SOCIETÀ ITALIANA DI TOSSICOLOGIA
SITOX
1972

21° Congresso Nazionale

Società Italiana di Tossicologia

Pericolo, rischio e rapporto rischio-beneficio

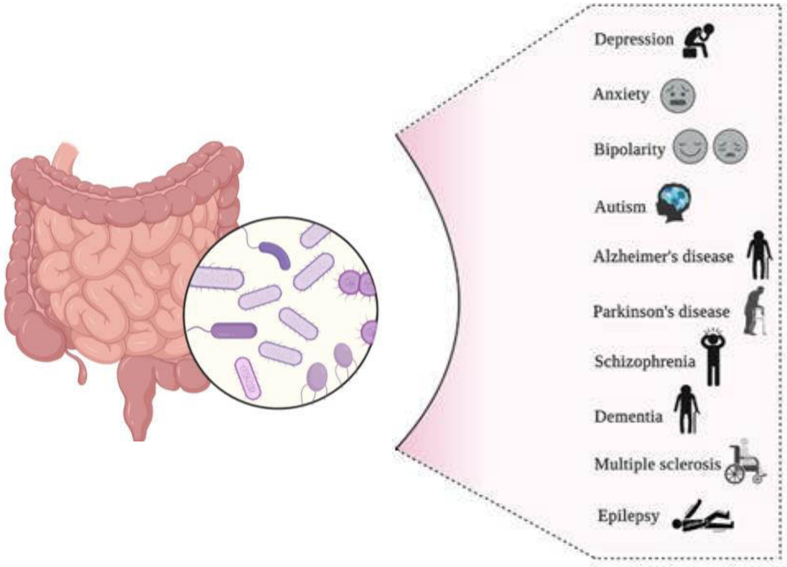
www.sitox.org

BOLOGNA
20-22 Febbraio 2023

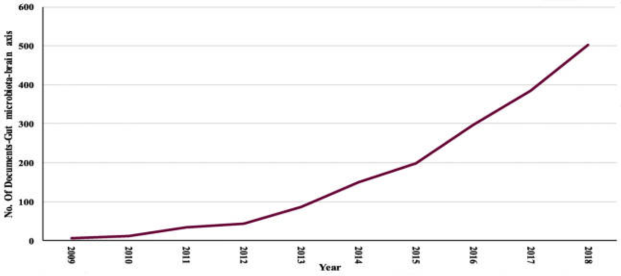
Analisi del microbiota orale e fecale in pazienti con disordini da uso di cocaina: stimolazione magnetica transcranica ripetitiva (rTMS) come nuovo potenziale trattamento per il ripristino della condizione di eubiosi

Dr. Simone Baldi, PhD
Department of Experimental and Clinical Medicine – University of Florence
simone.baldi@unifi.it

Gut microbiota and mood/neurological impairments

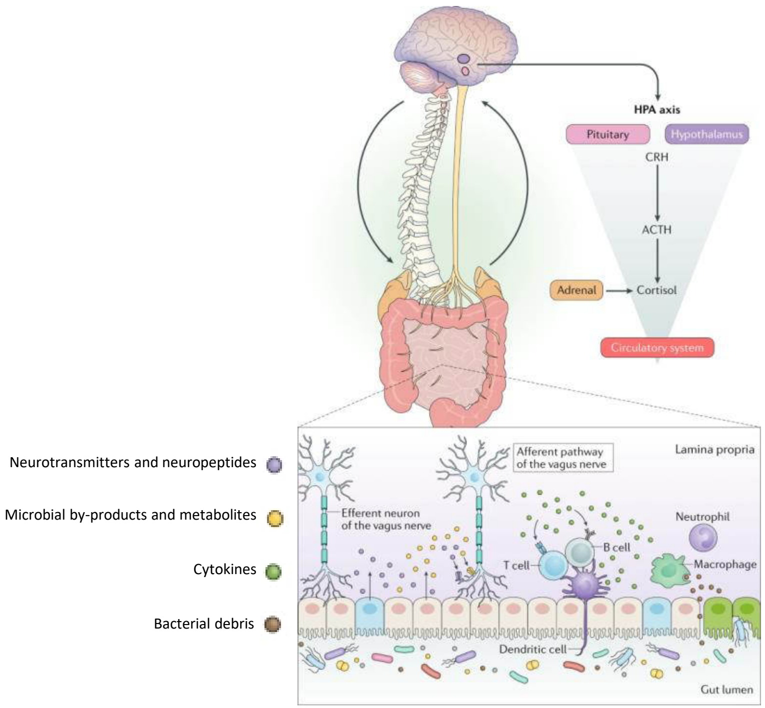


Global research trends in microbiota-gut-brain axis during 2009–2018: a bibliometric and visualized study



Sa'ed H. Zyoud et al. (2019)

Microbiota-Gut-Brain Axis

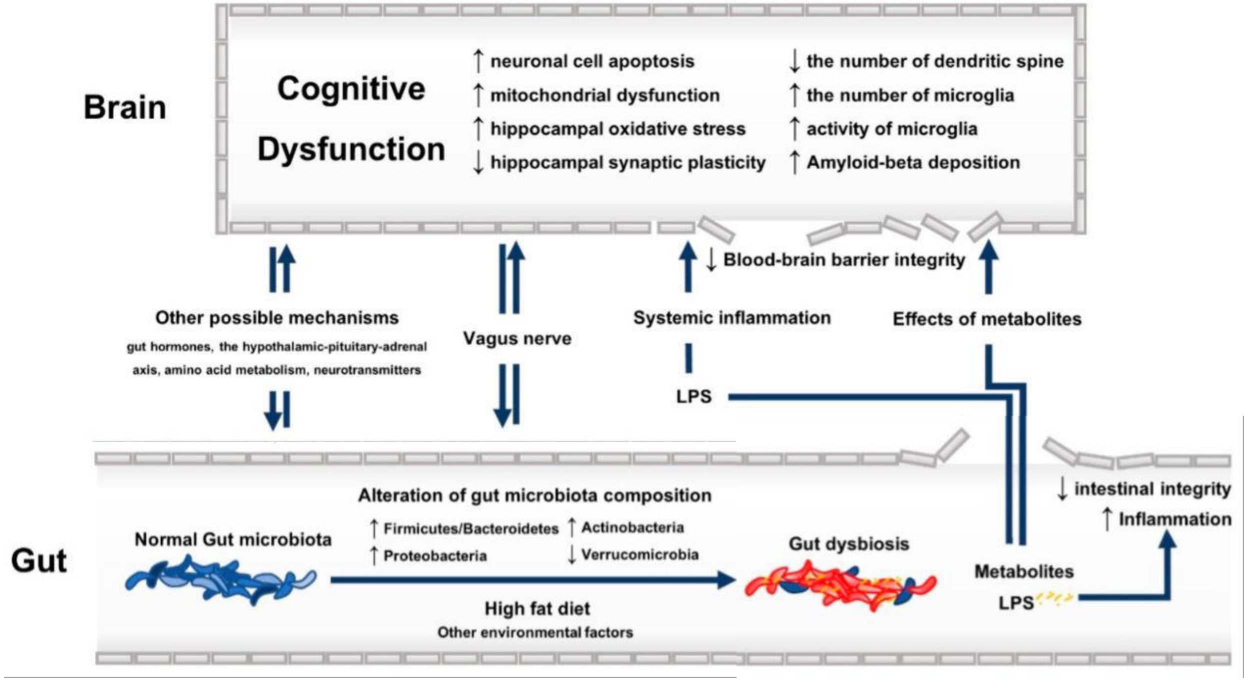


Livia H. Morais et al. (2021)

