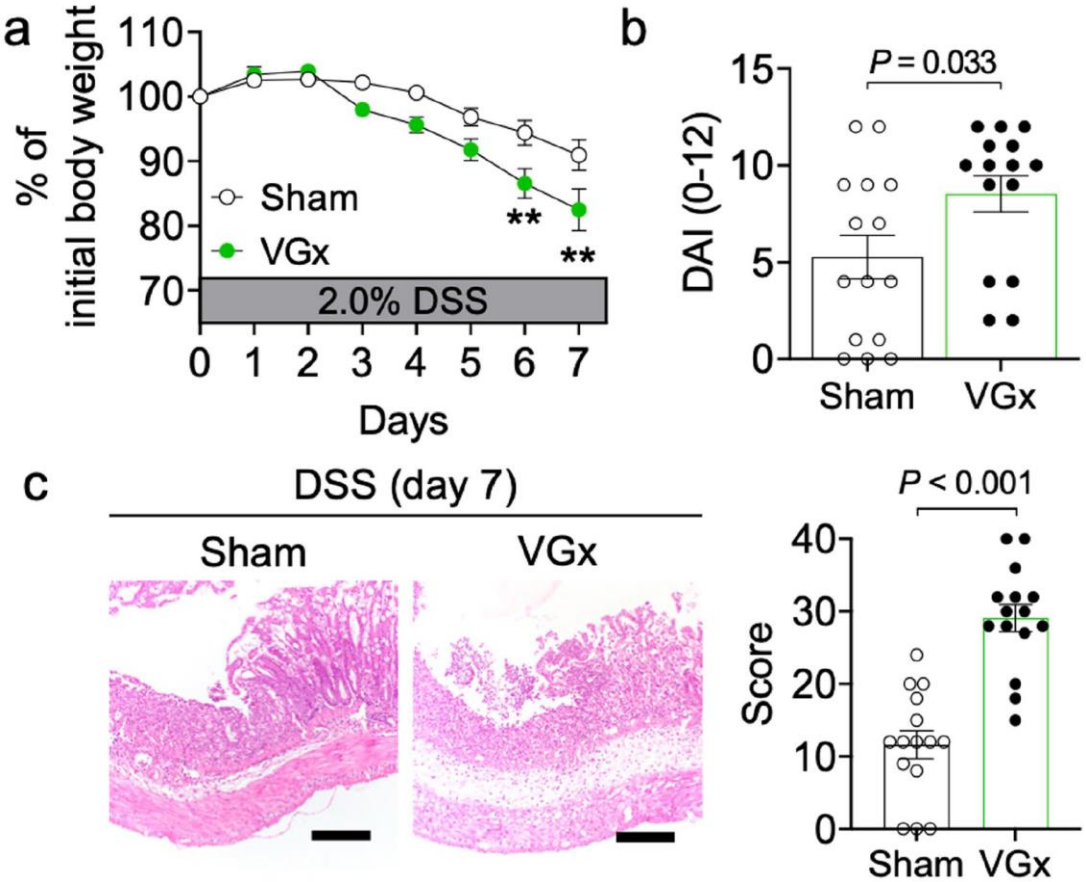


Eight-week-old male wild-type B6 mice were subjected to VGx or sham surgery.
Colonic T cell phenotypes and colonic gene expression were analysed 2 days later (n = 12 per group).
Frequency of ALDH+ cells among MHC-II+APCs (CD45+TCRβ−CD3−B220−NK1.1−MHC-II+) in the colon. Quantification of ALDH+ cells.
Frequency of FOXP3+ (Treg) cells among CD4+ T cells in colonic lamina propria.

→ frequency of $ALDH^+$ and **pTreg** were **decreased** by VGx

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

The effect of VGx (3)



WT mice were subjected to Sham or VGx and then were given DSS for 7 days, starting at day 2 after surgery.
Graphs show pooled data of three independent experiments (n = 15/group).
a, Relative body weight change during colitis. ** indicates P < 0.01. b, DAI.
c, Representative HE staining of colon sections (left panel, bar: 200 μm) and histological scores (right panel).

→ the **susceptibility** to DSS-induced colitis model was **increased** by VGx

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Short Summary

Colonical APCs:

- ***Chrm1*** was highly expressed
- ***Aldh1a1 , 2*** *was* increased by **Ach** and **Mus**
- **pTreg** was **enhanced** by APC with Mus
- mAchR was essential to this enhancement

VGx (perturbation of Vagus nerve):

- *Aldh1a1, 2* level and $ALDH^+$ cells were **decreased**
- **pTreg** were **decreased**
- the **susceptibility** to colitis was **increased**

