

METAGENOMICS

**Micrbiota and host determinants of
behavioral phenotypes in maternally
separated mice**

22/05

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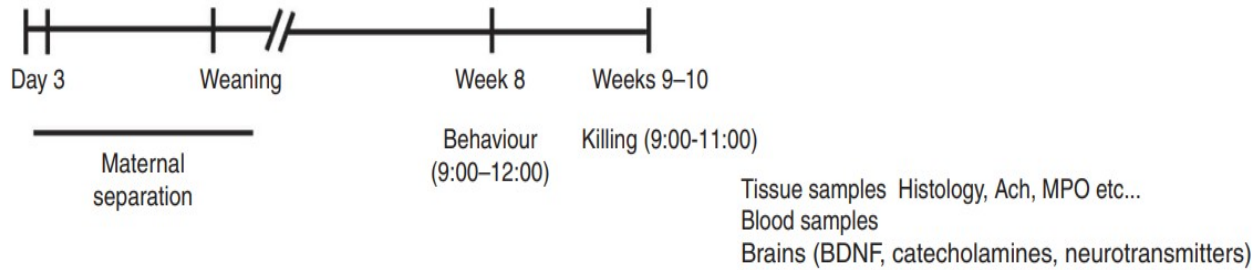
Blue box

- * MS - Maternal separation
- GF – Germ-free, is absence of gut microbiota
- SPF - Specific pathogen-free mice, denote the presence of gut microbiota
- Intestinal microbiota
- Colonization – transfer of microbiota from a SPF to a GF mice
- Intestinal dysbiosis – unbalance in the bacteria lining the GI tract
- Hypothalamic–pituitary–adrenal (HPA) axis
- OTU - Operational taxonomic units, diversity of microbiota

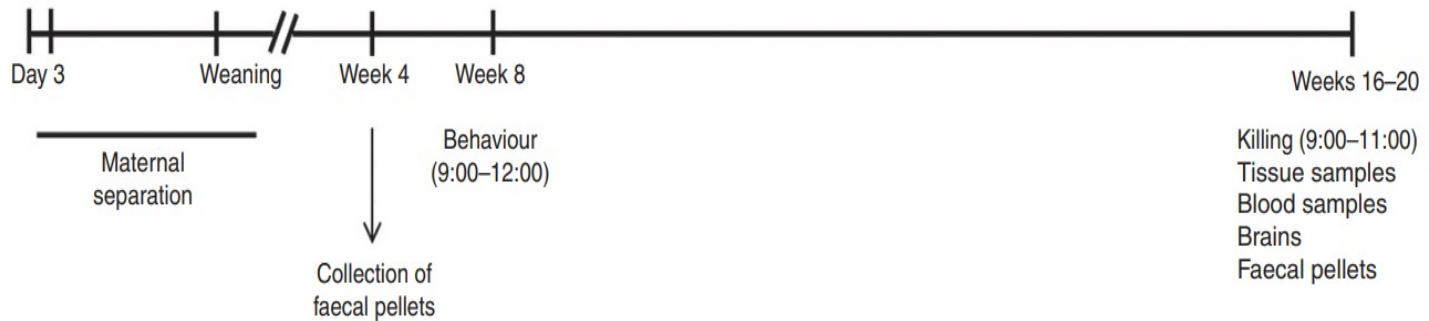
- There is growing evidence that intestinal microbiota can affect host behaviour.
- The absence of bacteria results in an abnormal HPA response to stress that can be reversed by colonization with commensal bacteria.
- Maternal separation (MS) in rodents is a well-established model of early-life stress that induces **long-lasting alterations** in behaviour and gut dysfunction.
- Therefore, the aim of this study was to investigate the relative contributions of gut commensal bacteria as well as **host factors** in the expression of **altered behaviour** in the MS model.