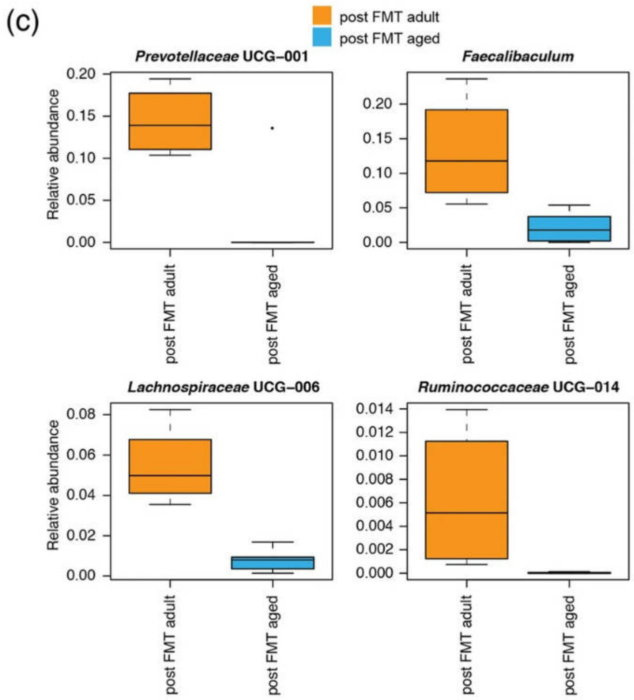
The intestinal microbiota regulates the gut-brain axis through:

- Vagus nerve
- Microbial metabolites (SCFAs)
- Neurotransmitters
- Cytokynes

Microbiota-Gut-Brain Axis



Microbiota-Gut-Brain Axis



Faecal microbiota transplant from aged donor mice affects spatial learning and memory via modulating hippocampal synaptic plasticity- and neurotransmission-related proteins in young recipients

Alfonsina D'Amato, Lorenzo Di Cesare Mannelli, Elena Lucarini, Angela L. Man, Gwenaelle Le Gall, Jacopo J. V. Branca, Carla Ghelardini, Amedeo Amedei, Eugenio Bertelli, Mari Regoli, Alessandra Pacini, Giulia Luciani, Pasquale Gallina, Annalisa Altera, Arjan Narbad, Massimo Gulisano, Lesley Hoyles, David Vauzour & Claudio Nicoletti

Microbiome **8**, Article number: 140 (2020) | Cite this article

- FMT from aged donors determined a strong reduction of bacteria associated with SCFAs production (Lachnospiraceae, Faecalibaculum, and Ruminococcaceae) and disorders of the CNS (Prevotellaceae and Ruminococcaceae)

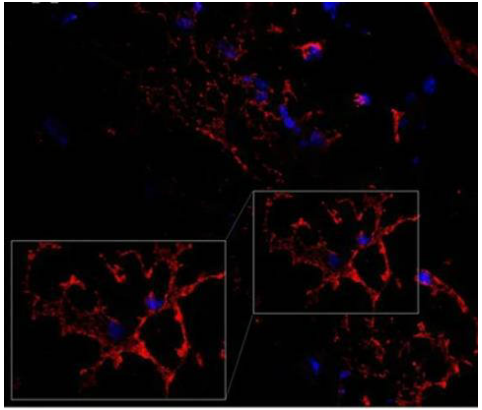
Microbiota-Gut-Brain Axis

- FMT from aged donors led to impaired spatial learning and memory in young adult recipients
- Determined an altered expression of proteins involved in synaptic plasticity and neurotransmission in the hippocampus.
- Microglia cells of the hippocampus fimbria, acquired an ageing-like phenotype

Faecal microbiota transplant from aged donor mice affects spatial learning and memory via modulating hippocampal synaptic plasticity- and neurotransmission-related proteins in young recipients

Alfonsina D'Amato, Lorenzo Di Cesare Mannelli, Elena Lucarini, Angela L. Man, Gwenaelle Le Gall, Jacopo J. V. Branca, Carla Ghelardini, Amedeo Amedei, Eugenio Bertelli, Mari Regoli, Alessandra Pacini, Giulia Luciani, Pasquale Gallina, Annalisa Altera, Arjan Narbad, Massimo Gulisano, Lesley Hoyles, David Vauzour & Claudio Nicoletti

Microbiome **8**, Article number: 140 (2020) | Cite this article



Microbiota-Gut-Brain Axis

> Front Aging Neurosci. 2022 Oct 31;14:1032494. doi: 10.3389/fnagi.2022.1032494. eCollection 2022.

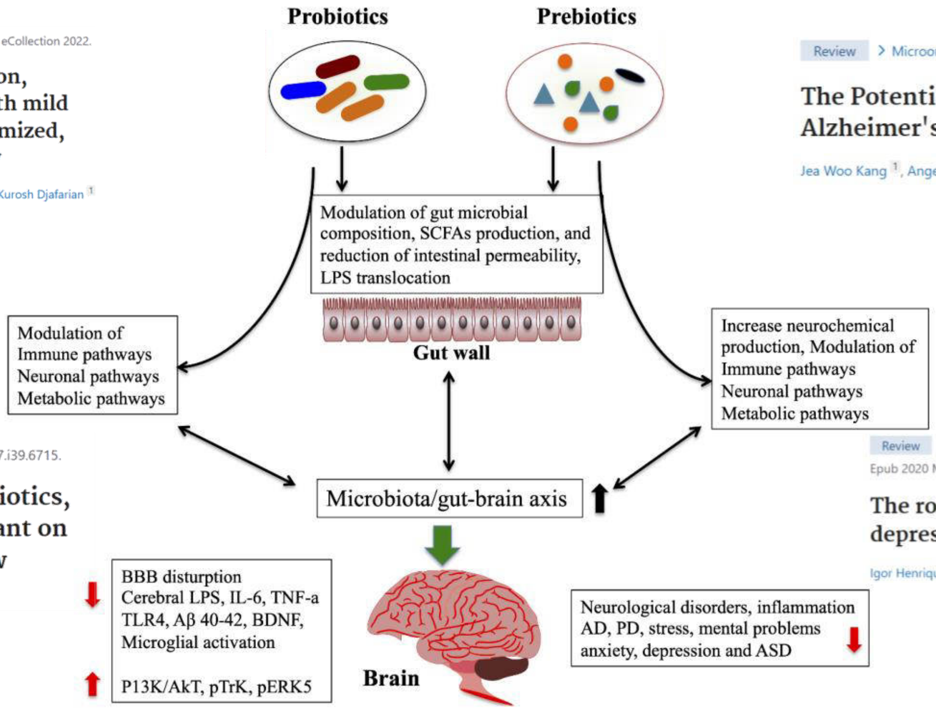
Effects of probiotic supplements on cognition, anxiety, and physical activity in subjects with mild and moderate Alzheimer's disease: A randomized, double-blind, and placebo-controlled study

Camellia Akhgarjand [1], Zahra Vahabi [2 3], Sakineh Shab-Bidar [4], Farnaz Etesam [5], Kurosh Djafarian [1]

> World J Gastroenterol. 2021 Oct 21;27(39):6715-6732. doi: 10.3748/wjg.v27.i39.6715.

