MELISSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

CHICORY ROOTS AS ANTIDOTE TO SPACEFLIGHT-INDUCED CHRONIC STRESS

A TRANSLATIONAL STUDY IN THE FRAMEWORK OF THE REBUS PROJECT

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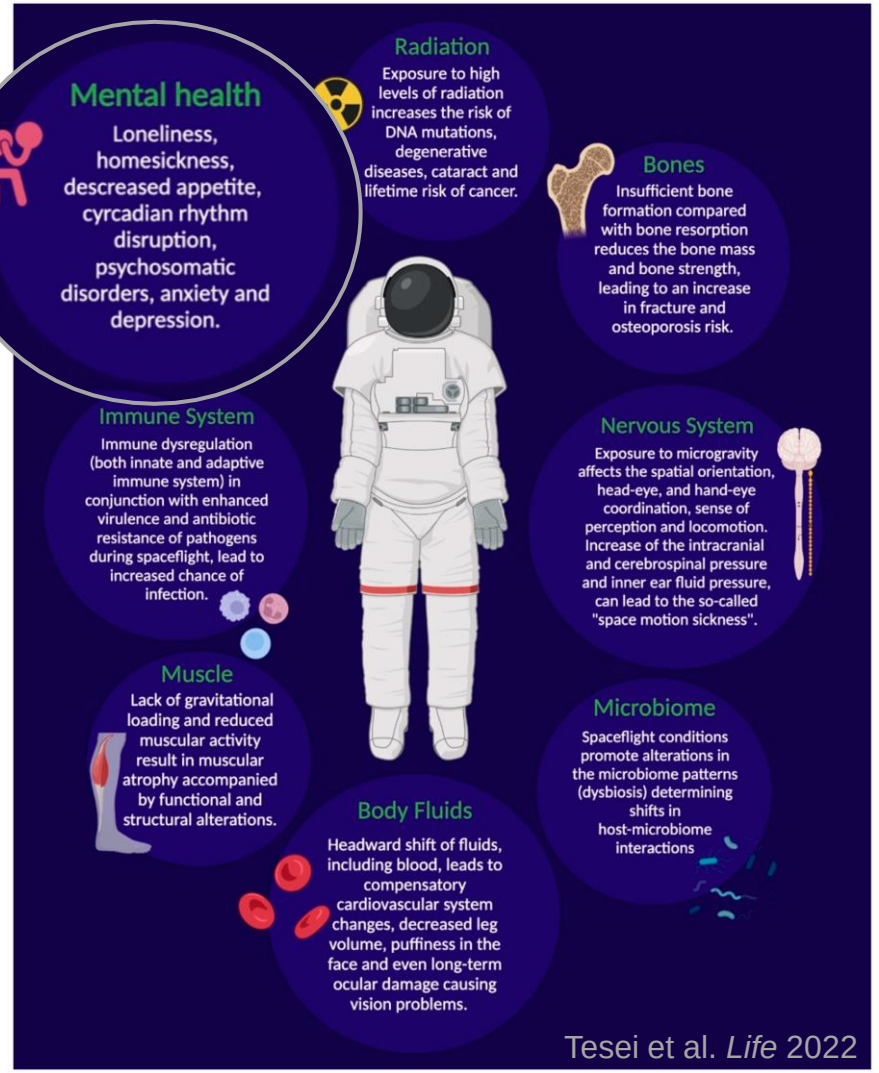
Rationale

→ During future long-duration space missions, astronauts will experience **prolonged confinement and isolation** and will be exposed to **environmental and psychosocial stressors** that may impair:

- physiological stress reactions
- psychological functioning
- neuropsychological capabilities

→ To favour the success of these missions, it is critical to minimise the potential consequences on the well-being of crewmembers

→ **the use of prebiotics may be a promising approach**



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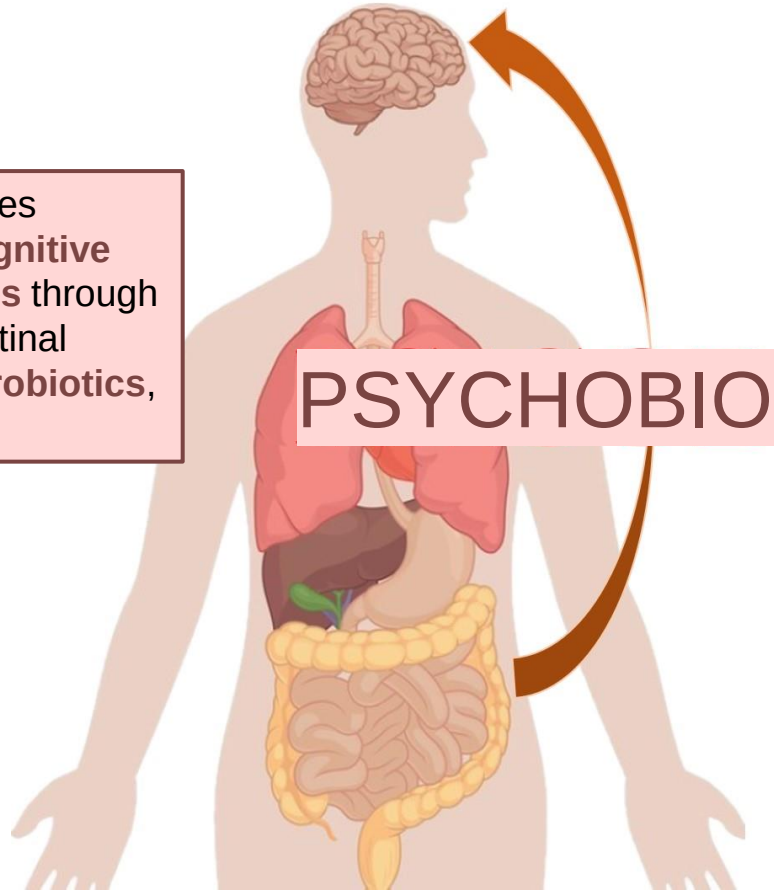
Exposure to space-related stressors causes alterations in various systems, including behavioural and microbiome changes

Prebiotics

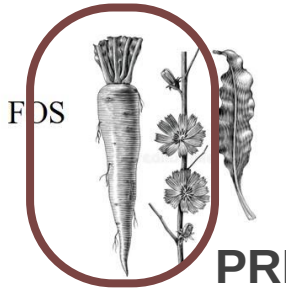
- → Prebiotics, such as **fructans**, are **selectively fermented by the intestinal bacterial flora** whereby they positively orient the composition of the gut microbiota
- → Their **degradation products** (**short-chain fatty acids**, SCFAs) are released into blood circulation, thus affecting not only the gastrointestinal tracts but also distant organs, including the **brain**

Fructans: prebiotics classified based on the degree of polymerization: short chain → fructo-oligosaccharides (**FOS**), long chain → **inulin**

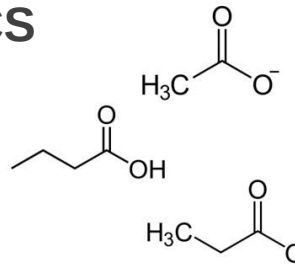
Psychobiotics: substances capable of modulating **cognitive and emotional responses** through a direct effect on the intestinal microbiota (**prebiotics**, **probiotics**, **postbiotics**)



PSYCHOBIOTICS

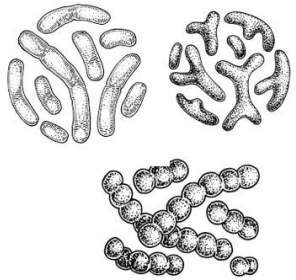


PREBIOTICS



POSTBIOTICS

PROBIOTICS



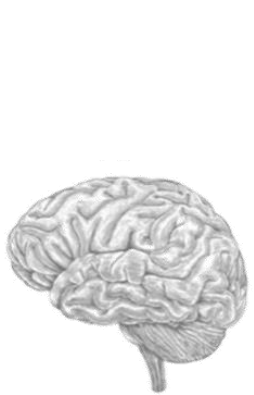
Short chain fatty acids (SCFA): postbiotics produced by fermentation of prebiotics by intestinal microorganisms

Aim

→ Verify whether the consumption of prebiotic-rich vegetables, cultivable within the **bioregenerative life support systems**, is able to **counteract the psychophysiological alterations induced by chronic stress**

→ To this aim, we tested whether, in a **mouse model of chronic stress**, the daily consumption of **chicory roots** prompts the recovery of stress-related behavioural and physiological alterations

- Behavioural tests
- Analyses of physiological parameters (e.g. stress hormones, caecal microbiota composition)



Behavioural tests and physiological parameters

Test battery «A»

→ Cognitive performance
- Fixed ratio test (FR; associative learning)

→ Motivation
- Progressive ratio test (PR)

→ Executive functions
- Attentional set shifting task (ASST; attentional capabilities and cognitive flexibility)

→ Biological samples
- Blood
- Caecal content
- Faeces
- Cerebral tissues

Test battery «B»

→ Cognitive performance
- Barnes maze test (spatial memory)
- Novel object recognition test (NOR; recognition memory)
- Sociability and social novelty test (SSN; social memory)

→ Sociality
- Sociability and social novelty test (SSN)

→ Anxiety
- Elevated zero maze test (ZM)
- Open field test (OF)