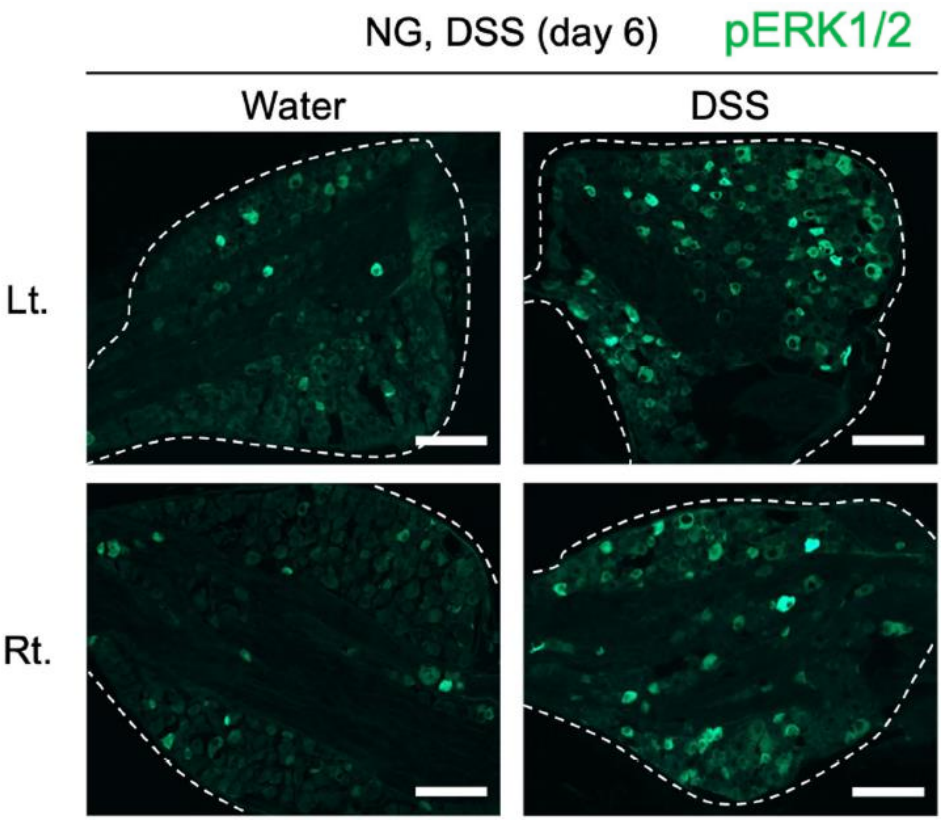
1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

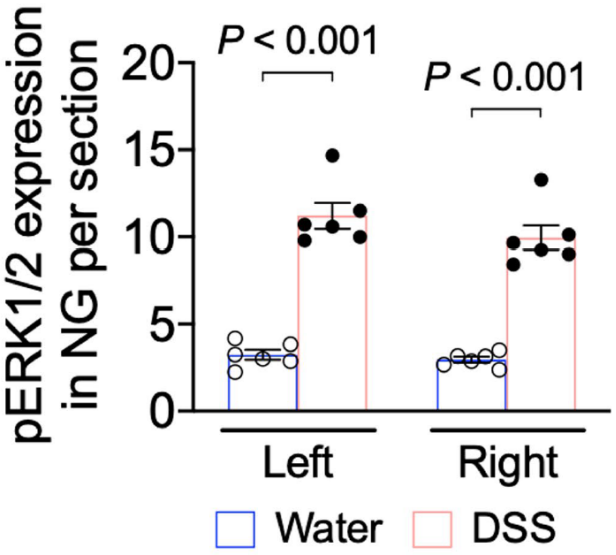
Contents of Main Paper

1. What is the role of colonic APCs in maintaining the pTreg?

2. How the pTreg homeostasis is regulated?

Exploration of the key neurons (1)





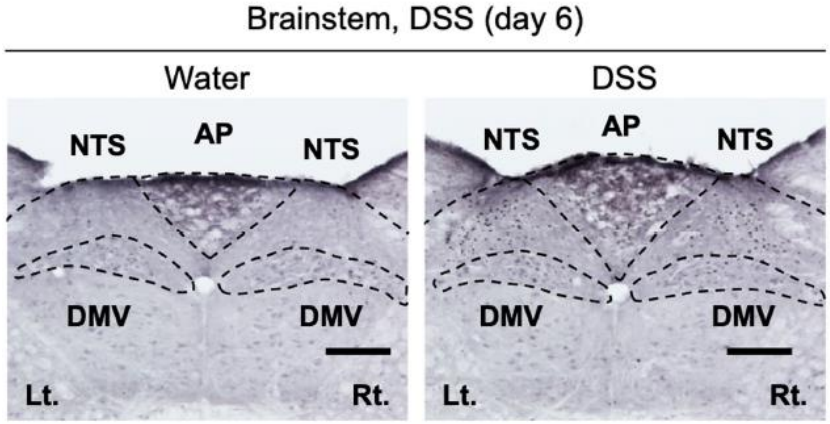
WT mice were given DSS or water for 6 days (n = 6/group).
Representative images of immunofluorescence staining for pERK1/2 (green) in NG (upper panel, bar: 100 μm). Quantification of pERK1/2-experssing neurons (lower panel).

pERK1/2:
Marker of the functional activity

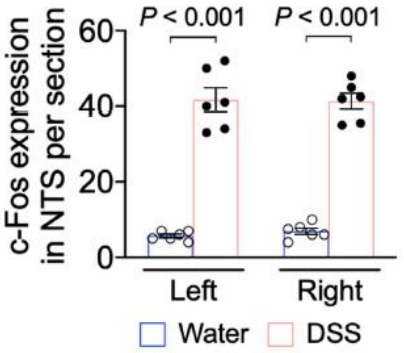
→ Nodose ganglions(**NGs**) were **activated** in colitis

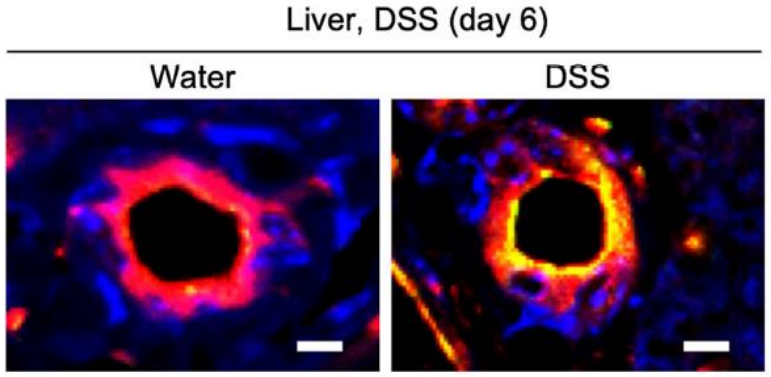
1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Exploration of the key neurons (2)

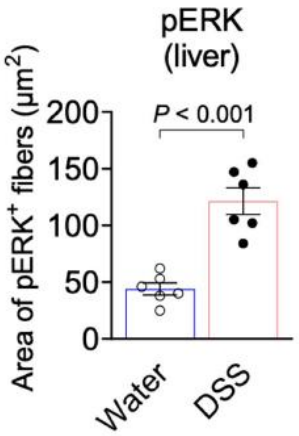


c-Fos, pERK1/2:
Marker of the functional activity





pERK1/2 PGP9.5 = marker of the neurons



WT mice were given DSS or water for 6 days (n = 6/group).
Representative images of c-Fos immunoreactivity in NTS (left panel, bar: 200 μm). Number of c-Fos immunoreactive neurons (right panel).
Representative images of immunofluorescence double-staining for pERK1/2 (green) and PGP9.5 (red) in murine liver sections. Co-stained sites are shown in yellow (left panel). Scale bar indicates 10 μm. Quantification of pERK1/2-experssing area in PGP9.5 positive nerve fibre (right panel).