Microbiota shaping - the effects of probiotics, prebiotics, and fecal microbiota transplant on cognitive functions: A systematic review

Simone Baldi [1], Tiziana Mundula [1], Giulia Nannini [1], Amedeo Amedei [1]



Review > Microorganisms. 2021 Nov 6;9(11):2310. doi: 10.3390/microorganisms9112310.

The Potential Utility of Prebiotics to Modulate Alzheimer's Disease: A Review of the Evidence

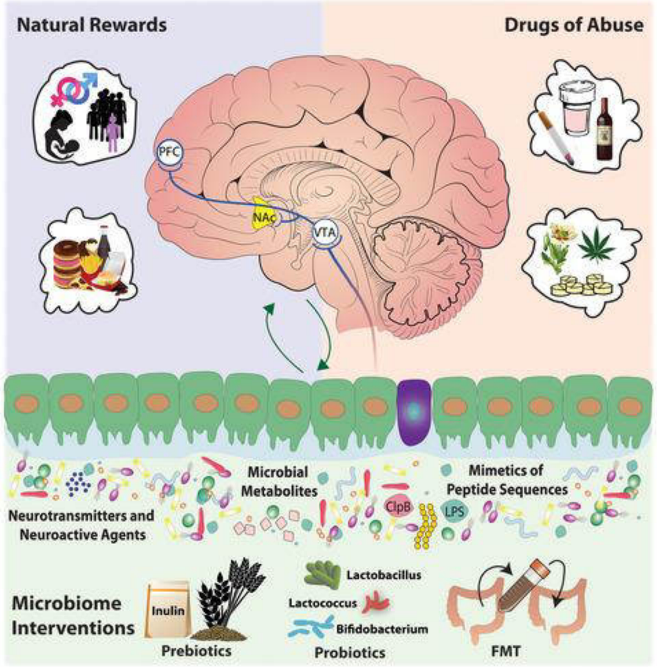
Jea Woo Kang [1], Angela M Zivkovic [1]

Review > Eur Neuropsychopharmacol. 2020 May;34:1-18. doi: 10.1016/j.euroneuro.2020.03.006. Epub 2020 Mar 30.

The role of prebiotics in cognition, anxiety, and depression

Igor Henrique R Paiva [1], Eduardo Duarte-Silva [2], Christina Alves Peixoto [3]

Gut microbiota and reward processes



Rubén García-Cabrerizo et al. (2020)

The intestinal microbiota is involved in regulating brain reward functions, both in natural and non-natural reinforcers

- Targeting the microbiota-gut-brain axis could be a promising therapeutic strategy for disorders associated with alterations in the reward system

GM and Cocaine-Animal Studies

Neurotox Res. 2019 Jan;35(1):111-121. doi: 10.1007/s12640-018-9936-9. Epub 2018 Jul 31.

Alterations in the Gut Microbiota of Rats Chronically Exposed to Volatilized Cocaine and Its Active Adulterants Caffeine and Phenacetin

Cecilia Scorza [1], Claudia Piccini [2], Marcela Martínez Busi [3], Juan Andrés Abin Carriquiry [3],

Sci Rep. 2019 Aug 21;9(1):12187. doi: 10.1038/s41598-019-48428-2.

Cocaine Induces Inflammatory Gut Milieu by Compromising the Mucosal Barrier Integrity and Altering the Gut Microbiota Colonization

Ernest T Chivero [1], Rizwan Ahmad [2], Annadurai Thangaraj [1], Palsamy Periyasamy [1], Balawant Kumar [2], Elisa Kroeger [1], Dan Feng [1], Ming-Lei Guo [1], Sabita Roy [3], Punita Dhawan [2, 4], Amar B Singh [2, 4], Shilpa Buch [5]

Brain Behav Immun. 2023 Jan;107:286-291. doi: 10.1016/j.bbi.2022.10.020. Epub 2022 Oct 28.

The gut microbiota alone and in combination with a social stimulus regulates cocaine reward in the mouse

Rubén García-Cabrerizo [1], Thaisa Barros-Santos [2], David Campos [2], John F Cryan [3]

Sci Rep. 2016 Oct 18;6:35455. doi: 10.1038/srep35455.

Alterations of the Host Microbiome Affect Behavioral Responses to Cocaine

Drew D Kiraly [1 2 3], Deena M Walker [1], Erin S Calipari [1], Benoit Labonte [1], Orna Issler [1], Catherine J Pena [1], Efrain A Ribeiro [1], Scott J Russo [1], Eric J Nestler [1 2]

Cell Host Microbe. 2022 Nov 9;30(11):1615-1629.e5. doi: 10.1016/j.chom.2022.09.014. Epub 2022 Nov 1.

Gut colonization by Proteobacteria alters host metabolism and modulates cocaine neurobehavioral responses

Santiago Cuesta [1], Paula Burdisso [2], Amir Segev [3], Saïd Kourrich [4], Vanessa Sperandio [5]

Neuropsychopharmacology. 2018 Dec;43(13):2606-2614. doi: 10.1038/s41386-018-0211-9. Epub 2018 Sep 10.

The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence

Kevin Lee [1], Helen E Vuong [2], David J Nusbaum [2], Elaine Y Hsiao [2], Christopher J Evans [1 3], Anna M W Taylor [4]