Design (a)

Germ-free C57BL/6 mice



SPF C57BL/6 mice

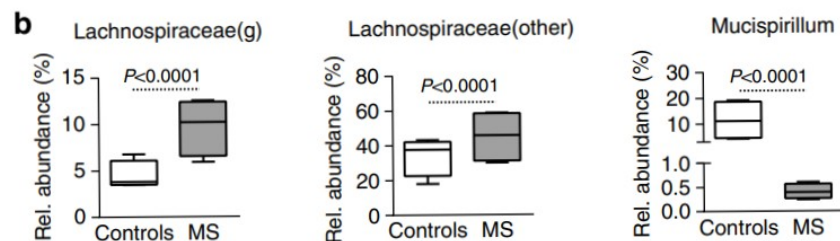
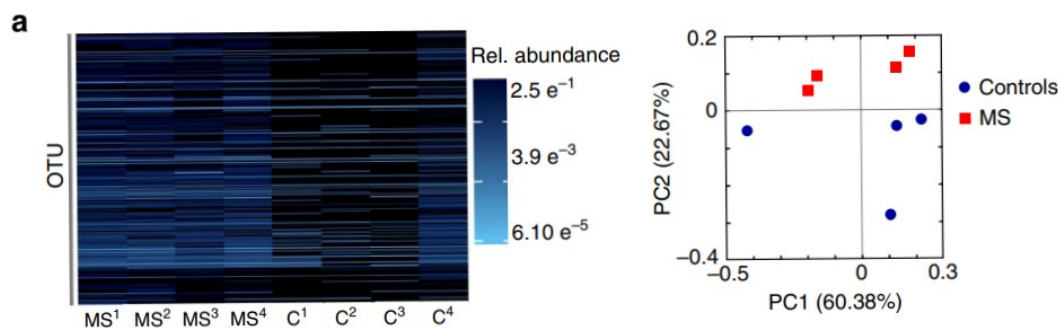


Results (3)

A Two-way analysis of variance

- One factor being the presence/absence of gut microbiota (SPF versus GF)
- The second factor being the treatment i.e MS versus control.

MS altered the colonic microbiota composition of SPF mice



- 16S ribosomal DNA-based method, to screen colonic microbiota composition profiles.

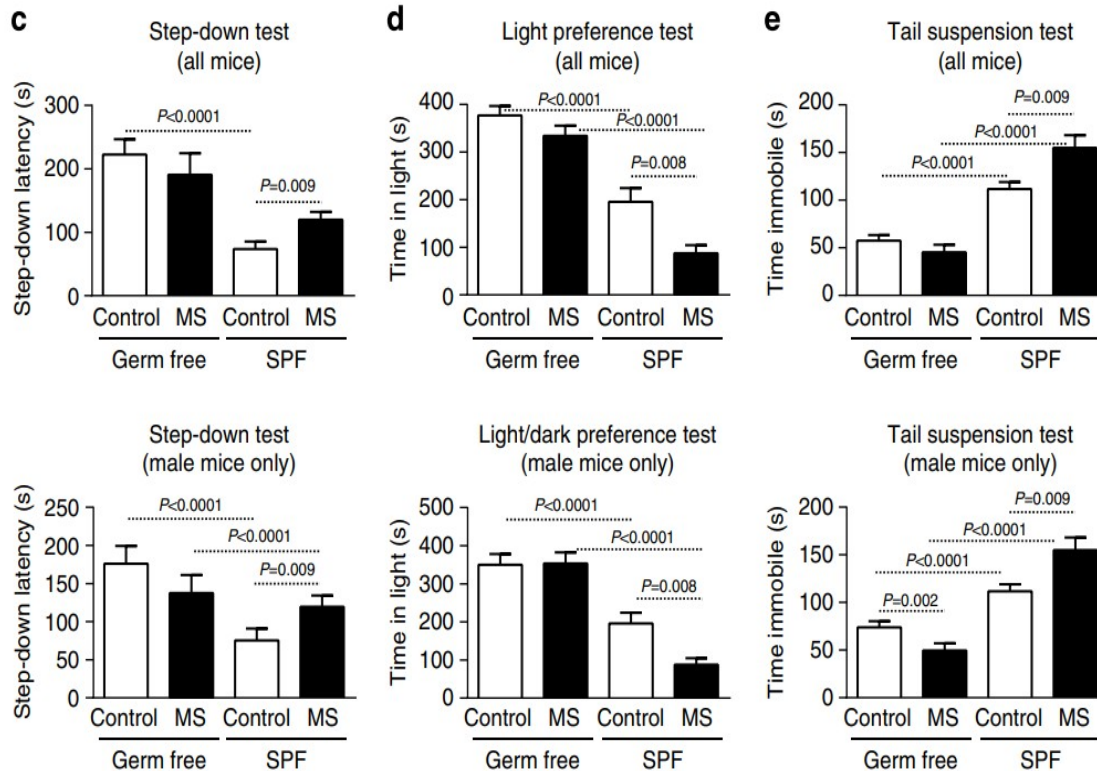
- MS was associated with an altered microbiota profile in 4-week-old MS mice.