→ Stress reactivity
- Response to restraint stress

→ Body measurements
- Body weight, food and liquid intake

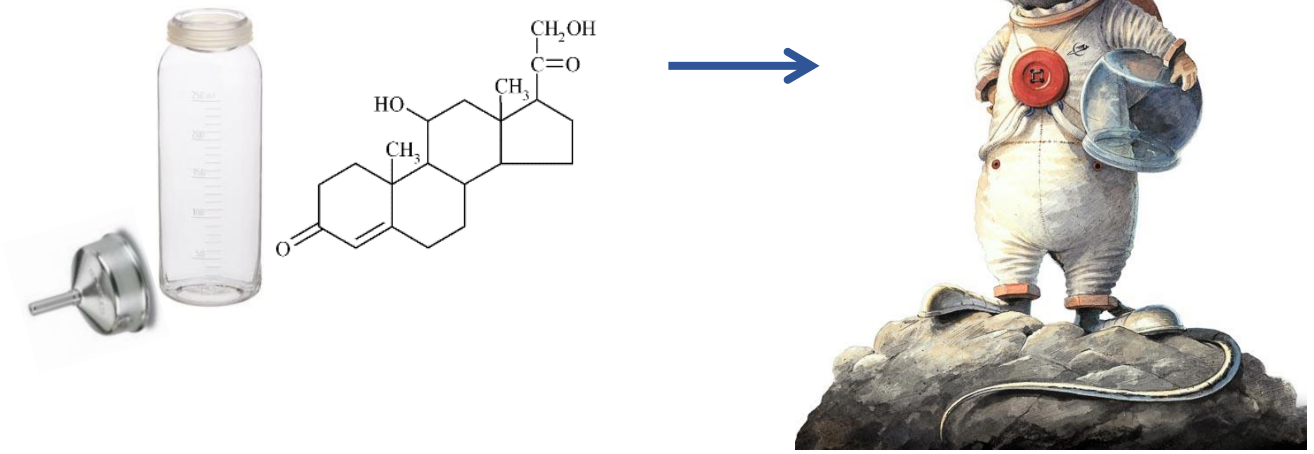
Stress exposure

Corticosterone

- Chronic treatment (8-11 weeks) with a **low dose of the stress hormone corticosterone** (35 µg/ml) through drinking water to induce a moderate and persistent elevation of glucocorticoid levels

  → **mimics the effects of chronic stress** to which astronauts will be subjected during **long-duration space missions**

  → **dosage:** 8.60 mg/kg/die



Prebiotic diet

Chicory roots (*Cichorium intybus* cv Chiavari)

- Chronic administration (9-12 weeks) of prebiotics (fructans) through a **diet containing 25% of chicory roots**
- Cultivated and analysed by the team of Prof. Battistelli (IRET-CNR) → fructans content 46.1 ± 0.6% of the dry weight

  → **dosage:** on average 0.42 g/die of fructans (range 13.5-16.0 g/kg/day)

CNR IRET
Istituto di Ricerca sugli Ecosistemi Terrestri

ISTITVTO SVPERIORE DI SANITÀ

Experimental design

Experimental groups

standard diet (CTRL)

control condition (H_2O)

H_2O - CTRL

stress condition (CORT)

CORT - CTRL

diet containing chicory roots (CIC)

control condition (H_2O)

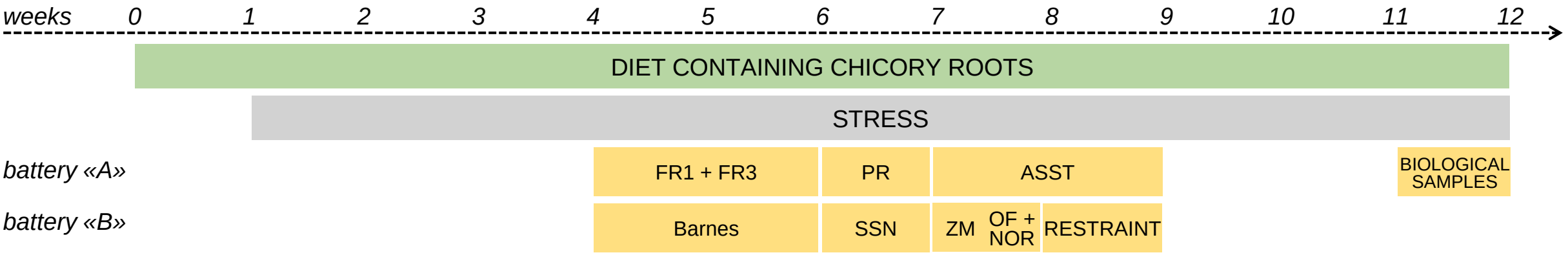
H_2O - CIC

stress condition (CORT)

CORT - CIC

= chicory roots

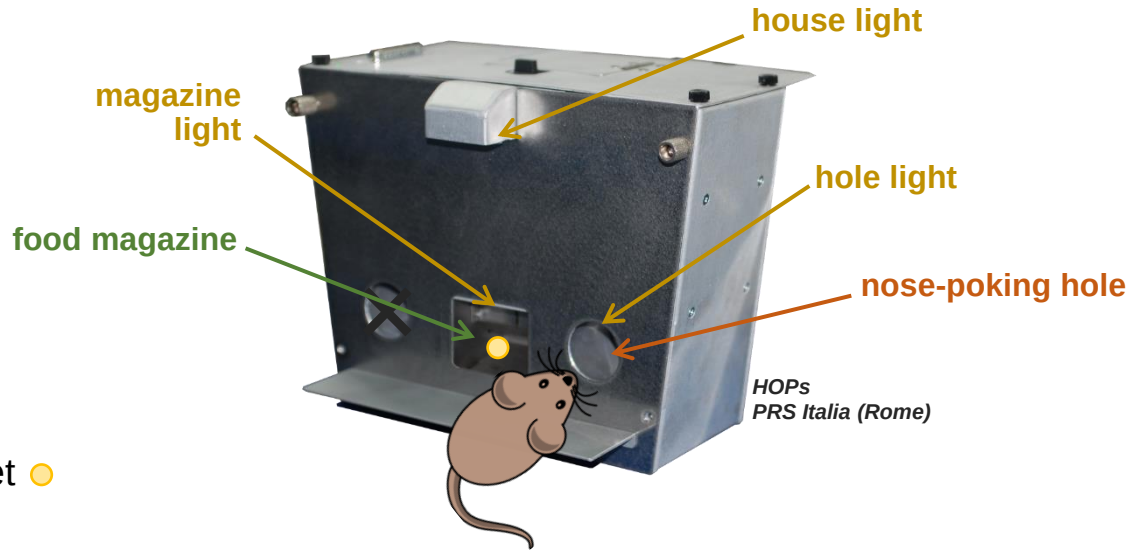
Timeline



Fixed and progressive ratio tests

- → **Fixed ratio (FR)**: subjects learn to perform 1 nosepoke to obtain 1 food reward
- → Used to measure the **learning of a task**
  - **FR1** (max 8 days, 30 min/day); **FR3** (max 2 days, 30 min/day)

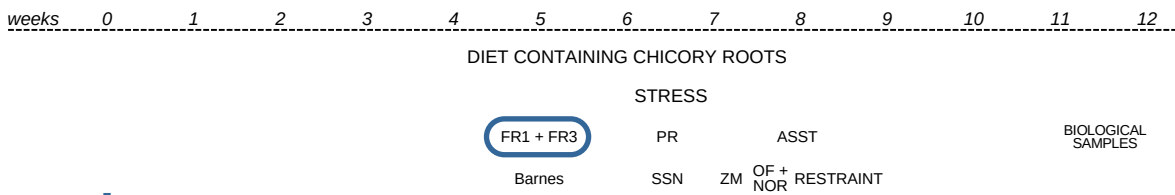
- → **Progressive ratio (PR)**: the number of nosepokes needed to obtain each subsequent reward is progressively increased
- → Used to measure the **effort a subject is willing to make to obtain 1 food reward**
  - **PR** (max 9 days, max 8 min/pellet)
  - **breaking point:** final ratio achieved → **index of motivation**



reward = 1 precision pellet
(20 mg, Bio-Serv®)

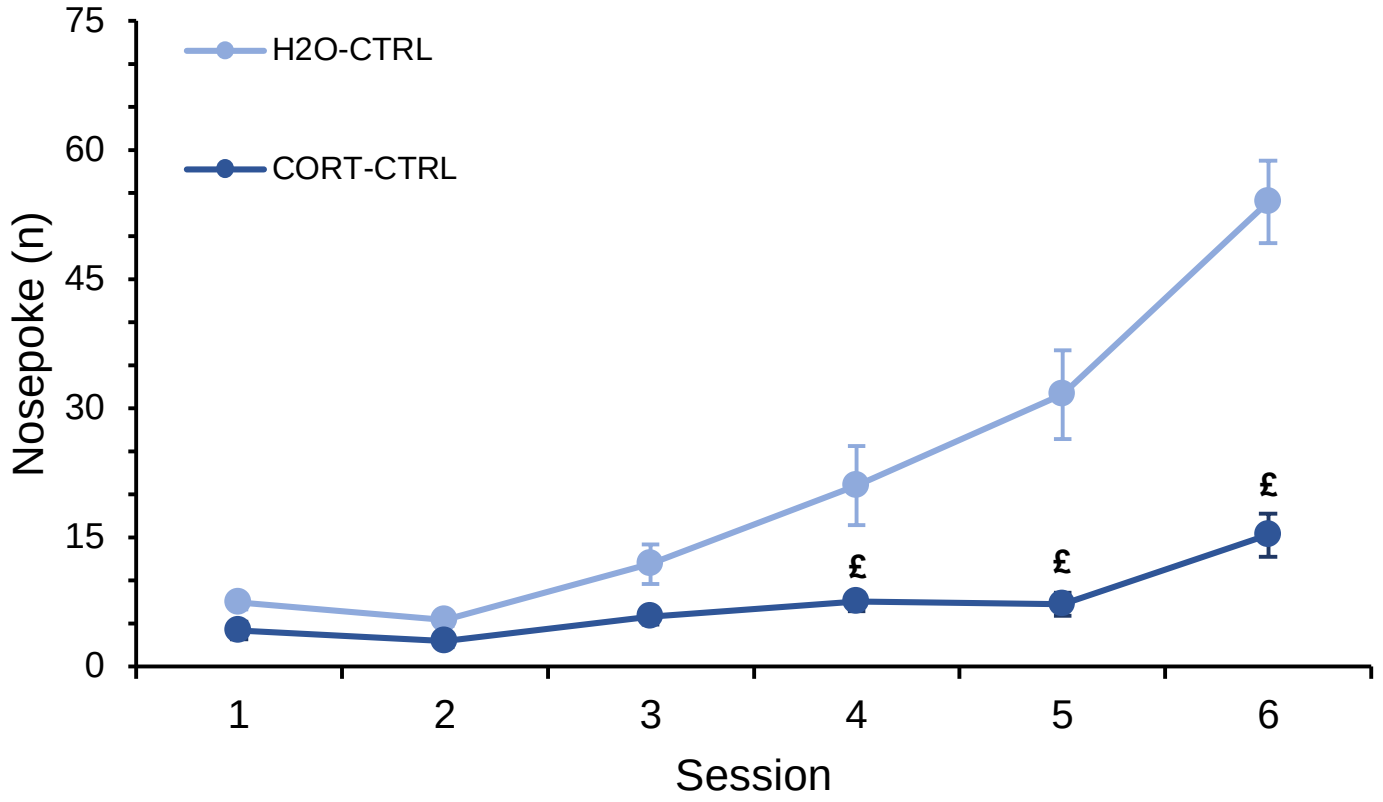
pellets	nosepokes per pellet (breaking point)	total nosepokes
1st	**3**	3
2nd	**3**	6
3rd	**6**	12
4th	**6**	18
5th	**10**	28
6th	**15**	43
7th	**21**	64
8th	**28**	92
9th	**36**	128
10th	**45**	173