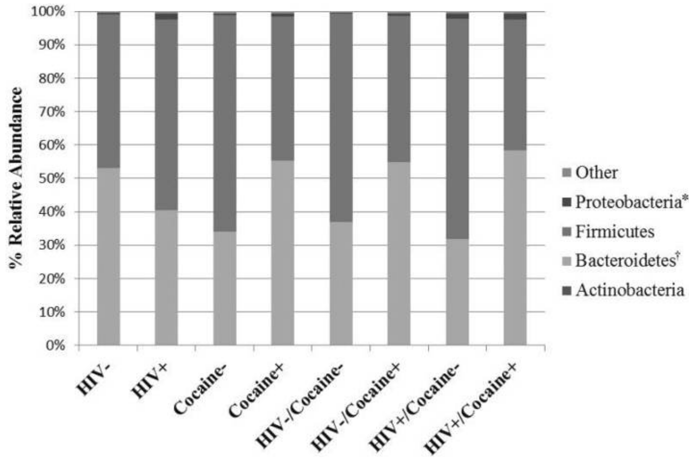
GM and Cocaine-Uman Studies

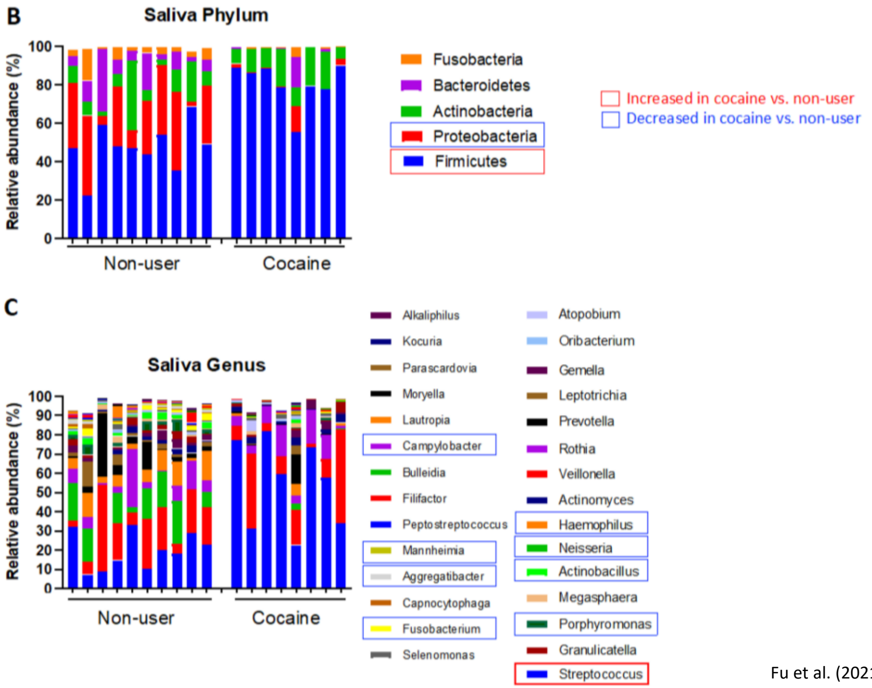
Associations of cocaine use and HIV infection with the intestinal microbiota, microbial translocation, and inflammation.

Volpe GE[1], Ward H[1], Mwamburi M[2], Dinh D[3], Bhalchandra S[3], Wanke C[1], Kane AV[1]

Author information ›

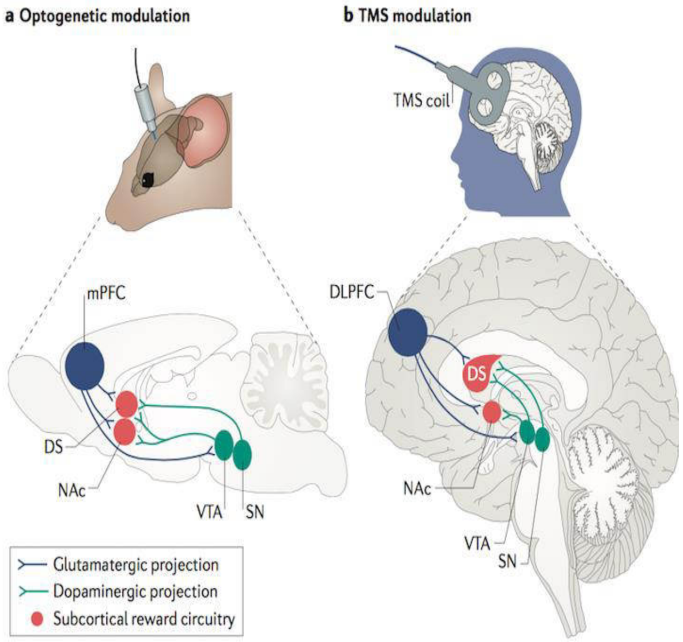
Journal of Studies on Alcohol and Drugs, 01 Mar 2014, 75(2):347-357





Fu et al. (2021)

repetitive Transcranial Magnetic Stimulation (rTMS)

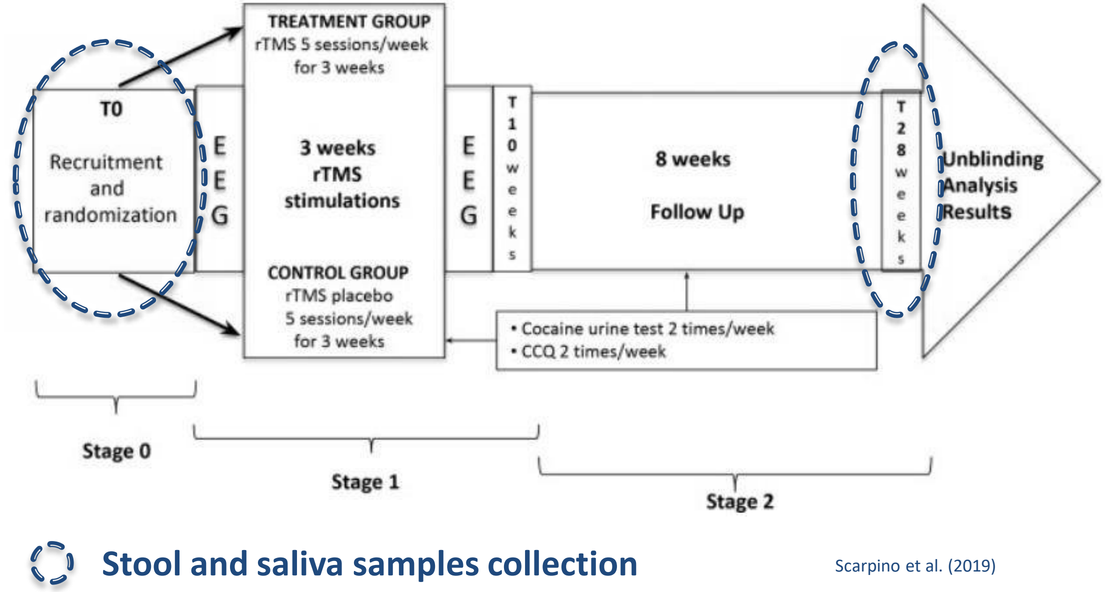


Diana, et al. Nat Rev Neurosci, 2017

Stimulation of the dorsolateral prefrontal cortex (DLPFC) with the repetitive Transcranial Magnetic Stimulation (rTMS) in humans is able to restore the normal functioning of the gratification circuits, returning the neurotransmitter systems to homeostasis.

- Pharmacological treatments
- Psychotherapeutic and socio-rehabilitative treatments
- Non-Invasive Brain Stimulation

Methods - Randomised Controlled Trial



Scarpino et al. (2019)

Enrolled subjects:

- 58 CUD (cocaine use disorder)
- 20 controls

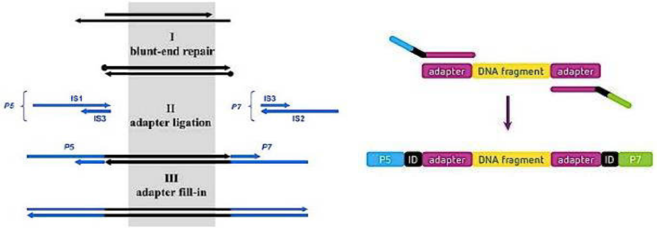
Intervention:

- 15 rTMS sessions
- Frequency of 15 *Hz* (40 trains per signal)
- 60 stimuli/train with a 15-sec pause, for a total of 2400 stimuli in a 13-minute time period.