- MS mice presented with higher abundance of unclassified Lachnospiraceae and lower abundance of the genus Mucispirillum.

Remarks: These results indicate that MS induces early-life dysbiosis, which persists into adulthood.

MS induced anxiety-like behaviour in SPF but not in GF mice

*** Mouse behaviour was assessed using step-down, light preference and tail suspension tests.



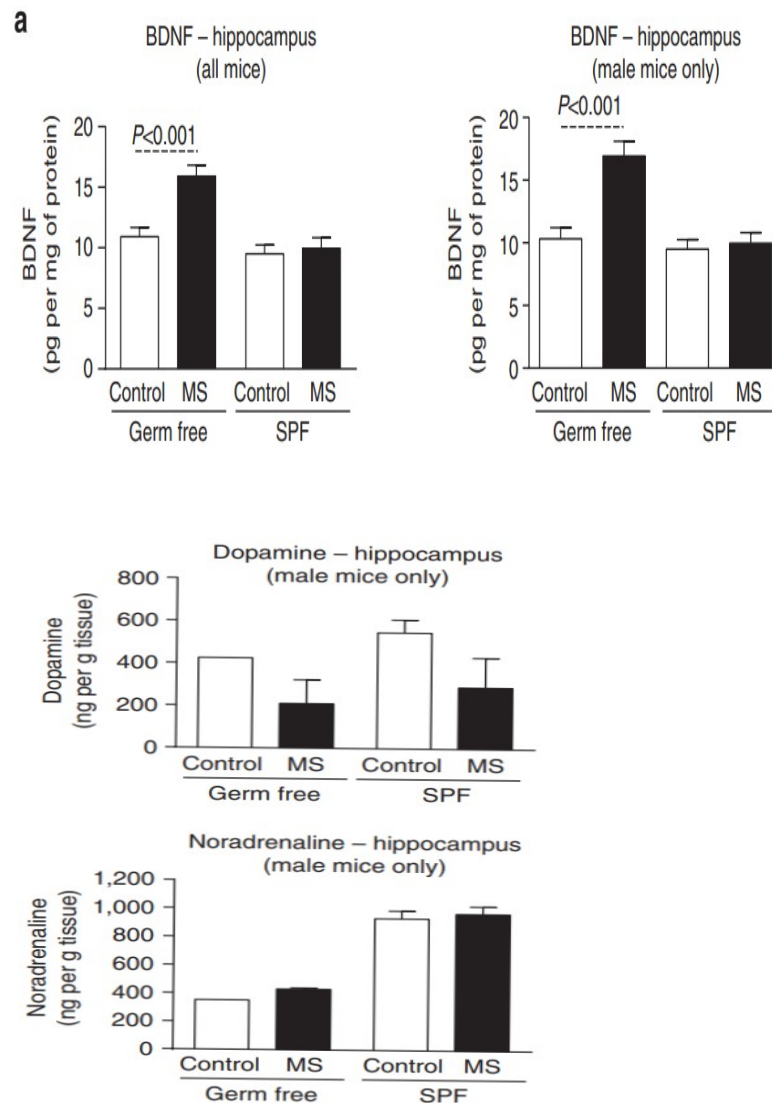
C. When analysing GF and SPF mice separately,
- MS induced anxiety-like behaviour in SPF mice as MS mice stepped down from the elevated platform with latency delayed by 70% compared with controls.

D. SPF MS mice spent 55% less time in the illuminated compartment and displayed longer latency to re-enter the illuminated compartment compared with controls.

E. Finally, in the tail suspension test, SPF MS mice were immobile for a longer time compared with control mice

Brain BDNF and catecholamine levels

- When analysing the two groups separately, SPF MS and control mice had similar levels of BDNF, serotonin, dopamine and noradrenaline.
- However, GF MS mice exhibited higher hippocampal BDNF levels than GF controls.