Fixed ratio test (FR)



Associative learning

learning to perform 1 nosepoke to obtain 1 food reward



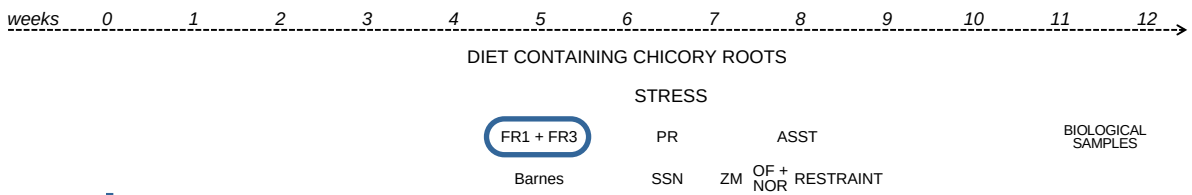
£ H_2O-CTRL vs. **CORT-CTRL**

Days to criterion:

H_2O-CTRL: 4.43 ± 0.29

£ **CORT-CTRL:** 6.82 ± 0.23

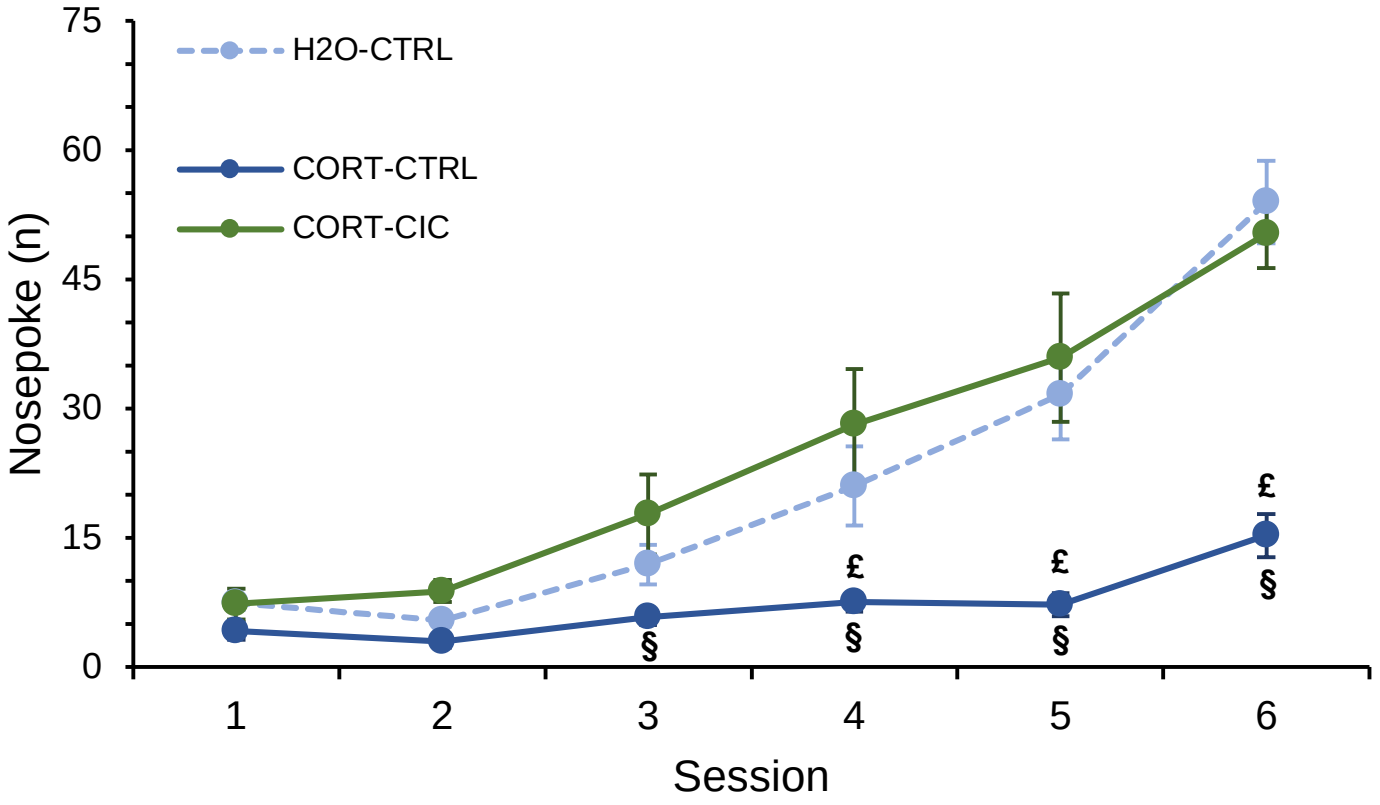
- Non-stressed animals fed with the standard diet showed a classic learning curve
- Stressed animals fed with the standard diet showed a clear impairment and learned the task more slowly



Fixed ratio test (FR)

Associative learning

learning to perform 1 nosepoke to obtain 1 food reward



£ H_2O-CTRL vs. CORT-CTRL

§ CORT-CTRL vs. CORT-CIC

Days to criterion:

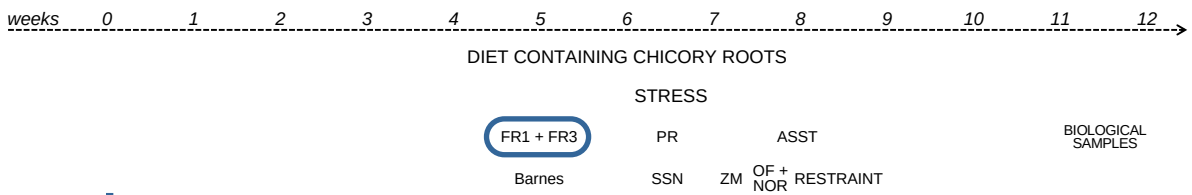
H_2O-CTRL: 4.43 ± 0.29

£ CORT-CTRL: 6.82 ± 0.23

§ CORT-CIC: 4.42 ± 0.50

- The learning curve was completely restored in stressed animals fed with the prebiotic diet → prebiotics led to a complete recovery from the negative effects induced by stress

Fixed ratio test (FR)



Associative learning

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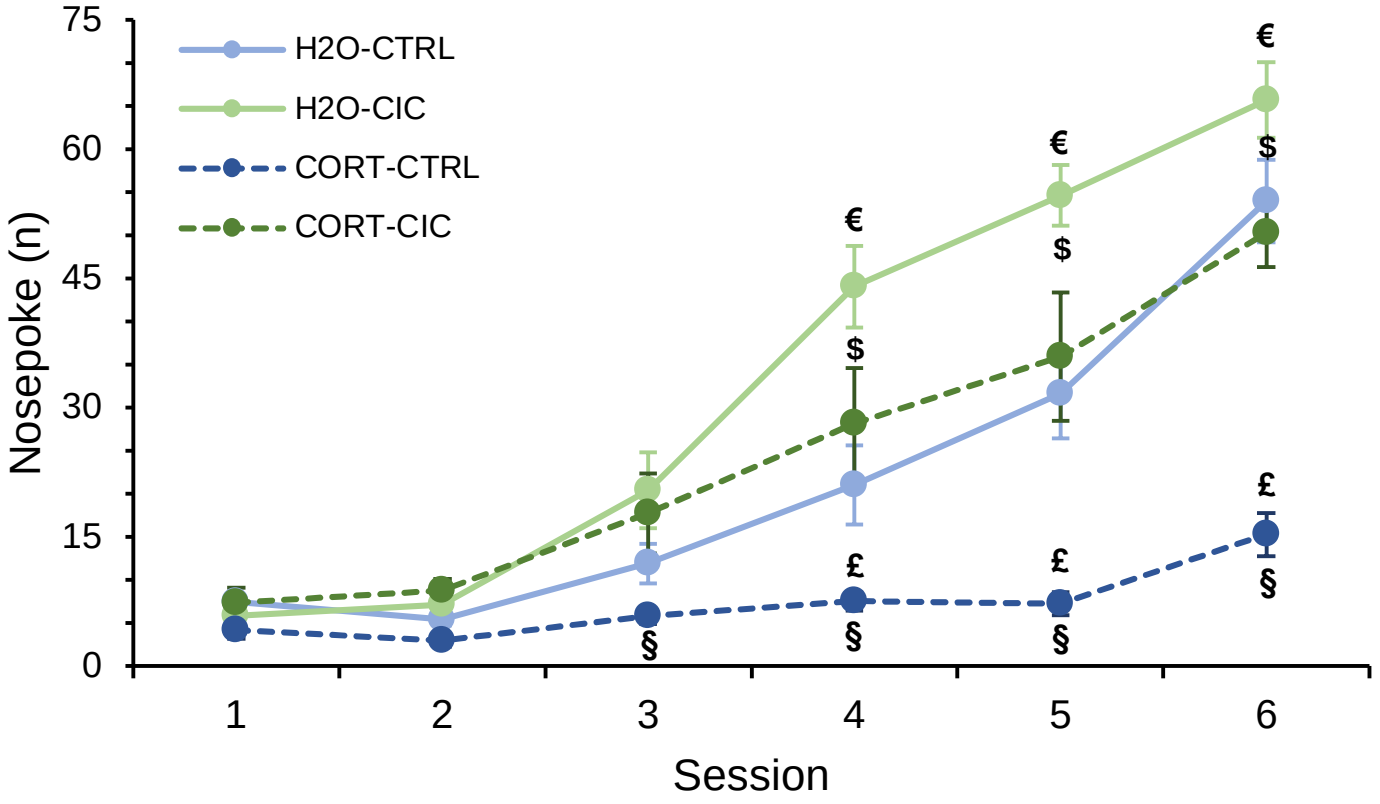


£ H_2O-CTRL vs. **CORT-CTRL**

$ H_2O-CIC vs. **CORT-CIC**

€ H_2O-CTRL vs. H_2O-CIC

§ **CORT-CTRL** vs. **CORT-CIC**

Days to criterion:

H_2O-CTRL: 4.43 ± 0.29

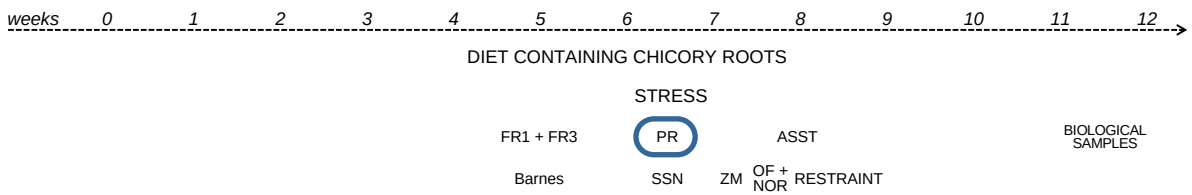
£ **CORT-CTRL:** 6.82 ± 0.23

§ **CORT-CIC:** 4.42 ± 0.50

H_2O-CIC: 3.73 ± 0.21

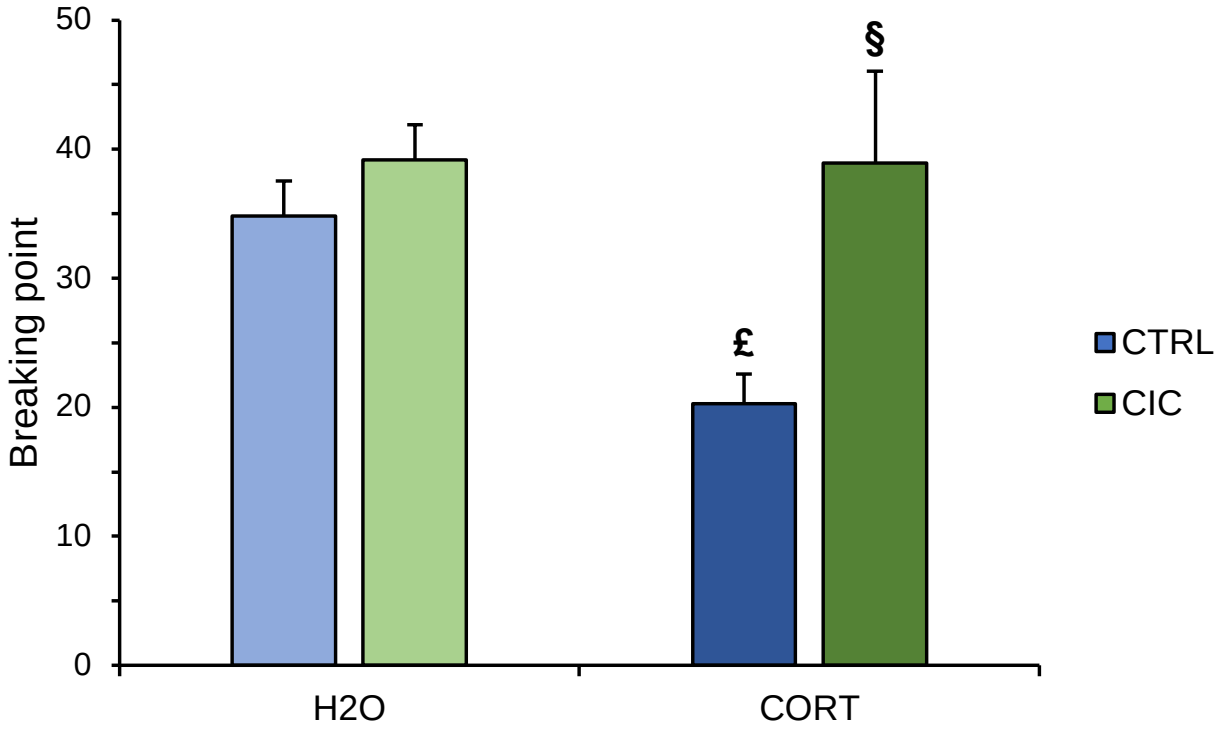
- Non-stressed animals fed with the chicory diet showed a further improvement in their cognitive performance → prebiotics exert a beneficial effect not only in the presence of stress-induced alterations but also in a normal condition

Progressive ratio test (PR)



Motivation

effort the subject is willing to make to obtain a reward



£ H_2O-CTRL vs. CORT-CTRL

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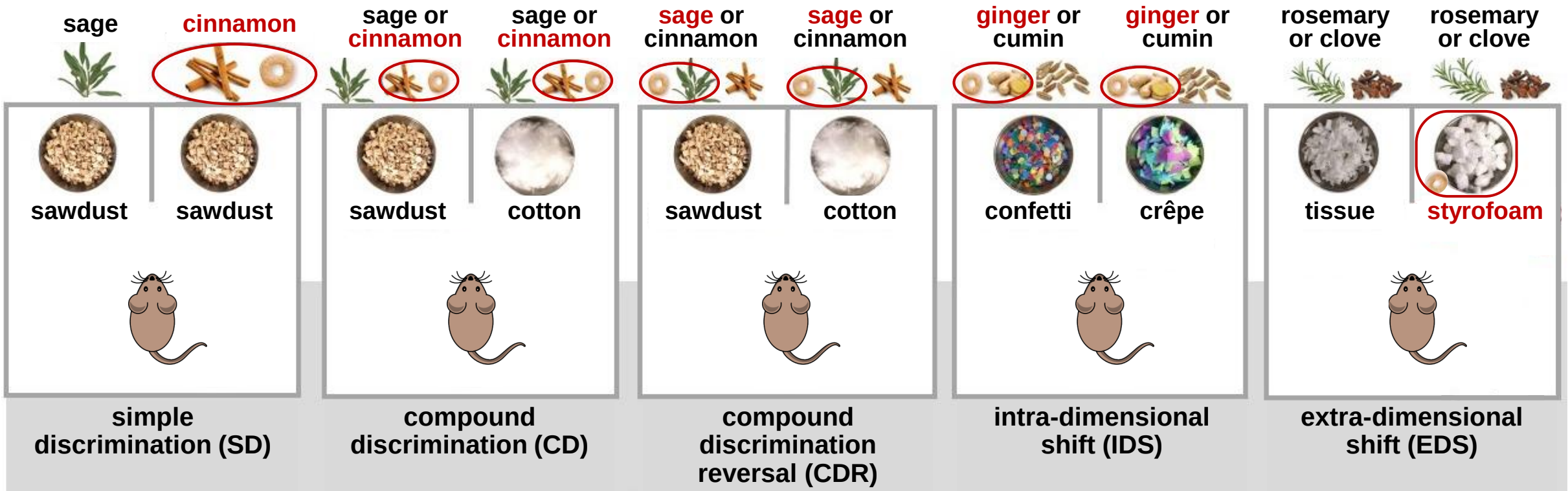
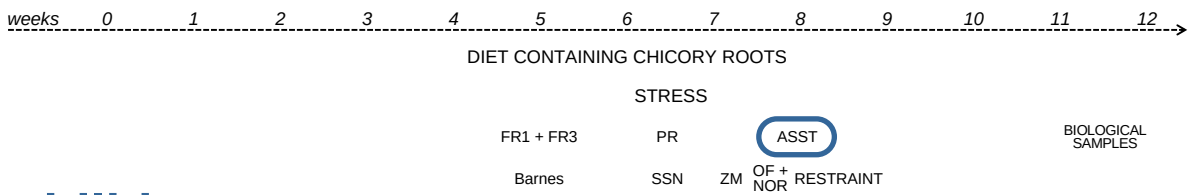
- Stressed animals fed with the standard diet showed a considerably lower level of motivation
- The prebiotic diet was able to significantly counteract the negative effects induced by stress, restoring the level of motivation

Attentional set-shifting task

→ Evaluates individual **capability to acquire a reward-associated rule** and to subsequently **disregard such rule in favour of a new one**, once the learning criterion has been attained by trial and error

- 5 stages, with different discriminations involving stimuli belonging to 2 dimensions: **olfactory and tactile-visual**
- in each stage, mice must understand in which of the 2 bowls they must dig to obtain the reward
- the reward is indicated by a particular stimulus (odour or digging medium) whilst the other stimulus acts as a confounder
- to complete each stage, the mouse must reach a criterion of **8 correct trials in 10 consecutive trials**





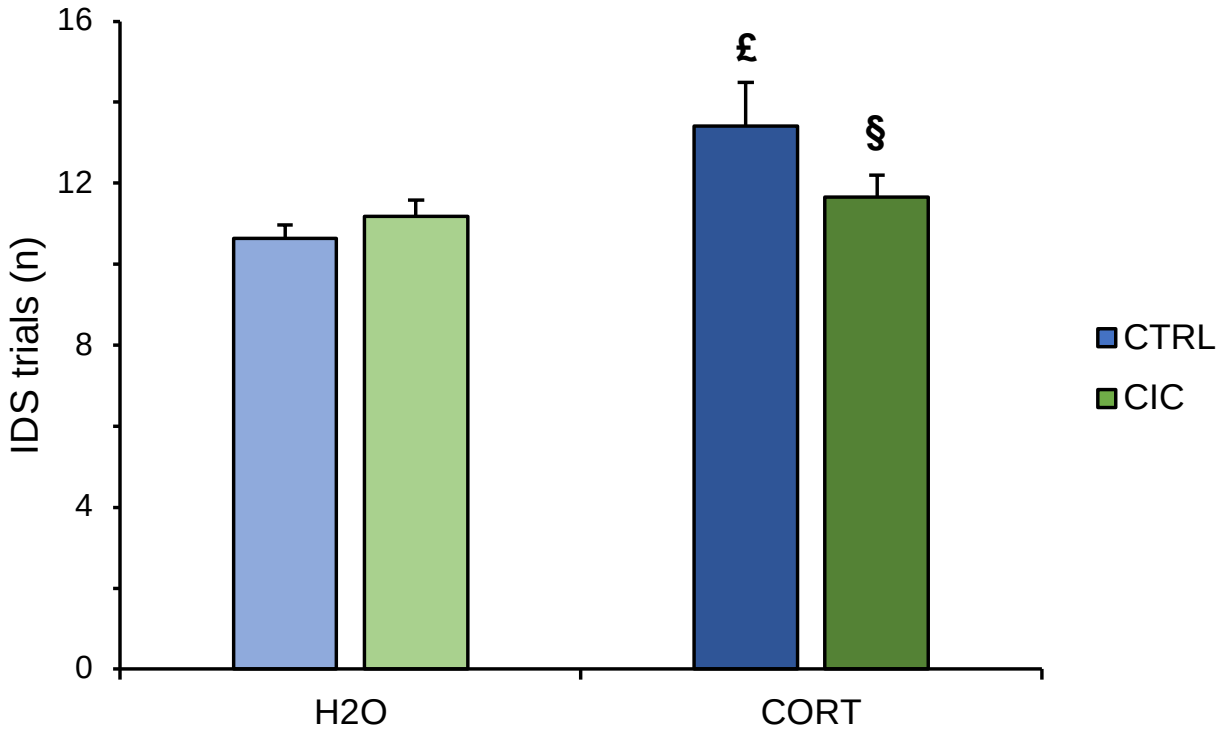
Attentional set-shifting task

Attentional capabilities

number of trials performed to reach the criterion in the intra-dimensional shift (IDS)