Methods - 16SrRNA sequencing of stool and saliva samples

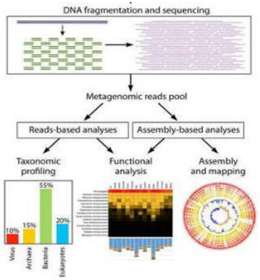
Sampling

DNA extraction



Library preparation

16S rRNA sequencing on Miseq Illumina platform

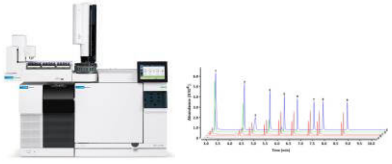


Bioinformatic and statistical analysis

Methods - Analysis of fecal SCFAs and MCFAs profile with GC/MS

- Collection of stool samples
- Storage al -80°C

- Addition of sodium bicarbonate (buffer) 10mM (1:1 w/v)
- Ultrasonication (5’) and centrifugation (5000 rpm for 10’)
- Collection of the supernatants



- 200 µL of sample + 50µL of ISTDs mixture + 1000 µL of MTBE + 100 µL of HCl 6 M + 0,5 M NaCl
- Vortexing (5’), centrifugation (1000 rpm for 3’) and collection of supernatants
- GC/MS Analysis

Results - Toxicological and psychiatric assessments

Randomized Controlled Trial > Neurophysiol Clin. 2019 Feb;49(1):1-9.
doi: 10.1016/j.neucli.2018.10.002. Epub 2018 Oct 26.

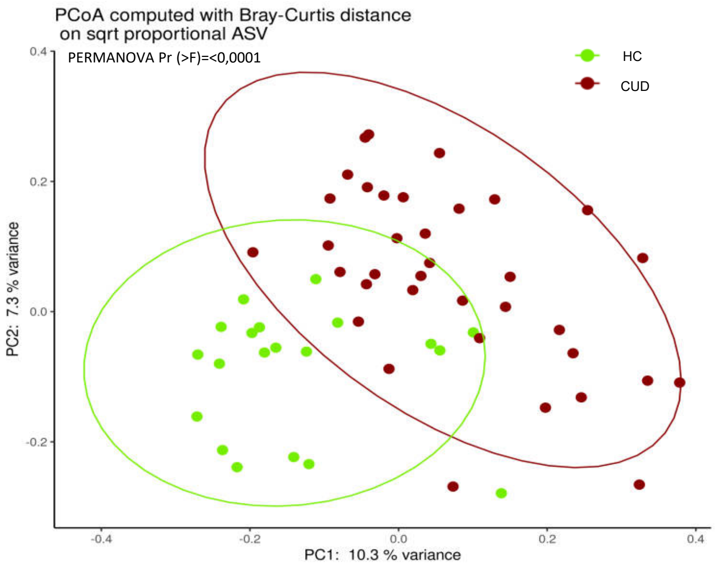
Efficacy of high-frequency (15Hz) repetitive transcranial magnetic stimulation (rTMS) of the left premotor cortex/dorsolateral prefrontal cortex in decreasing cocaine intake (the MagneTox study): A study protocol for a randomized placebo-controlled pilot trial

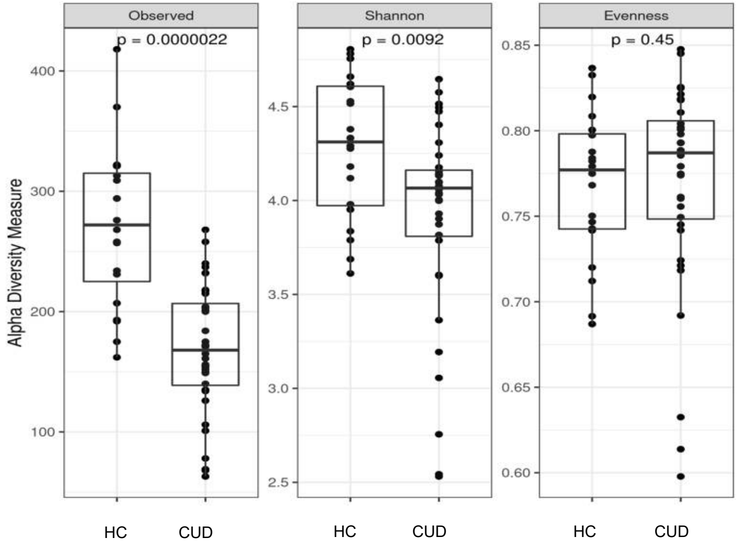
Maenia Scarpino [1], Giovanni Lanzo [2], Maya Salimova [3], Francesco Lolli [4], Amedeo Del Vecchio [3], Cesarina Cossu [2], Maria Bastianelli [2], Brunella Occupati [5], Cecilia Lanzi [5], Stefano Pallanti [3], Aldo Amantini [2], Guido Mannaioni [3], Antonello Grippo [6]

- The severity of craving mediated by cocaine-related cues was decreased in the rTMS-treated patients
- rTMS-treated patients reported a significant reduction of daily cocaine use
- Psychometric impulsivity parameters and depression scales improved in rTMS-treated patients

Results - Microbiota composition in CUD and HC

Stool samples

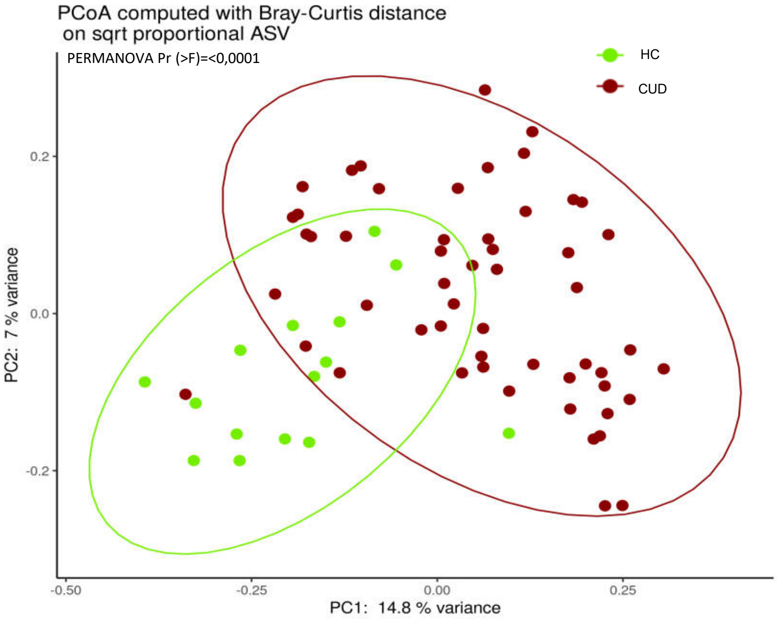


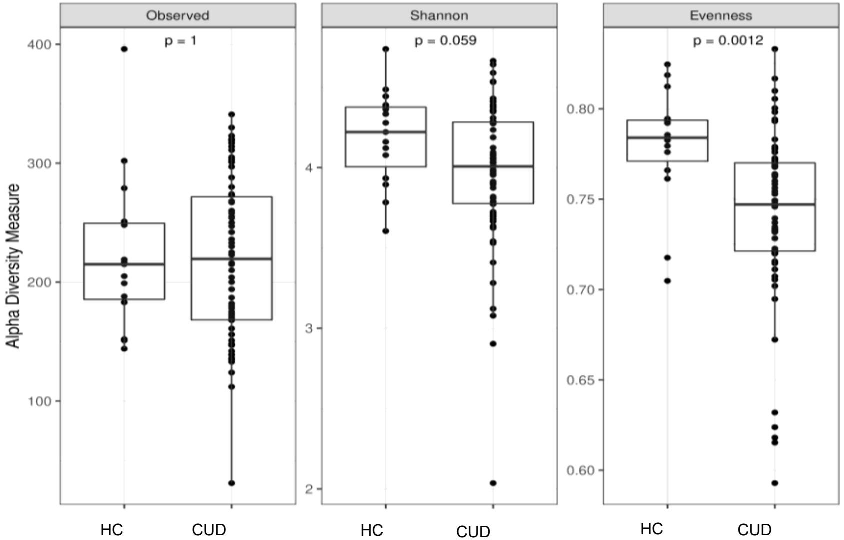


Principal coordinates analysis (PCoA) and alpha-diversity indices both highlighted clearly differences between HC and CUD samples