→ **NTS** and **vagus nerve in the liver** were **activated** in the colitis

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

HVx (Hepatic vagotomy)

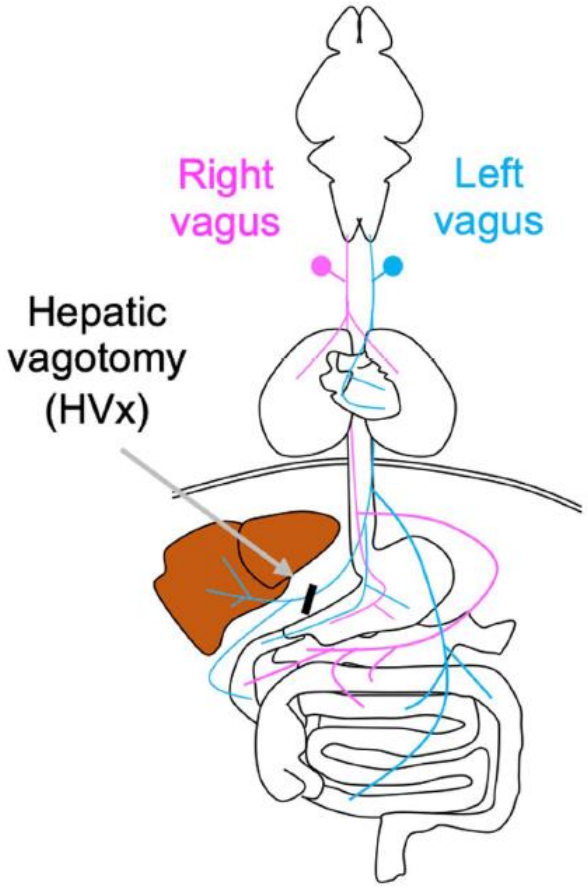
Vagus nerve in the liver was **activated** in colitis

⬇

Surgical perturbation of **the common hepatic branch** of the vagus nerve
= **HVx**