£ H_2O-CTRL vs. CORT-CTRL

§ CORT-CTRL vs. CORT-CIC

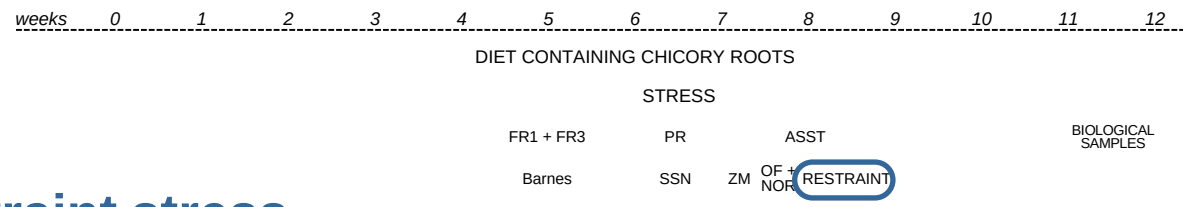


- Stressed mice fed with the standard diet showed worse attentional capabilities, requiring more trials to reach the criterion
- The chicory diet led to a significant improvement in the performance of stressed animals, restoring the level of attention

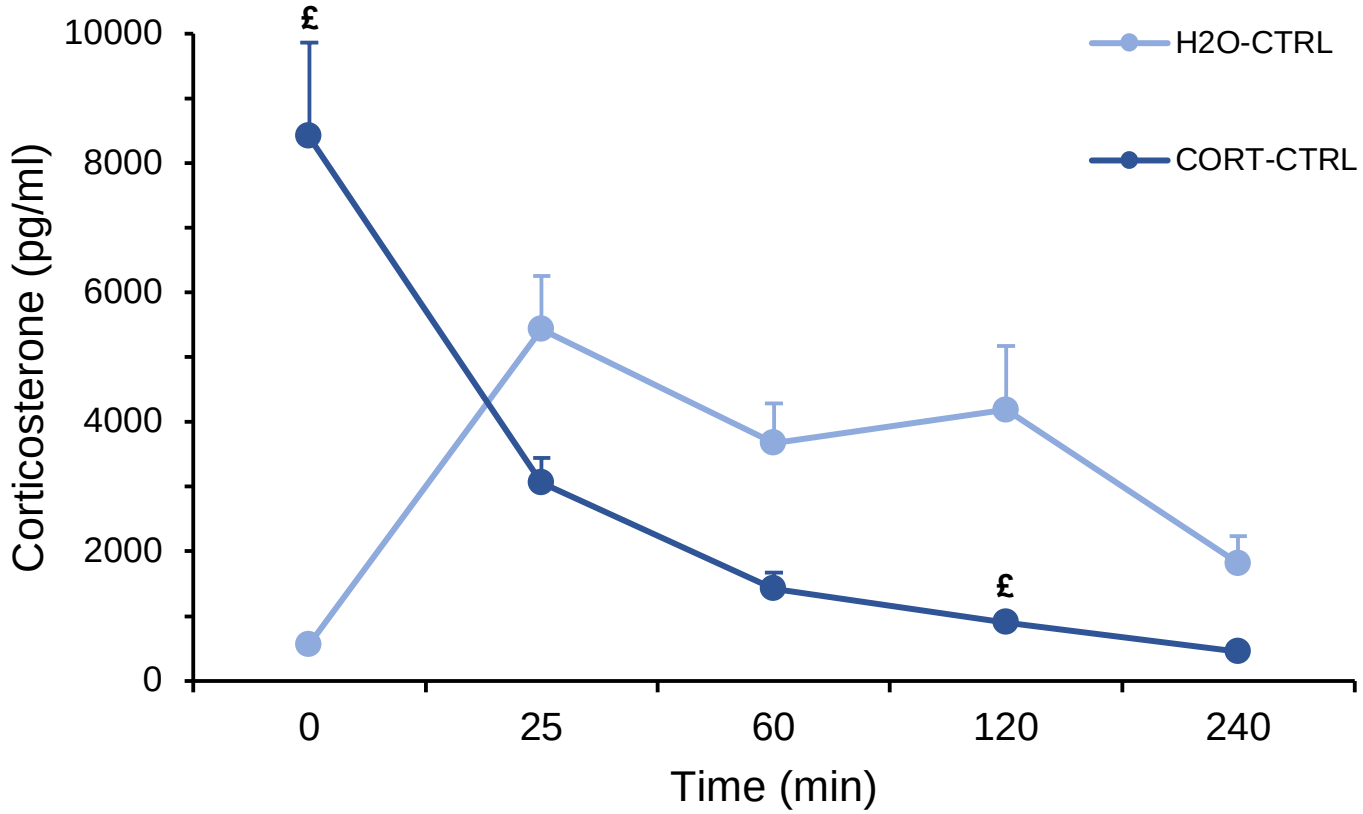
Stress reactivity

- To evaluate the **reactivity of the stress axis** in response to an **acute stress procedure**, plasma samples were collected immediately before and then 25, 60, 120 and 240 min after the beginning of a **25-min restraint stress**
- A small amount of blood (50 microliter) is collected using the tail nick technique
- The concentration of corticosterone in plasma was measured using commercially available ELISA kits

Stress reactivity



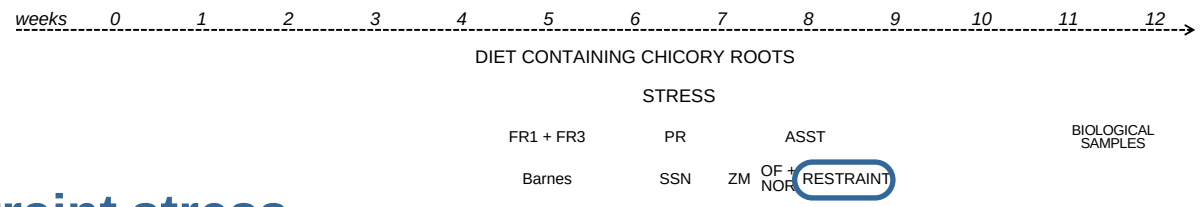
Response to restraint stress
plasma corticosterone concentrations, at baseline and in response to an acute stress



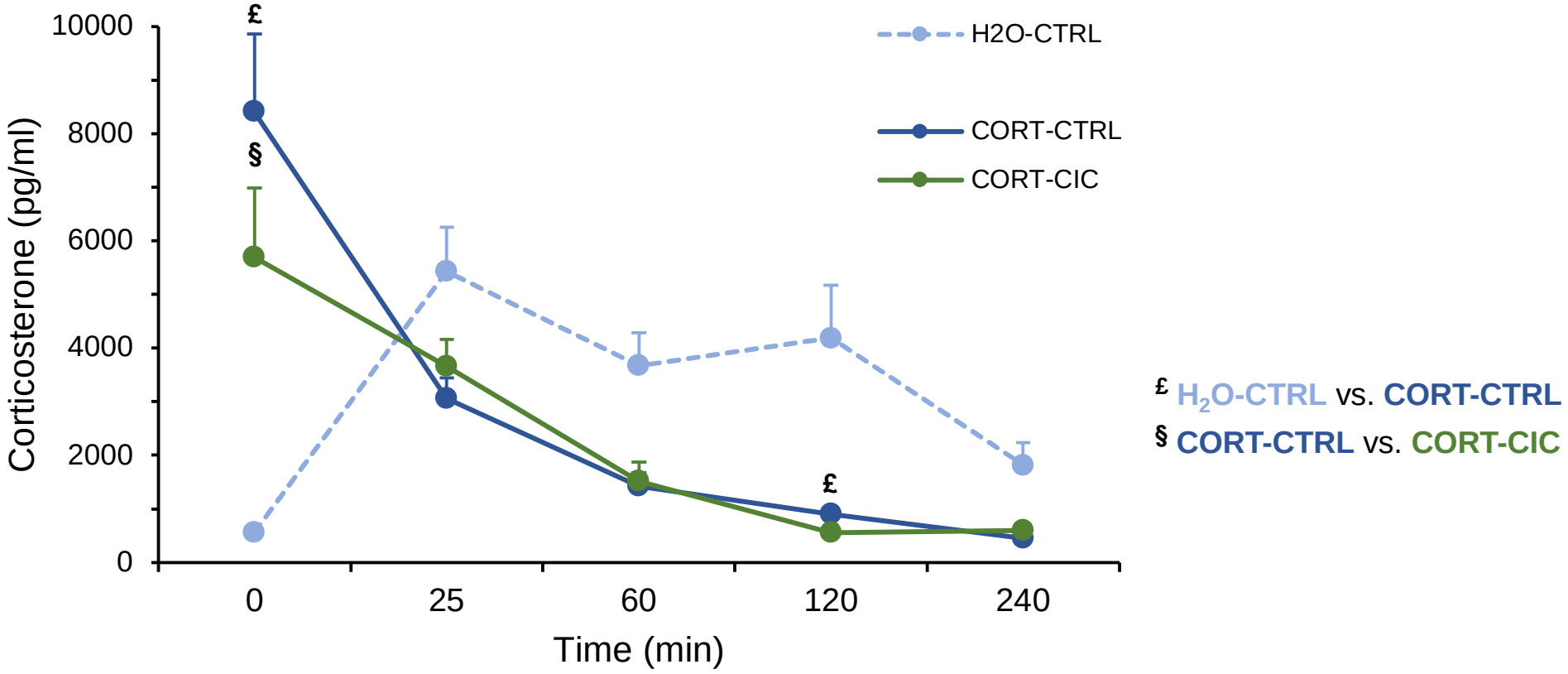
£ H_2O-CTRL vs. CORT-CTRL

- Non-stressed mice fed with the standard diet showed a typical response curve, with very low concentrations at baseline and a peak of response immediately after the end of the restraint
- The profile of stressed mice fed with the standard diet differed considerably, with the highest concentrations before the restraint