Results - Microbiota composition in CUD and HC

Saliva samples

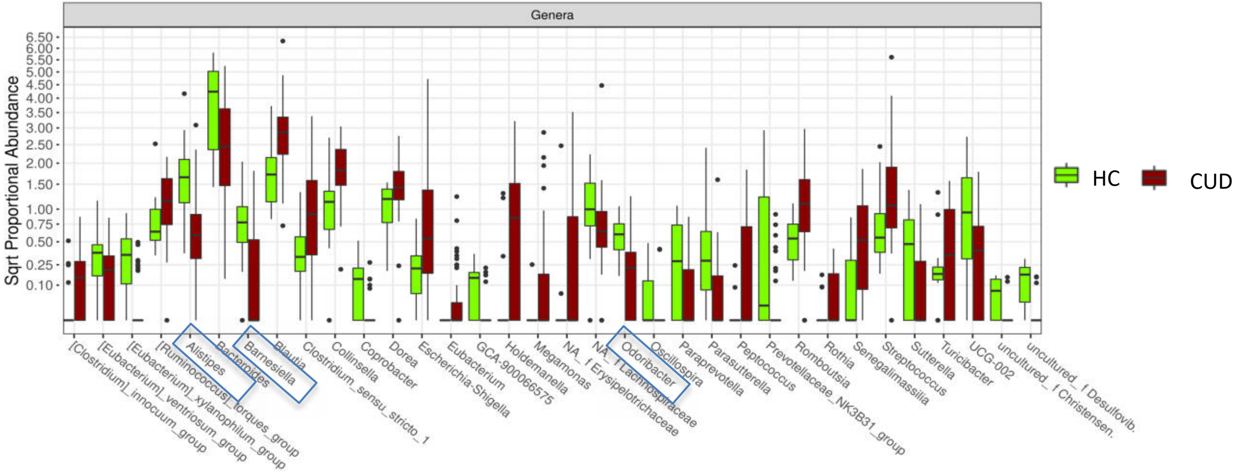




Principal coordinates analysis (PCoA) and alpha-diversity indices both highlighted clearly differences between HC and CUD samples

Results - Microbiota composition in CUD and HC

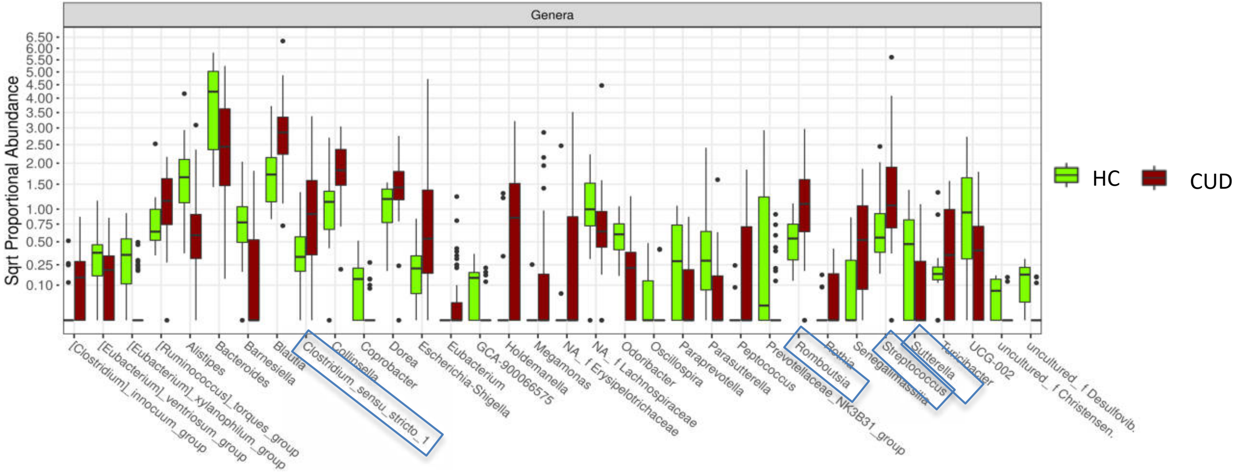
Stool samples



<u>Cud patients showed lower abundances of health-promoting, Alistipes spp., Barnesiella spp. and Odoribacter spp.</u>

Results - Microbiota composition in CUD and HC

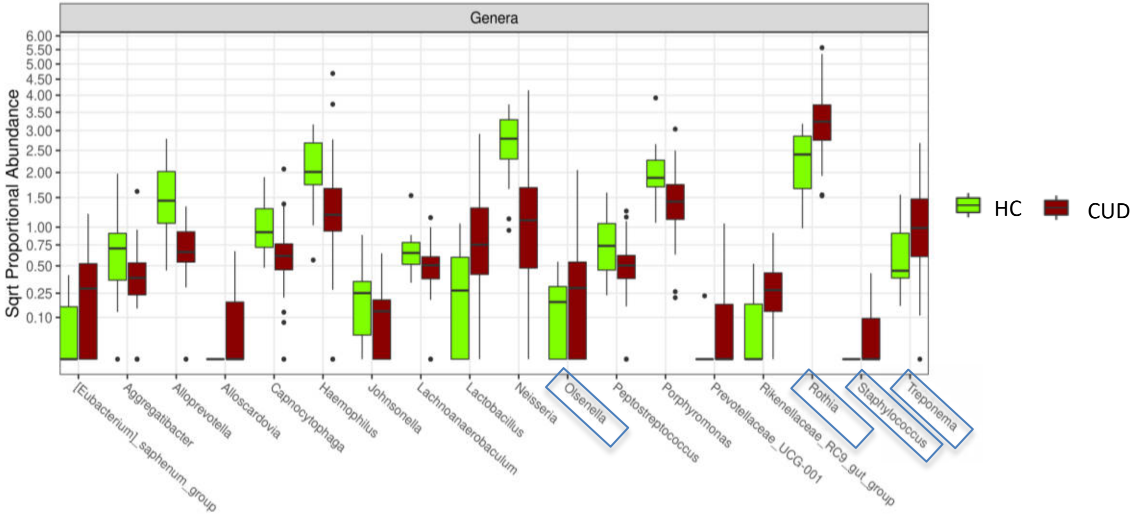
Stool samples



<u>**Cud patients showed higher levels of pro-inflammatory *Clostridium*, *Romboutsia*, *Streptococcus* and *Sutterella* genera**</u>