Germ-free C57BL/6 mice - colonization

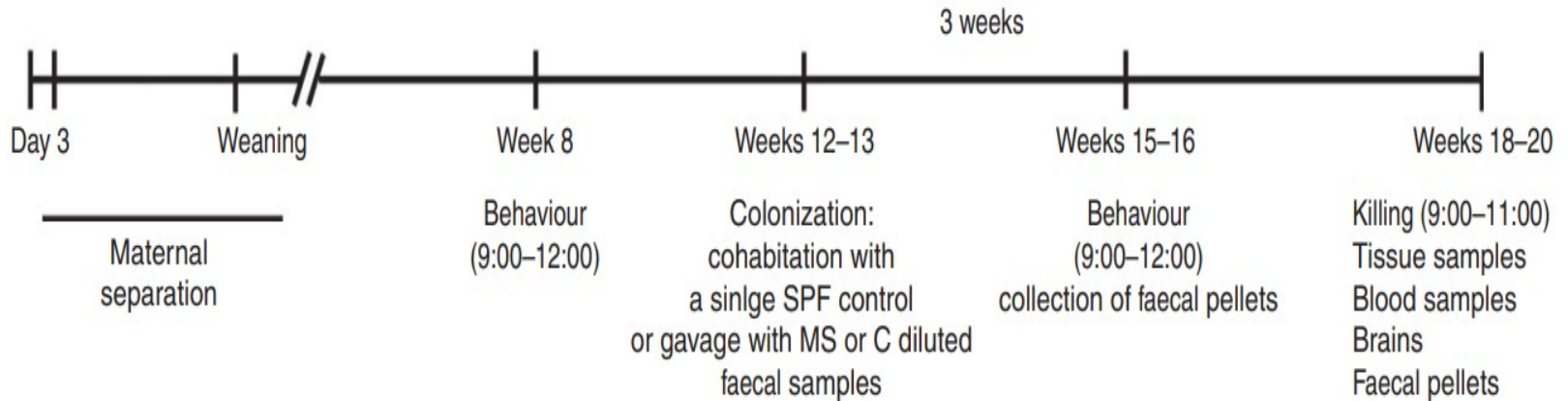


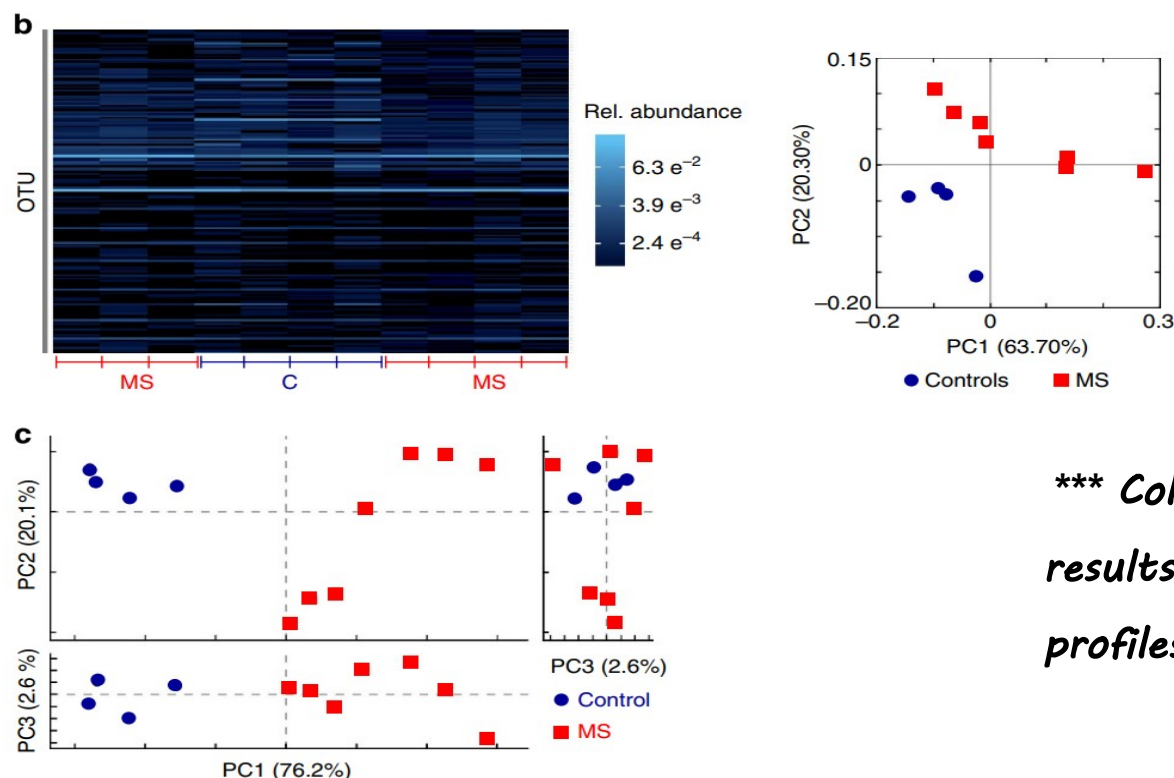
Figure 7 | Study design timeline. Schematic timeline representation of the GF, SPF and colonization experiments performed in the current study.