Stress reactivity

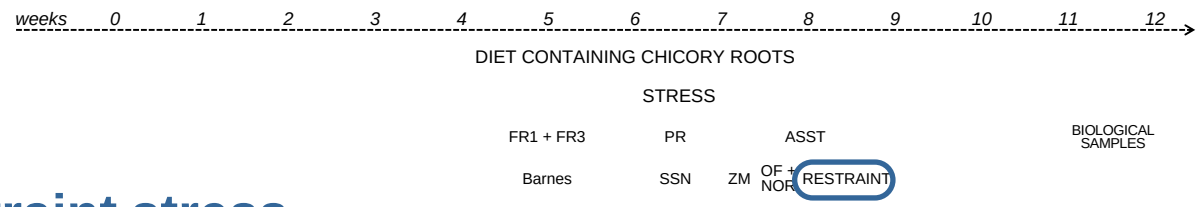


Response to restraint stress
plasma corticosterone concentrations, at baseline and in response to an acute stress



- Corticosterone concentrations at baseline were significantly decreased in stressed mice fed with the chicory diet

Stress reactivity



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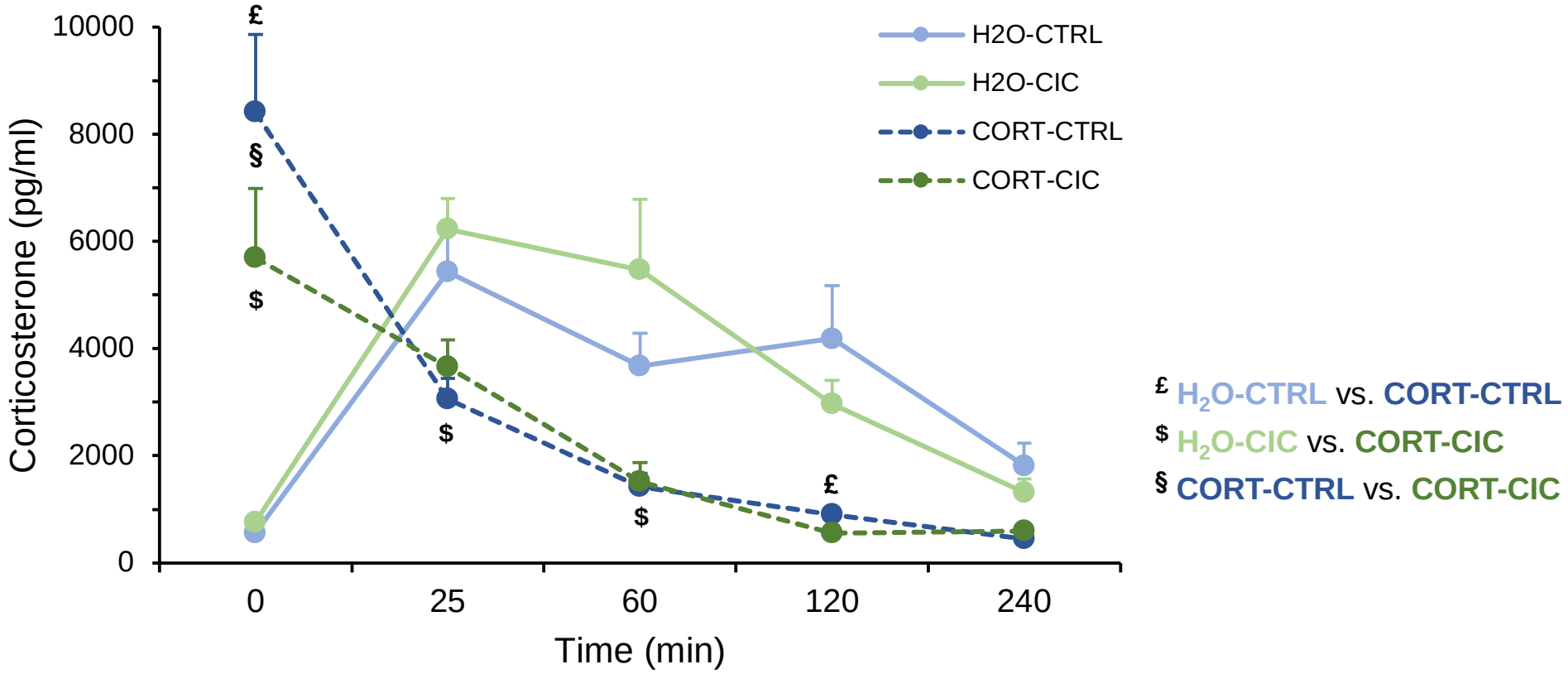
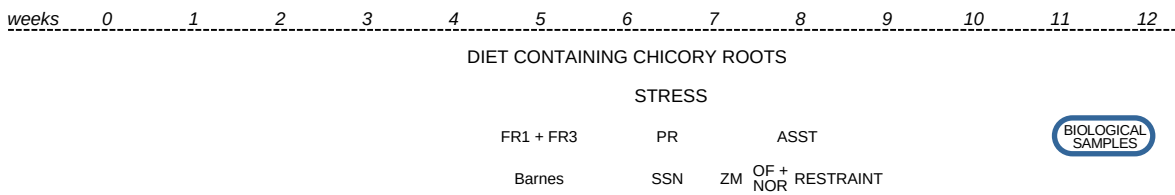


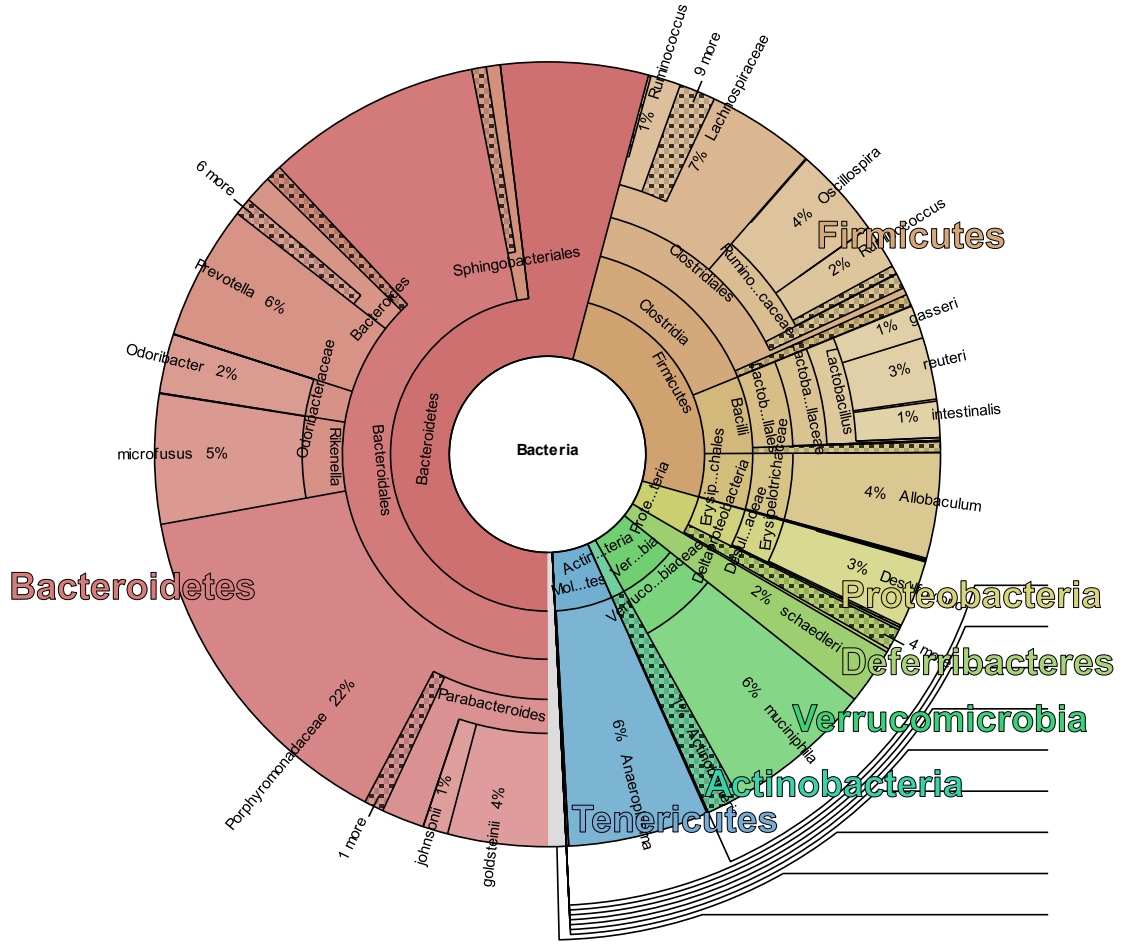
- No significant effects of chicory administration emerged within the non-stressed group

Summary

	CORTICOSTERONE **Detrimental effects** (H_2O-CTRL vs. CORT-CTRL)	PREBIOTIC DIET **Beneficial effects** (CORT-CTRL vs. CORT-CIC)
→ Associative learning (FR)	✓	✓
→ Motivation (PR)	✓	✓
→ Attentional capabilities (ASST)	✓	✓
→ Cognitive flexibility (ASST)	✗	n/a
Spatial memory (BARNES)	✓	(✓)
Anxiety (ZM and OF)	✓	✗
Recognition memory (NOR)	(✓)	✗
Sociability (SSN)	✗	n/a
Social memory (SSN)	✗	n/a
Body weight	✓	✓
→ Basal corticosterone	✓	✓