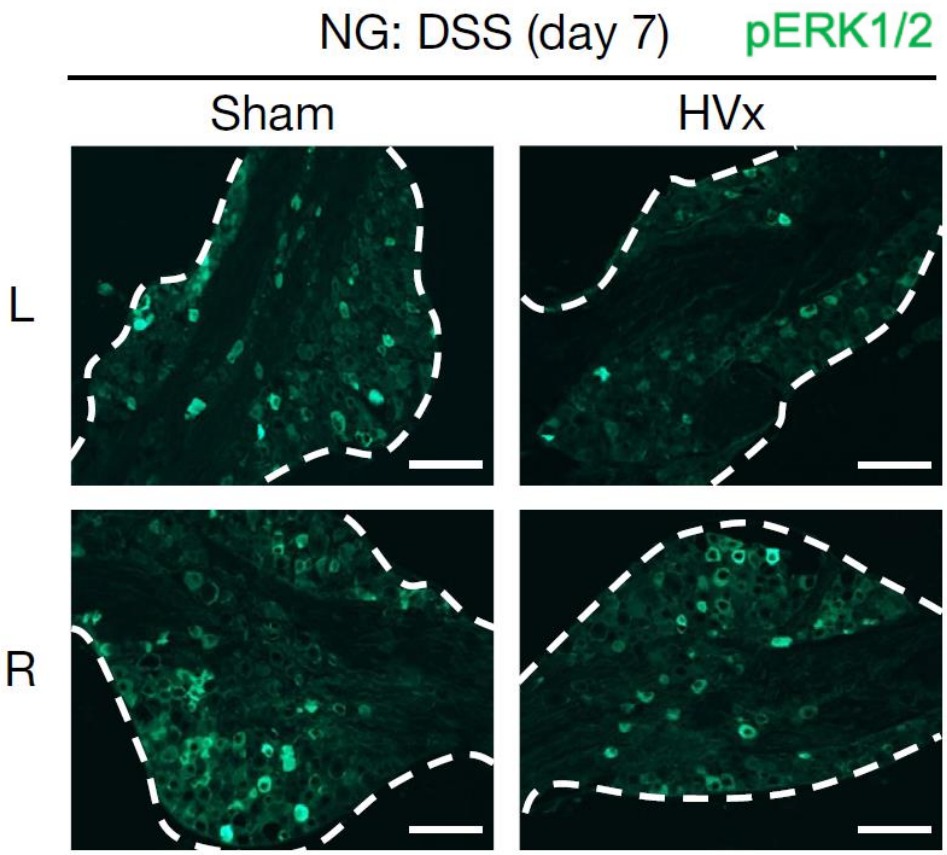
⬇

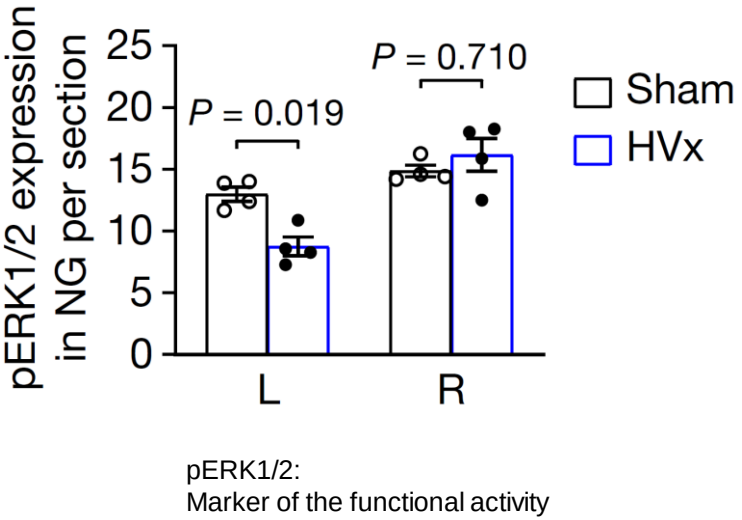
pERK1/2, c-Fos, pTreg ?



1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Effect of the HVx (1)





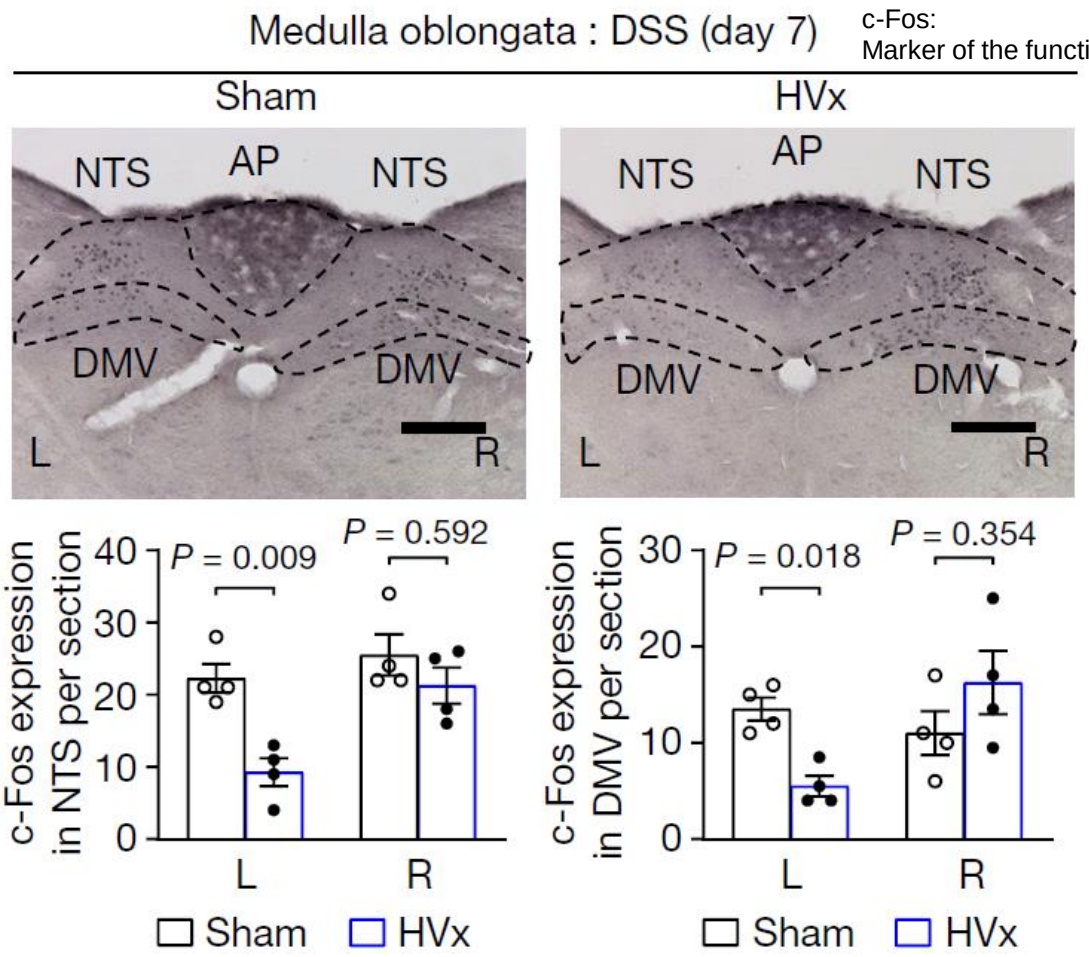
pERK1/2:
Marker of the functional activity

Wild-type mice were subjected to HVx or sham surgery and were then given DSS for 7 days, starting at day 2 after surgery (n = 4 per group). DMV, dorsal motor nucleus of the vagus; AP, area postrema.
Representative immunostaining for phosphorylated ERK1/2 (pERK1/2) in NG. Scale bars, 100 μm. Relative pERK1/2 level in NG per section.

→ The **only Left NG** was **deactivated** by the HVx

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Effect of the HVx (2)