Results - Microbiota composition in CUD and HC

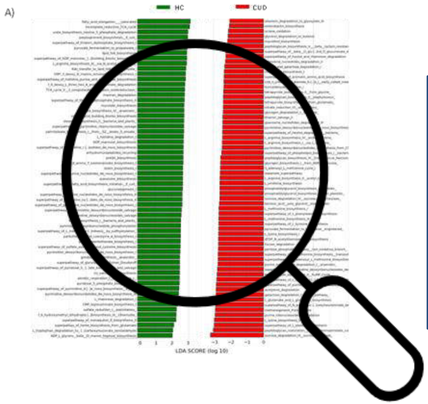
Saliva samples



***Treponema* spp., *Staphylococcus* spp., *Rothia* spp. and *Olsenella* spp., all genera related to periodontal inflammation and other oral disease, resulted overrepresented in CUD patietns**

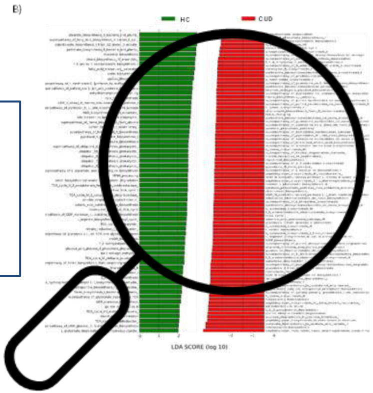
Results - Functional evaluation of gut microbiota: PICRUSt2 analysis

Stool samples



- ↑ **Several inflammatory pathways**
- ↑ **Glutamate and glutamine biosynthesis**
- ↑ **Tyrosine, lysine, arginine, phenylalanine biosynthesis**

Saliva samples

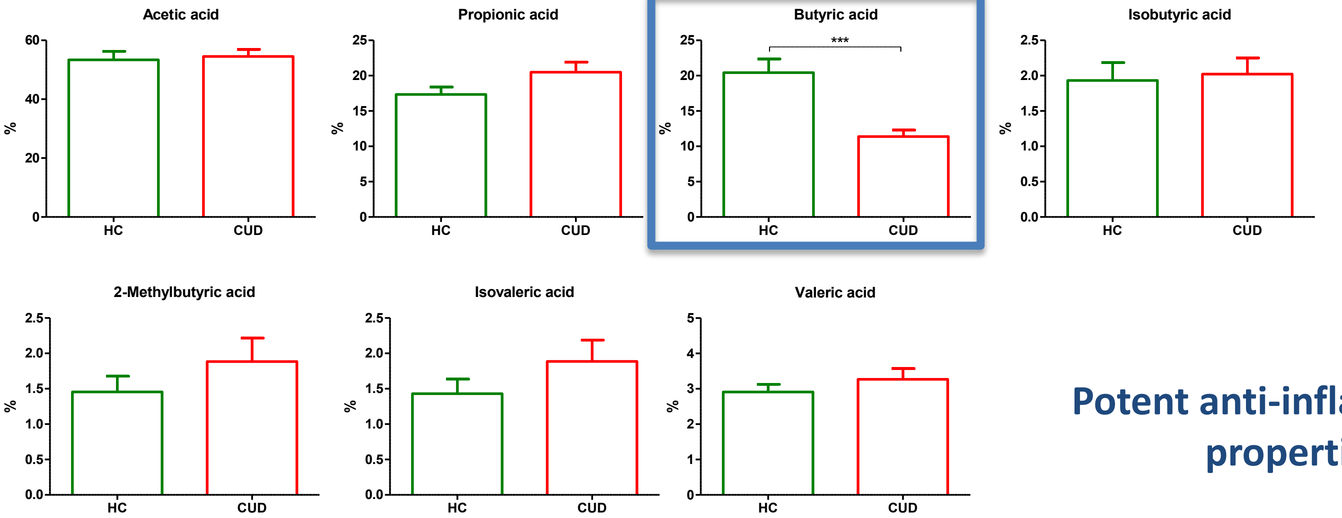


- ↑ **Isoleucine biosynthesis**
- ↑ **Valine biosynthesis**
- ↑ **Pentose phosphate pathway**
- ↑ **Pyruvate fermentation**

<u>Various metabolic functions of either salivary and intestinal microbiota resulted differentially expressed between CUD patients and HC</u>