Remarks

Open field test was conducted to evaluate GF mice behavior, number of faecal pellets excreted was greater, indicative of an enhanced stress-induced colonic motility.

...colonization of adult GF MS and control mice

- The impact of microbial colonization of GF MS and control mice using microbiota from an SPF mouse that had not been subjected to MS (SPF control mouse) was examined.
- Colonisation of both control and MS mice was performed by short-term cohabitation with a single SPF control mouse (two SPF control mice were used for two rounds of colonization experiments).



******* *Colonization of adult GF mice results in different colonic microbial profiles in MS and control mice.*

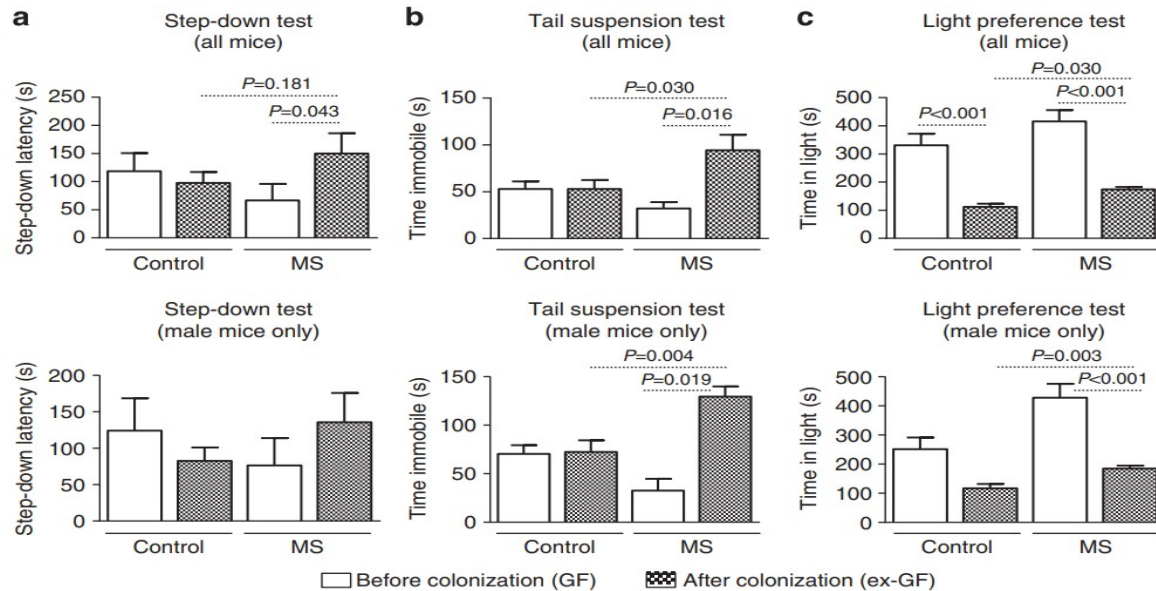
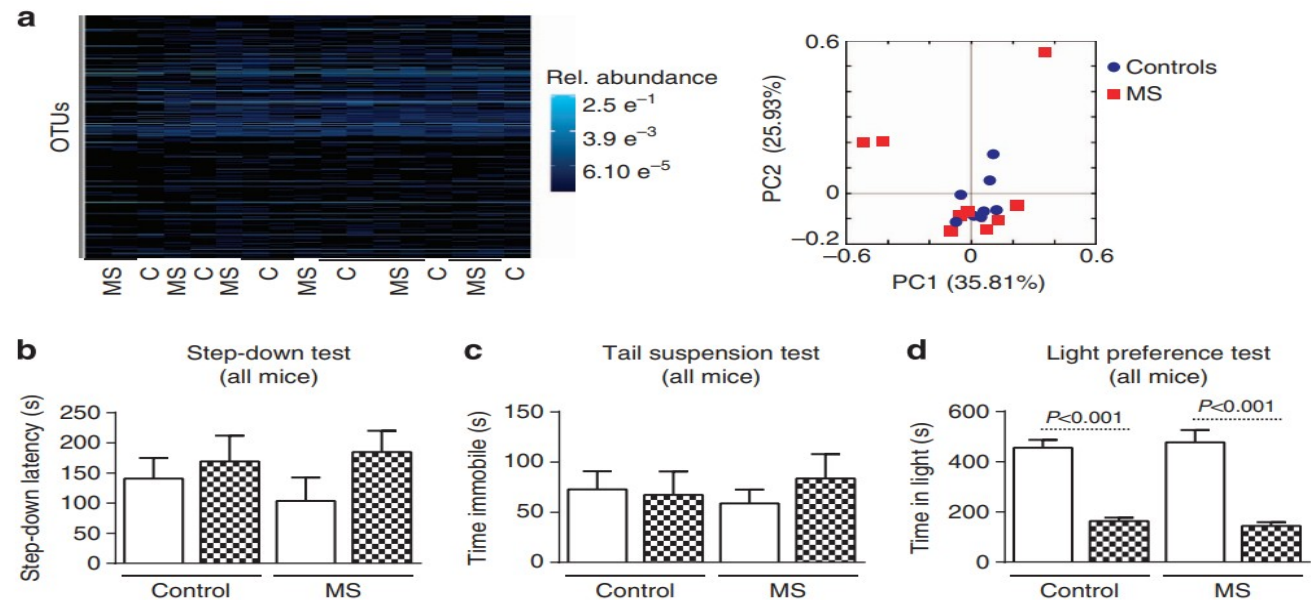