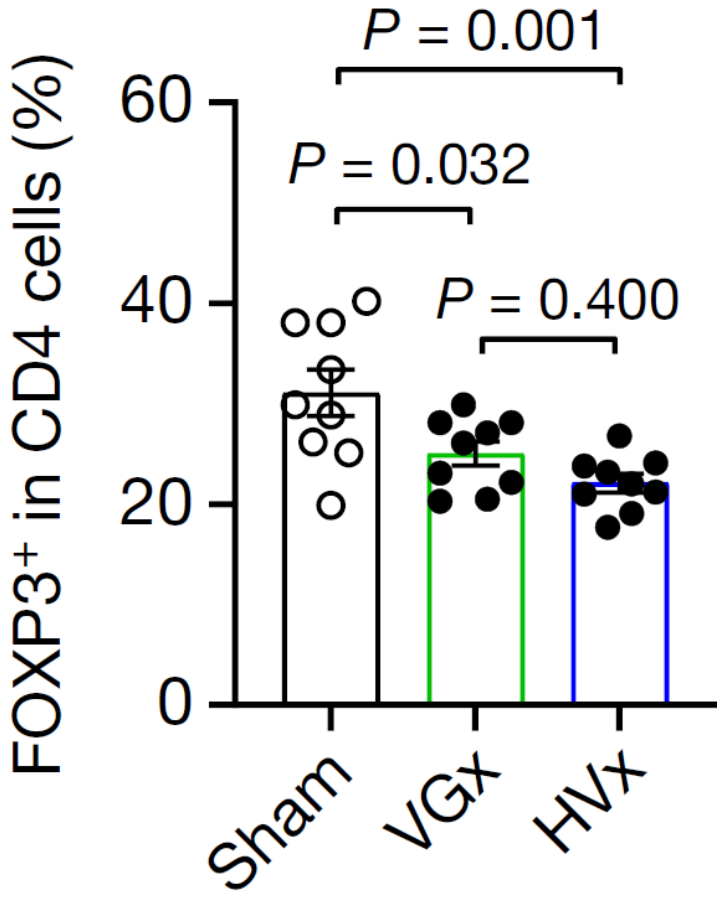


Wild-type mice were subjected to HVx or sham surgery and were then given DSS for 7 days, starting at day 2 after surgery (n = 4 per group). DMV, dorsal motor nucleus of the vagus; AP, area postrema.
Top, representative immunostaining for c-Fos in medulla oblongata. Scale bars, 200 μm. Bottom, relative c-Fos expression in NTS and DMV per section. L, left; R, right.

→ **Left NTS** and **DMV** were **deactivated** by the HVx

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Effect of the HVx (3)

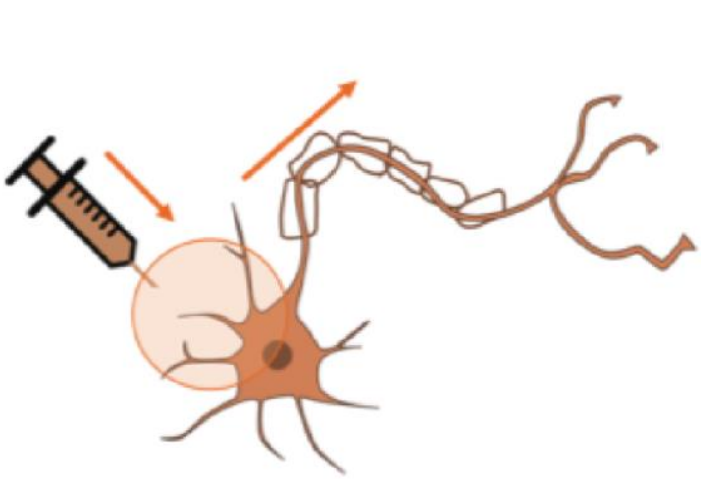


Wild-type mice were subjected to VGx, HVx or sham surgery (n = 9 per group).

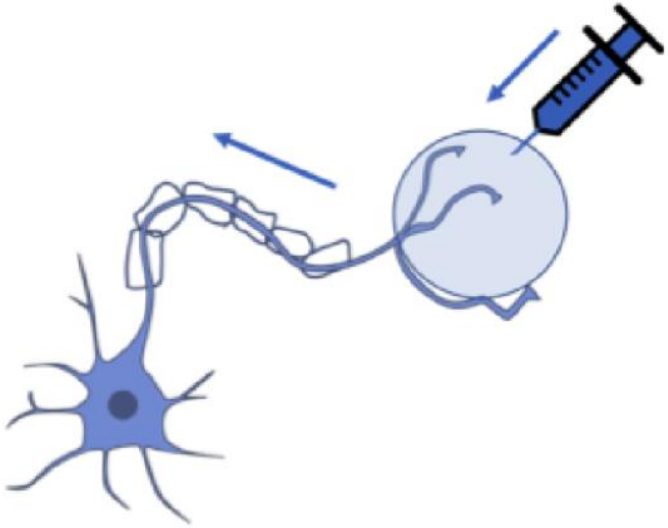
→ frequency of **pTreg** was **decreased** by HVx as same level as VGx