Microbial distribution

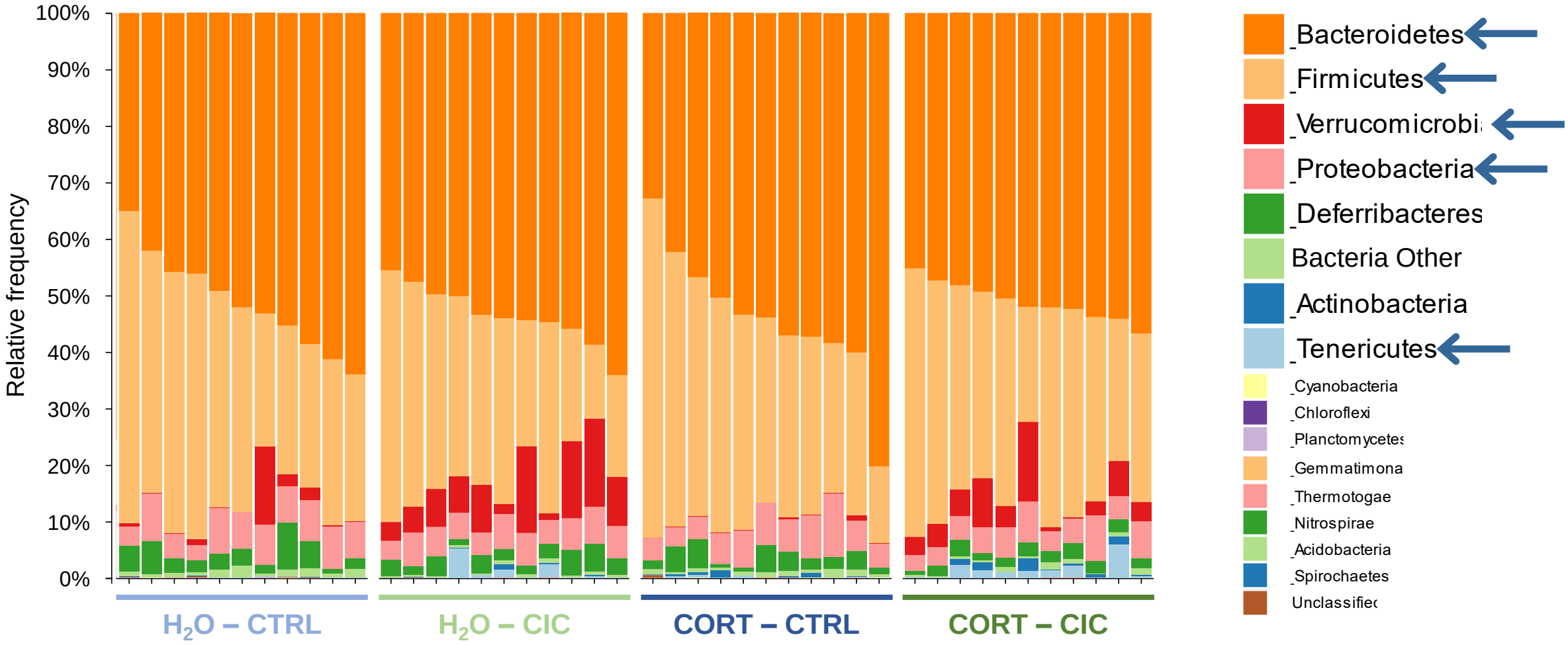




CORT - CTRL

CORT - CIC

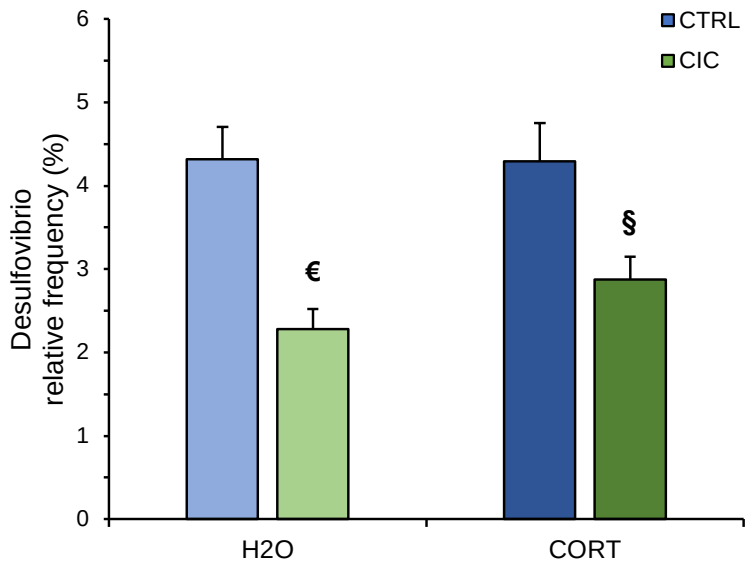
Microbial distribution at phylum level



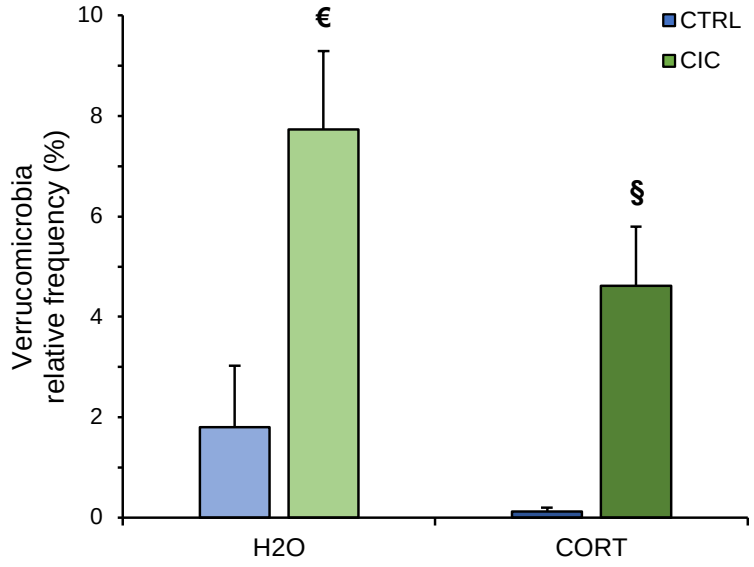
- Prebiotic supplementation effectively shifted microbiota composition in both non-stressed and stressed animals

Relative abundance of phyla Proteobacteria, Verrucomicrobia and Tenericutes

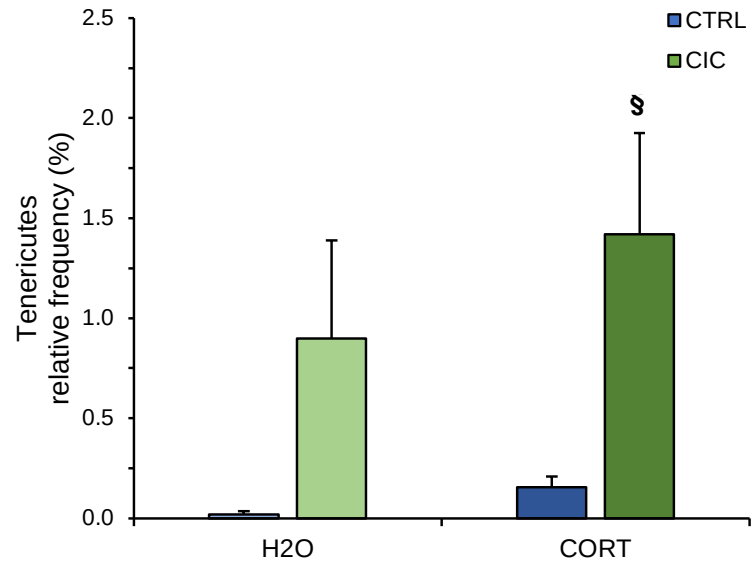
genus *Desulfovibrio* (main representative of phylum Proteobacteria)



phylum Verrucomicrobia (entirely composed of species *Akkermansia muciniphila*)

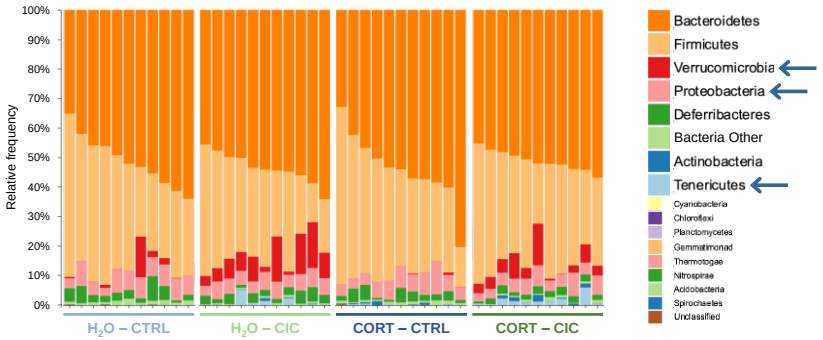


phylum Tenericutes (entirely composed of class Mollicutes)



€ H_2O-CTRL vs. H_2O-CIC

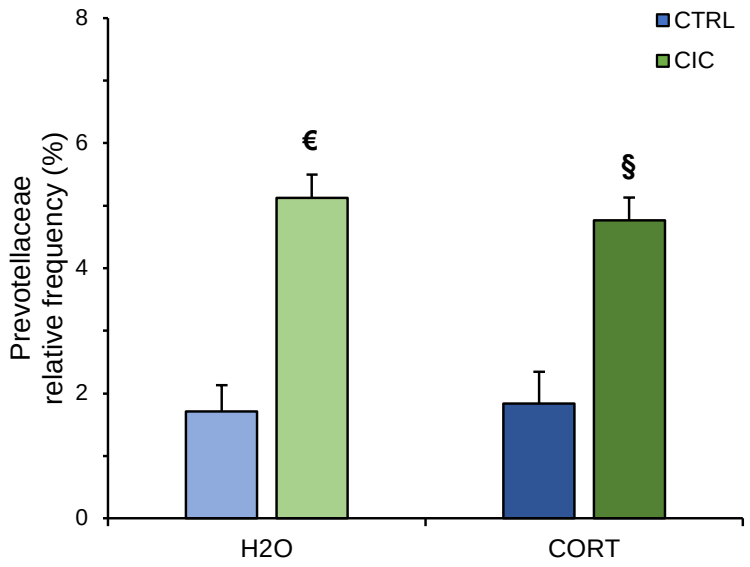
§ CORT-CTRL vs. CORT-CIC



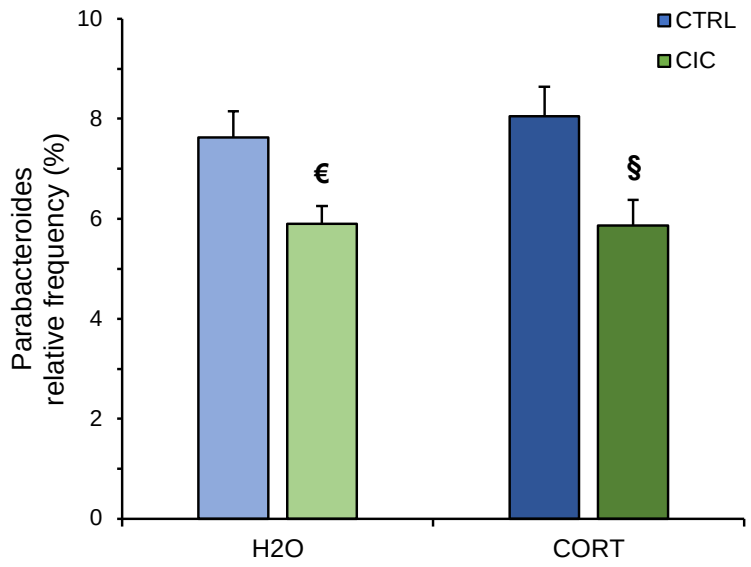
- Significant decrease in the phylum Proteobacteria and increase in the phyla Verrucomicrobia and Tenericutes between control and prebiotic-treated mice, in both non-stressed and stressed groups