Figure 5 | Colonization of adult GF mice induces anxiety-like behaviour and behavioural despair in MS mice but not in controls.

- **Behavioural despair** in MS mice was assessed by step-down and tail suspension tests at 3 weeks post colonization.
- MS mice displayed **longer latency** to step down from the elevated platform compared with before colonization, whereas control mice did not alter their behaviour
- Similarly, colonized MS mice, but not controls, spent **more time immobile** during the tail suspension test compared with before colonization

- To determine whether the altered microbiota associated with MS mice is sufficient to induce the MS behavioural phenotype, we **colonized** adult control GF mice with microbiota from SPF MS or control mice.
- The healthy mice that were gavaged with the gut microbiota of the **dysbiotic mice** were not able to **maintain that microbiota profile**, which appears to shift towards normal in the healthy recipient mice.



- These results indicate that **host factors present in MS mice**, but absent in control mice, are required to **select and maintain** the microbiota associated with MS.

- For the first time, the critical contribution of the intestinal microbiota in the expression of the MS behavioural phenotype is demonstrated.
- The importance of host factors, acting in **conjunction** with the microbiota, is further illustrated by our demonstration that the MS behavioural phenotype cannot be induced by simply colonizing control GF mice with the microbiota from MS mice.
- Thus, it is evident that the behavioural phenotype of this model of early-life stress reflects a **convergence** of microbial and host factors.
- While it is well established that MS produces long-lasting abnormalities in emotion-related behaviour in rodents with conventional microbiota, the abnormal behavioural profile depends on the species, strain, gender and experimental condition used.

- ❑ An elevated serum corticosterone levels in MS GF mice was observed, confirming that MS also induces a long-lasting vulnerability to stress under GF conditions, meaning that a microbial stimulus is not needed to alter the HPA axis response.
- ❑ Gut microbiota influences brain's monoamine metabolism, GF mice have lower dopamine and noradrenaline turnover rates compared to ex-GF mice.
- ❑ Interestingly, GF MS mice also displayed increased faecal pellet output during the open-field test.
- ❑ MS leads to increased activity of cholinergic nerves in the colon independently of the presence of bacteria.
- ❑ We speculate that altered enteric cholinergic function may contribute to the abnormal gut motility.
- ❑ It is also plausible that **increased** cholinergic activity of enteric nerves alters colonic motility and secretion, changing the physico-chemical environment within the colon, which results in the selection of a modified microbiota.

- When GF MS and control mice were colonized with the same control SPF microbiota, MS mice selected a different microbiota profile compared with control mice and exhibited an altered behavioural profile.
- The precise mechanisms, by which gut bacteria affect behaviour, are unclear, many hypothesis suggest that, bacteria can produce or alter the metabolism of neurotransmitters.
- The critical host factors are those that shape the habitat and therefore the composition of the intestinal microbiota.

GRAZIE

Team:

1. Rustemil **Konul**
2. Tamegye **Kouchou** Boris

Ready to take the quiz ??? (6)

- **Instruction**
- For Q1 - Q2, answer **True** or **False**, 3 – 5, select the right answer.

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