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Retrograde tracing



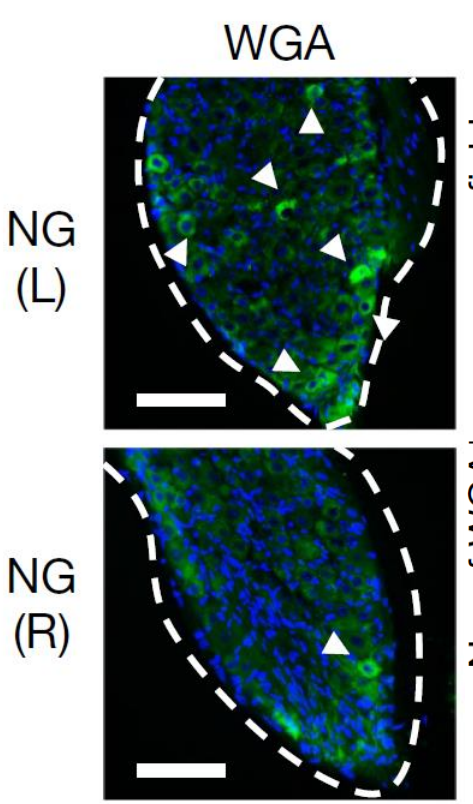
Anterograde

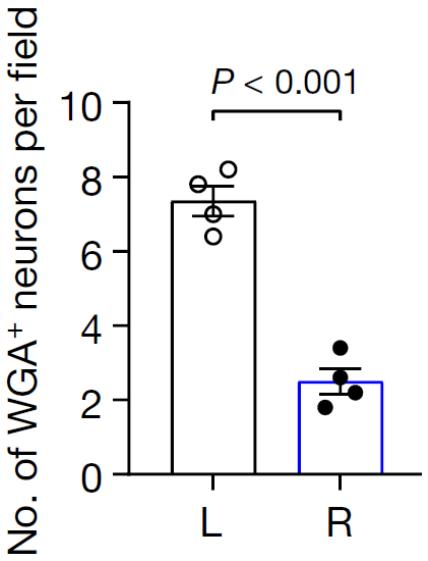


Retrograde

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

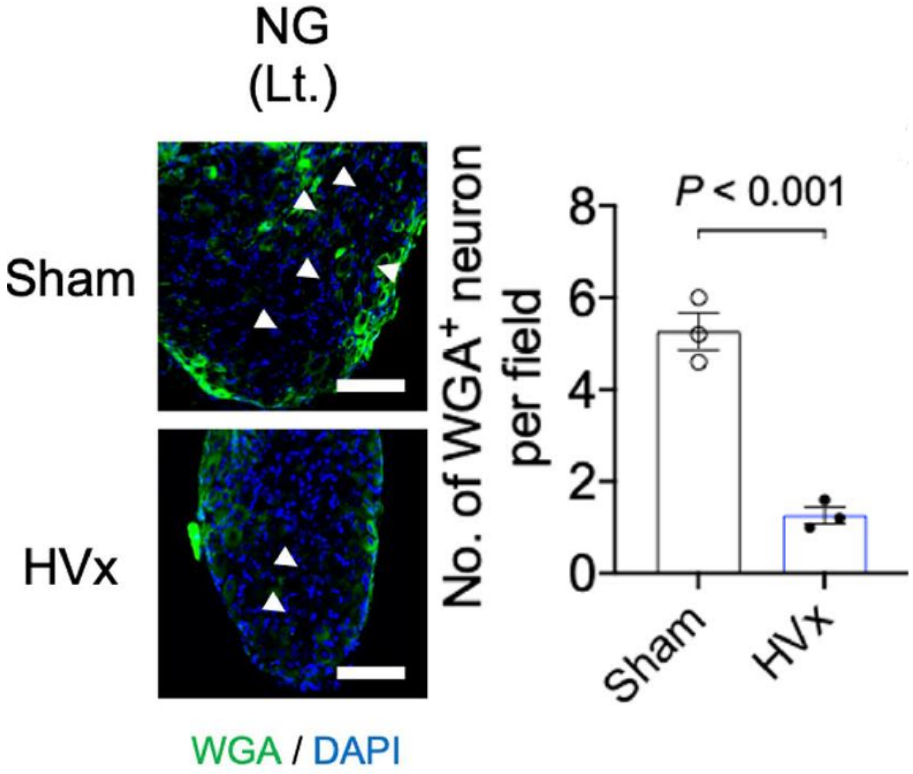
Hepatic retrograde tracing





WGA / DAPI = marker of the DNA
= retrograding marker of the neurons

Wheat germ agglutinnin (WGA) retrograde tracing. Representative fluorescence images of WGA–Alexa Fluor 488 (green) and DAPI (blue) in NG at 1 week after injection of WGA in liver. Arrowheads indicate WGA+ neurons in NG. Scale bars, 100 μm.



WGA retrograde tracing. WT mice were subjected to Sham or HVx and then were injected with Alexa Fluor 488 conjugated WGA at day 2 after surgery (n = 3/group). Fluorescence image of Alexa Fluor 488+ neuron (green) and DAPI (blue) in NG (f) at 1 week after injection of WGA in liver. Representative images (left panel, bar: 50 μm). Number of WGA+ neurons (right panel).

→ the liver transmits signals via the **left NG** and the signals were **reduced** by the **HVx**

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Functional asymmetry

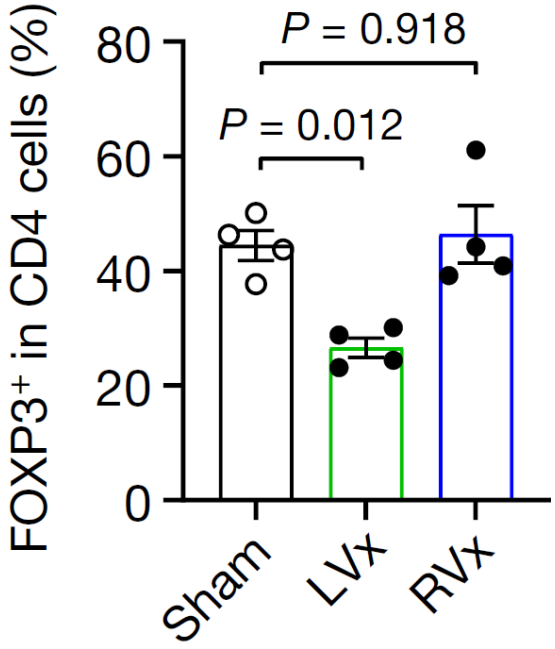
Left NG, NTS and DMV were affected by the HVx

⬇

The **functional asymmetry** ?