Relative abundance of selected genera of phyla Bacteroidetes and Firmicutes

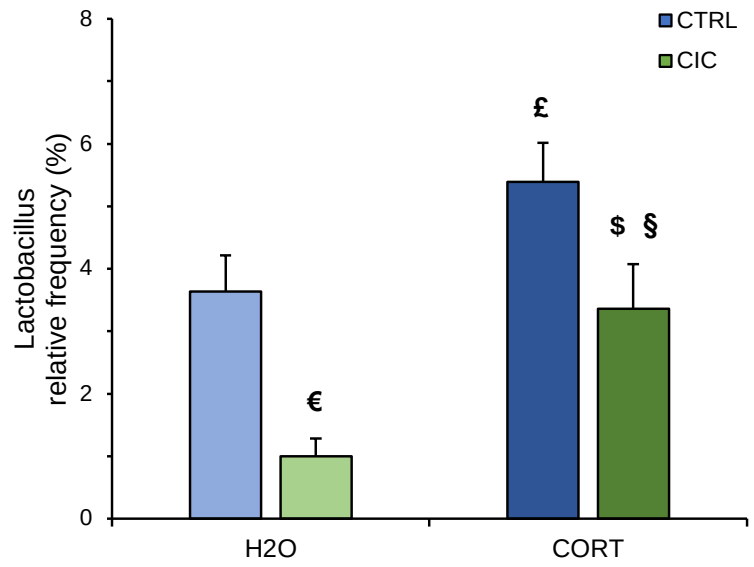
family Prevotellaceae
(belonging to phylum Bacteroidetes)



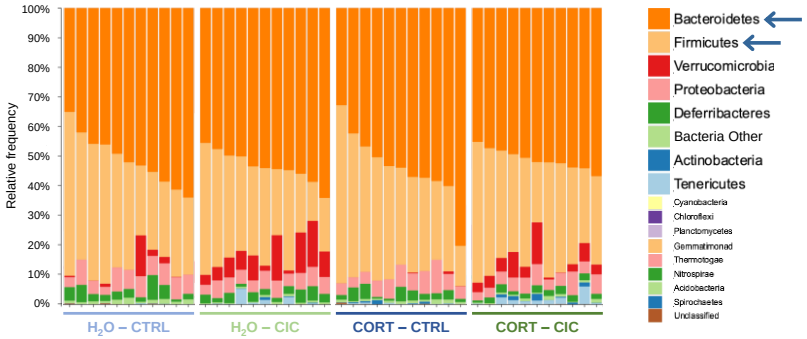
genus *Parabacteroides*
(belonging to phylum Bacteroidetes)



genus *Lactobacillus*
(belonging to phylum Firmicutes)



£ H_2O-CTRL vs. CORT-CTRL $ H_2O-CIC vs. CORT-CIC € H_2O-CTRL vs. H_2O-CIC § CORT-CTRL vs. CORT-CIC



- Prebiotic supplementation significantly increased Prevotellaceae and decreased Parabacteroides, regardless of stress exposure.
- Lactobacillus was significantly affected not only by the chicory diet but also by chronic stress → the stress-induced increase was fully normalized by prebiotics

Conclusions

- Fructans, by supporting microbial communities and/or through the release of metabolites (SCFAs), determine the recovery of numerous alterations induced by chronic stress, at both the behavioural and the physiological level
- Vegetables with prebiotic value can represent a valid aid to counterbalance the detrimental effects of stress and support astronauts' performances
  - The addition to the diet of vegetables rich in prebiotics can represent a valid and simple aid to maintain psychophysical well-being in the general population

Our findings provide a basis for future efforts to optimize the cultivation of prebiotic-rich vegetables within the bioregenerative life support systems to counteract the psychophysiological effects of stress and nurture a beneficial gut microbiome during long-duration human expeditions into outer space

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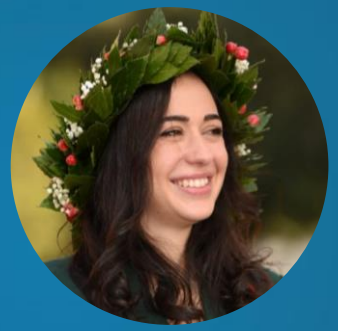

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THANK YOU.

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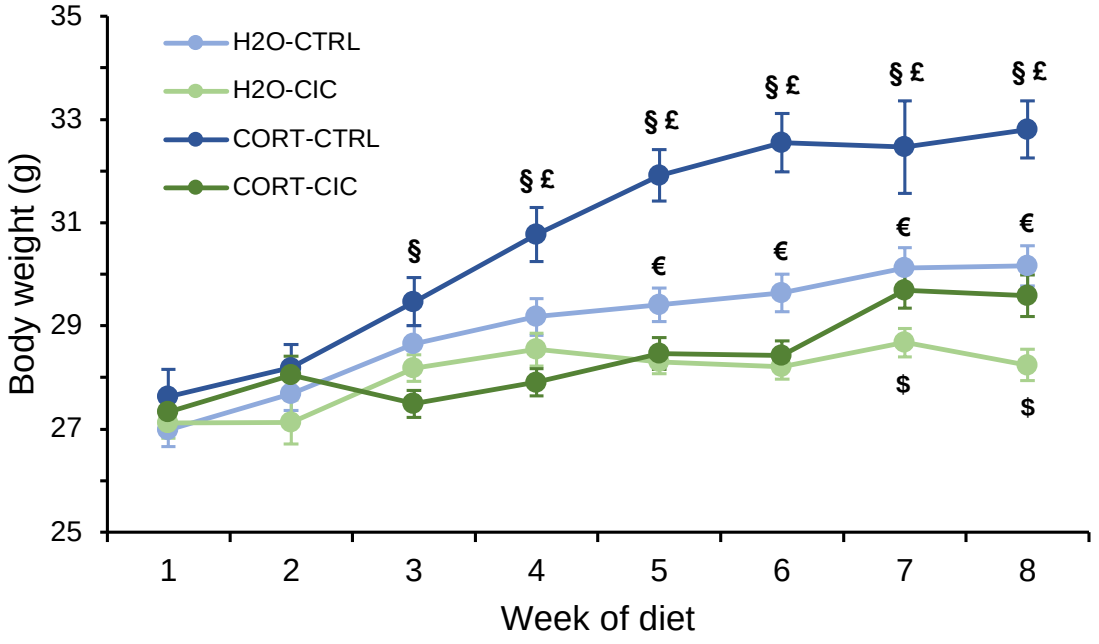
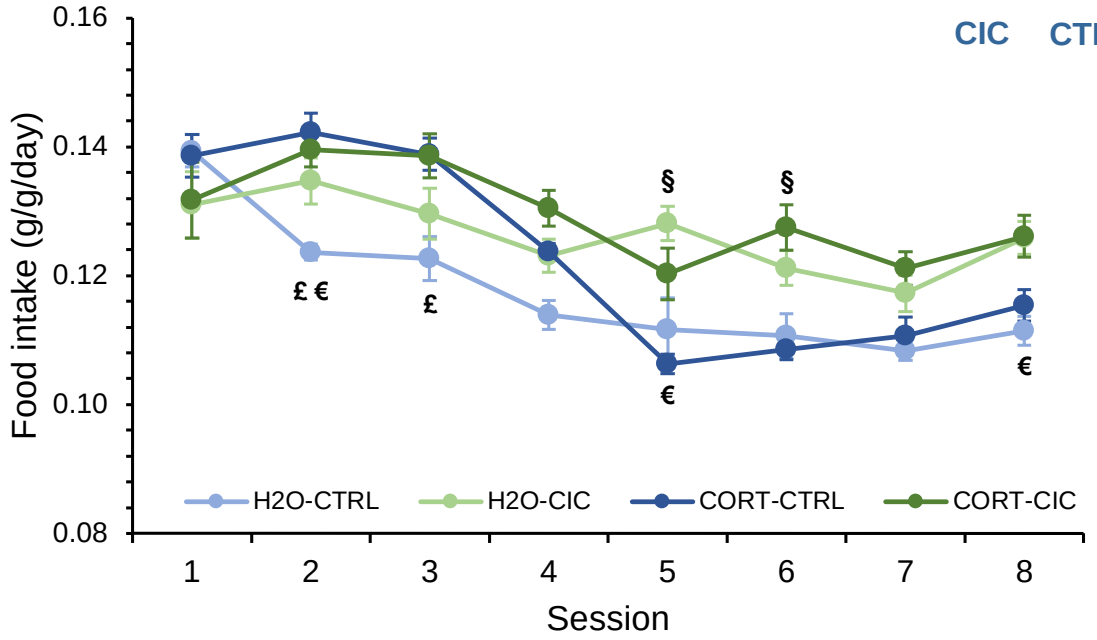
Physiological parameters

Body weight and food intake



Body weight



Food intake

£ H_2O-CTRL vs. CORT-CTRL

€ H_2O-CTRL vs. H_2O-CIC

$ H_2O-CIC vs. CORT-CIC

§ CORT-CTRL vs. CORT-CIC