Results - Functional evaluation of gut microbiota: SCFAs and MCFAS analysis

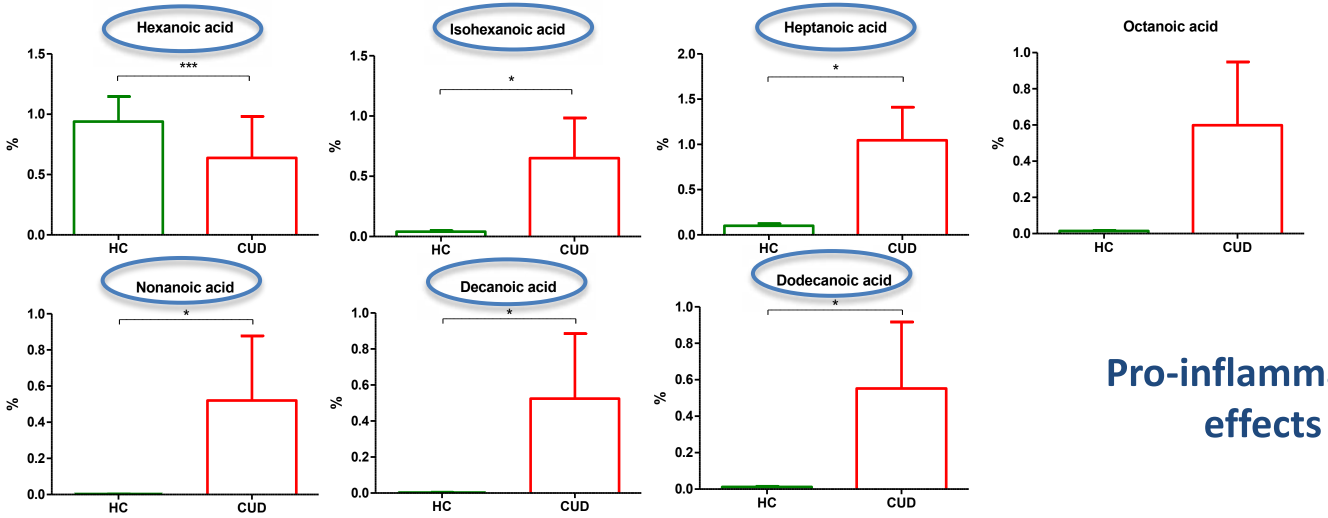
SCFAs



Potent anti-inflammatory properties

Functional evaluation of gut microbiota: SCFAs and MCFAS analysis

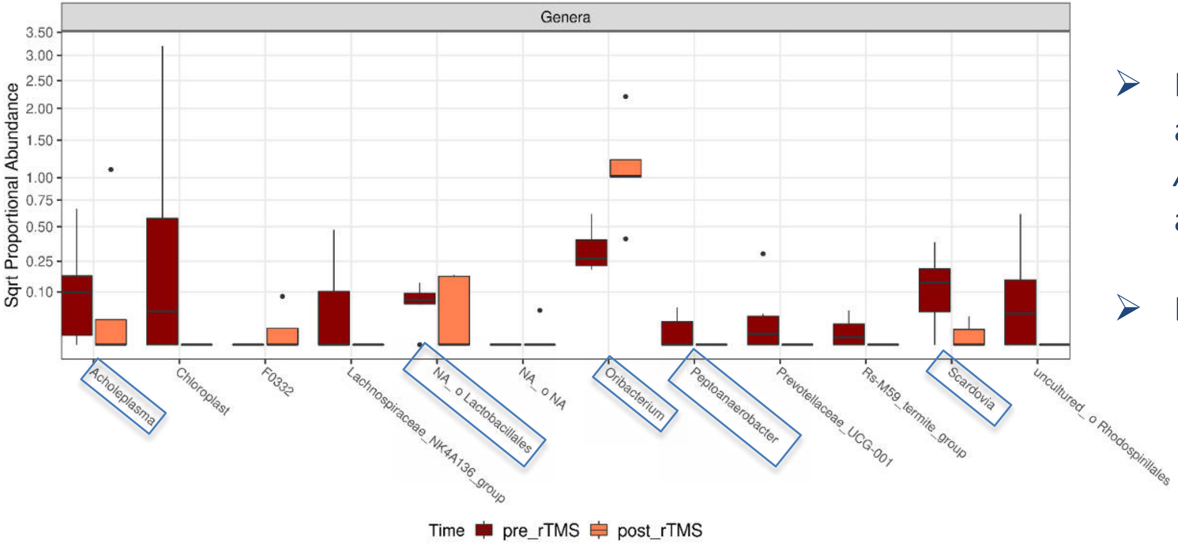
MCFAs



Pro-inflammatory effects

Results – rTMS effects on fecal and oral microbiota

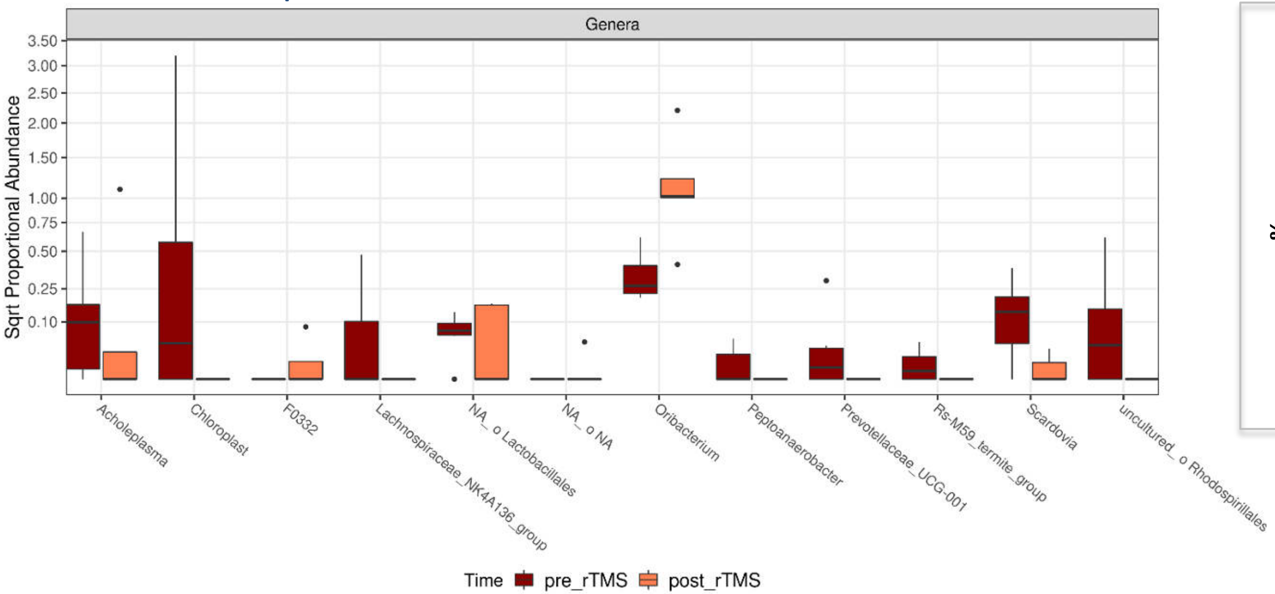
Saliva samples



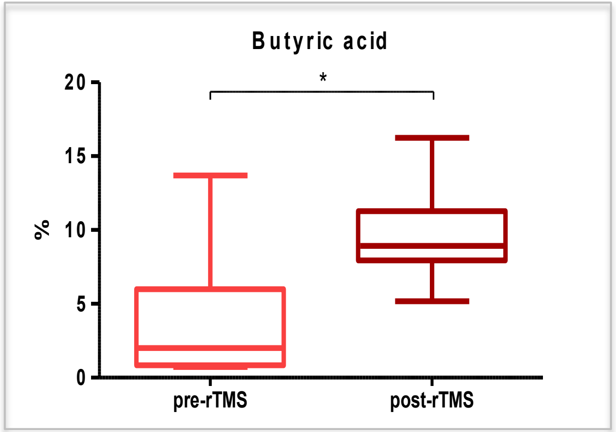
- Beneficial reduction of periodontal disease associated bacteria like Lactobaccillales, *Acholeplasma* spp., *Peptoanaerobacter* spp. and *Scardovia* spp.
- Beneficial increase of *Oribacterium* spp.

Results – rTMS effects on fecal and oral microbiota

Saliva samples



Fecal SCFA



Conclusions

- ✓ CUD patients showed a profound dysbiotic fecal and oral microbiota composition and function, confirming the evidence suggesting the important role of microbes in the pathogenesis of neuropsychiatric pathologies, including reward processes and substance-related disorders
- ✓ rTMS-induced cocaine abstinence and craving may represent a promising avenue for future therapeutic development.
- ✓ Diet rich in butyrate or psychobiotic supplementations could represent a supporting strategy to rTMS, modulating the gut brain communication and improving cognitive functions

UNIVERSITÀ DEGLI STUDI FIRENZE
DIPARTIMENTO DI MEDICINA SPERIMENTALE E CLINICA

UNIVERSITÀ DEGLI STUDI FIRENZE
NEUROFARBA
DIPARTIMENTO DI NEUROSCIENZE, PSICOLOGIA, AREA DEL FARMACO E SALUTE DEL BAMBINO

Toxicology & Neurophysiology Units

Prof. Guido Mananioni
Dr. Elisabetta Gerace
Dr. Virginia Cimino
Dr. Maya Salimova
Dr. Brunella Occupati
Dr. Cecilia Lanzi
Dr. Maenia Scarpino
Dr. Giovanni Lanzo
Dr. Antonello Grippo
Prof. Francesco Lolli

Microbiota-Immunity Axis Unit

Prof. Amedeo Amedei
Dr. Elena Niccolai
Dr. Edda Russo
Dr. Giulia Nannini
Dr. Simone Baldi
Dr. Leandro di Gloria

Mass spectrometry Unit

Prof. Gianluca Bartolucci
Dr. Marco Pallecchi

Bioinformatic Unit
Prof. Matteo Ramazzotti