⬇

Left or **Right** Surgical perturbation = **LVx**, **RVx**



→ the frequency of the pTreg was **reduced** by the **LVx**, but **not RVx**

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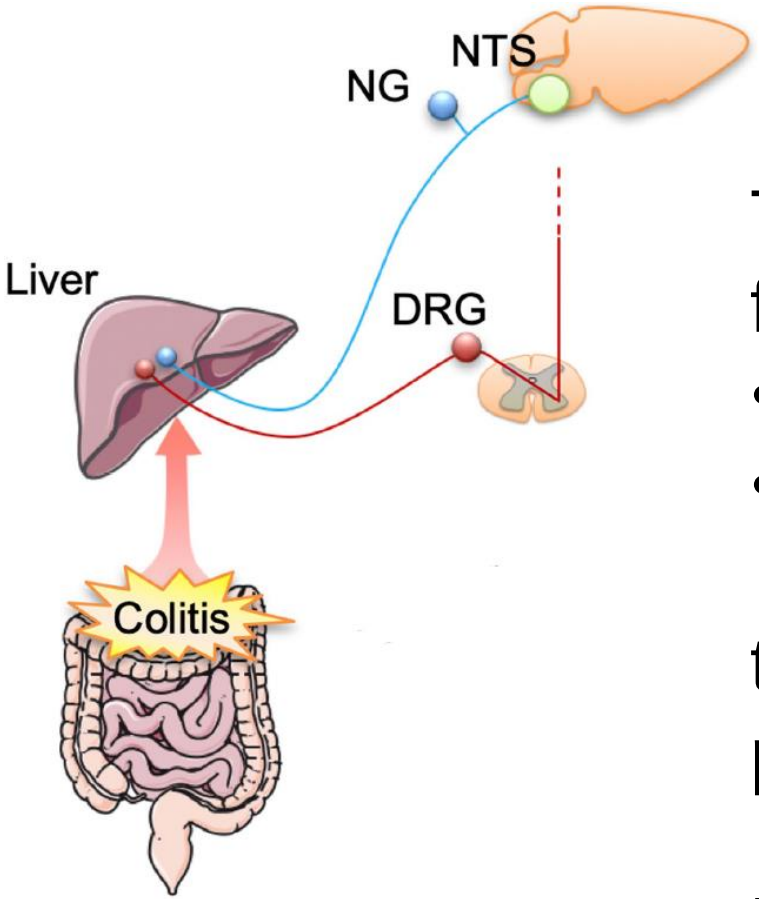
Left vagal sensory afferents from the liver tunes the pTreg

Wild-type mice were subjected to ventral subdiaphragmatic vagotomy (LVx), dorsal subdiaphragmatic vagotomy (RVx) or sham surgery (n = 4 per group). Frequency of FOXP3+ cells among CD4+ cells in colon at day 2 after surgery.

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Another pathway?



NTS: nucleus tractus solitarius
NG: Nodose ganglion
DRG: Dorsal root ganglion

There are **two** kown pathways from liver to brain

- via NG pathway
- via DRG pathway

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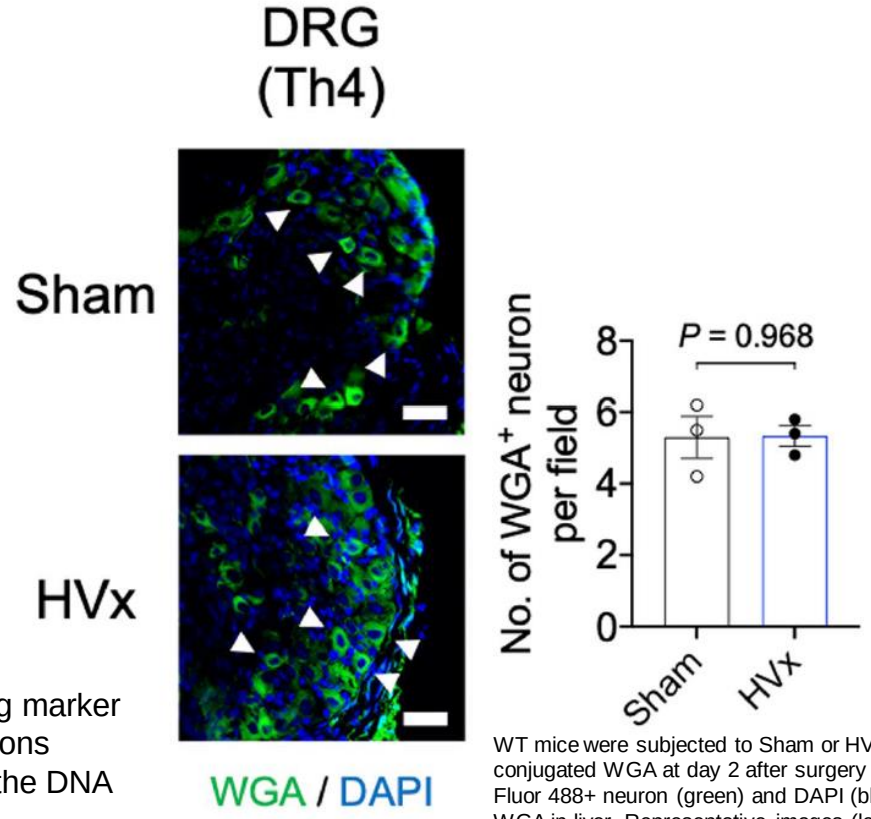
the DRG pathway affect the pTreg as well?

DRG: **TRPV1(+)** neuron,
Generate the **CGRP**

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Retrograde tracing



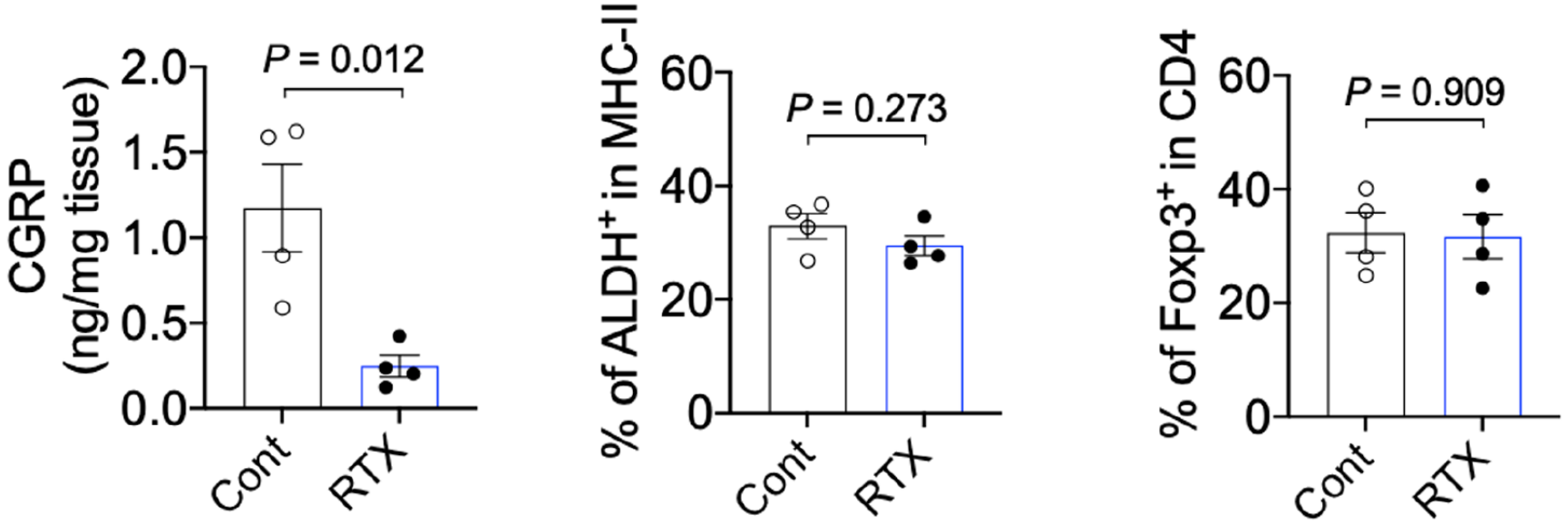
WGA: retrograding marker of the neurons
DAPI : marker of the DNA

WT mice were subjected to Sham or HVx and then were injected with Alexa Fluor 488 conjugated WGA at day 2 after surgery (n = 3/group). Fluorescence image of Alexa Fluor 488+ neuron (green) and DAPI (blue) in Th4 DRG at 1 week after injection of WGA in liver. Representative images (left panel, bar: 50 μm). Number of WGA+ neurons (right panel).

→ the liver transmits signals via the **DRG**
but the signals were **not affected** by the **HVx**
= **DRG pathway** is **independent** for **the common hepatic branch** of the vagus nerve

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Experiments with RTX



Eight-weekold WT type were intrathecally injected with resiniferatoxin (RTX) (n = 4/group). TRPV1+ nerves in spinal cord (Th4-7 and Th13) and colonic immune cells were analysed at day 7 after administration.
Colonic CGRP levels. Frequency of Foxp3+ cells among CD4+ cells. Frequency of ALDH+ cells among MHC-II+ colonic APCs.

→ CGRP was **reduced** by RTX
= **DRG** was **deactivated** by RTX
but, $ALDH^+$ cells and pTreg were **not reduced**

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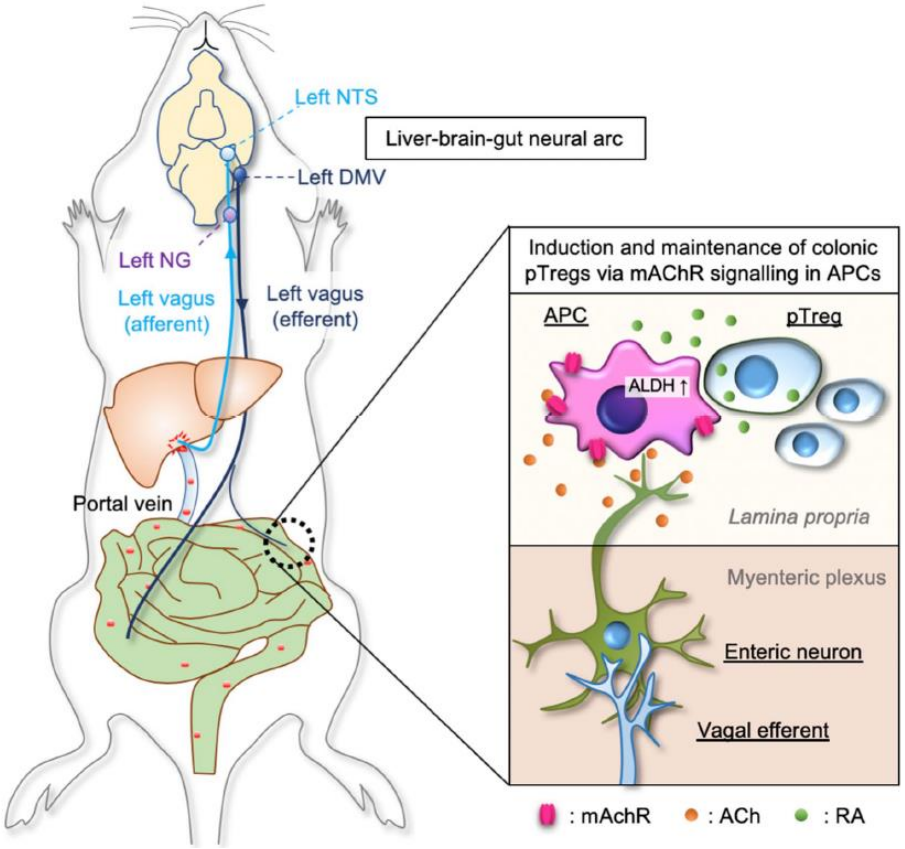
DRG pathway **did not affect** the number of pTreg

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Summary



liver-brain-gut neural arc:
Liver→Left vagus (afferent) →Left NG→Left NTS→Left DMV→Left vagus (efferent) →gut

APC and pTreg:
Ach↑ →mAchR↑(APC) →ALDH↑ →RA↑ →pTreg↑

1) T. Kanai, et al. *Nature*, **2020**, *585*, 591-596.

Future expectation

1. New treatment for IBD (Ulcerative colitis, Crohn's disease)

2. Control the excessive immune response induced by virus. Ex.) COVID-19