

Supporting Information for Are Neuromodulation Interventions Associated with Changes in the Gut Microbiota? A Systematic Review.

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Table S1. Search terms.

Database	Search string(s)
MEDLINE	<ol style="list-style-type: none"> 1. Deep brain stimulation/ 2. ((brain? adj3 stimulation) or deep brain or DBS).ab,jw,kf,ti. 3. Electroconvulsive Therapy/ 4. (Electroshock? or electroconvuls* or electro convuls* or electric shock? or ECT or est therap* or est treatment?).ab,jw,kf,ti. 5. transcranial direct current stimulation/ or transcranial magnetic stimulation/ 6. ((transcranial adj3 stimulation) or tms or rtms or tdc).ab,kf,ti. 7. (electrostimulation or electro stimul*).mp. 8. Vagus Nerve Stimulation/ 9. ((vag* nerve adj3 stimulat*) or VNS).ab,kf,ti. 10. ((vagal nerve or vagus nerve) and electric* impuls*).mp. 11. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 12. microbiota/ or gastrointestinal microbiome/ 13. (Microbial or Microbiome or Microbiota?).mp. 14. (Bacter* or Dysbiosis or Faeces or Feces or Fecal or Flora or Gut).mp. 15. 12 or 13 or 14 16. 11 and 15
PsycINFO	<ol style="list-style-type: none"> 1. Deep brain stimulation/ 2. ((brain? adj3 stimulation) or deep brain or DBS).ab,id,jx,ti. 3. exp Electroconvulsive shock/ 4. (Electroshock? or electroconvuls* or electro convuls* or electric shock? or ECT or est therap* or est treatment?).ab,jx,id,ti. 5. transcranial direct current stimulation/ or transcranial magnetic stimulation/ 6. ((transcranial adj3 stimulation) or tms or rtms or tdc).ab,id,ti. 7. (electrostimulation or electro stimul*).mp. 8. Vagus Nerve/ 9. (vagal nerve or vagus nerve or vns).mp. 10. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 11. microorganisms/ or gastrointestinal system/

	<p>12. (Microbial or Microbiome or Microbiota?).mp.</p> <p>13. (Bacter* or Dysbiosis or Faeces or Feces or Fecal or Flora or Gut).mp.</p> <p>14. 11 or 12 or 13</p> <p>15. 10 and 14</p>
Google	"DBS AND microbio", "ECT AND microbio", "VNS AND microbio", "TMS AND microbio", "tDCS AND microbio"
International Clinical Trials Registry Platform / clinicaltrials.gov	<p>"deep brain stimulation, microbiota", "deep brain stimulation, microbiome", "DBS, microbiota", "DBS, microbiome",</p> <p>"electroconvulsive therapy, microbiota", "electroconvulsive therapy, microbiome", "ECT, microbiota", "ECT, microbiome",</p> <p>"transcranial magnetic stimulation, microbiota", "transcranial magnetic stimulation, microbiome", "TMS, microbiota", "TMS, microbiome",</p> <p>"transcranial direct current stimulation, microbiota", "transcranial direct current stimulation, microbiome",</p> <p>"tDCS, microbiota", "tDCS, microbiome", "vagus nerve stimulation, microbiota", "vagus nerve stimulation, microbiome",</p> <p>"VNS, microbiota", "VNS, microbiome"</p>

Table S2. Identified clinical trials that mention use of a neuromodulation intervention and gut microbiota assessment.

Identifier	Associated Institute	Date first posted	Title	Disorder / condition	Neuromodulation intervention
NCT04855344	The First Affiliated Hospital with Nanjing Medical University	April 2021	Deep Brain Stimulation Therapy and Intestinal Microbiota	Parkinson's Disease	DBS
NCT03703414	Ziv Hospital	October 2018	Characterization of Microbial, Immune and Epigenetic Biomarkers for Major Depressive Disorder and ECT Treatment	Major Depressive Disorder	ECT
NCT04956159	University of Calgary	July 2021	TMS in Inflammatory Bowel Disease	Inflammatory Bowel Disease	TMS
NCT05140356	University of Electronic Science and Technology of China	December 2021	Therapeutic Intervention Effects of rTMS on Children With Autism Spectrum Disorder	Autism Spectrum Disorder	rTMS
NCT05177601	University of Calgary	January 2022	iTBS-DCS in Obsessive Compulsive Disorder	Obsessive Compulsive Disorder	TMS (iTBS-DCS)
NCT04783103	Universitair Ziekenhuis Brussel	March 2021	Neuromodulation in the Elderly Depressed: a Brain Imaging Pilot Study	Elderly depressed	tDCS combined with dTMS
NCT03601117	Stanford University	July 2018	Accelerated Intermittent Theta Burst Stimulation for Depressive Symptoms (aTBS)	Major Depressive Disorder	TMS (accelerated intermittent theta burst stimulation)
NCT04497493	Tianjin Anding Hospital	August 2020	Efficacy of Transcranial Direct Current Stimulation (tDCS) for the Treatment of Major Depressive Disorder	Major Depressive Disorder	tDCS
NCT03953768	University of Louisville	May 2019	VNS Prospective Neuromodulation of Autonomic, Immune and Gastrointestinal Systems (VNSAIG)	Epilepsy	VNS

NCT05090267	University of Florida	October 2021	The Path Study: Cognitive and Inflammation Targeted Gut-brain Interventions in Alcohol; Probiotics, Alcohol, Transcutaneous Vagus Nerve Stimulation, and HIV Study	Cognitive impairment and HIV	Transcutaneous VNS
DRKS00024823	Goethe Universität Frankfurt Psychiatrie	October 2021	Modulation of reward responsiveness, gut microbial composition and immune response in major depressive disorder through transcutaneous vagus nerve stimulation (MODULATE-DEPRESSION) - MODULATE-DEPRESSION	Major Depressive Disorder	Transcutaneous VNS
https://ufhealth.org/research-study/investigation-microbiota-changes-patients-dbs	University of Florida Health	Unknown	Investigation of Microbiota changes in patients with DBS	DBS	PD

DBS = deep brain stimulation, ECT = electroconvulsive therapy, iTBS-DCS = intermittent Theta-Burst direct current stimulation, PD = Parkinson's Disease, rTMS = repetitive transcranial magnetic stimulation, tDCS = transcranial direct current stimulation, TMS = transcranial magnetic stimulation, VNS = vagal nerve stimulation

Table S3. Methods of fecal microbiome analysis in included studies.

Reference	Fecal sample collection	Sample storage	DNA analysis	DNA extraction kit	Target region (primer set)	Sequence platform	Sequence read comparison tool and reference database	Beta diversity assessment
<i>Human studies</i>								
Kanayama <i>et al.</i> (2019)	Collected promptly after defecation	-20.0 °C	16S ribosomal RNA gene (Terminal restriction length polymorphism method)	NR	NR	NR	NR	-
Artifon <i>et al.</i> (2020)	Collected by stool swab	NR	16S ribosomal RNA gene	NR	NR	NR	NR	-
Ferrulli <i>et al.</i> (2021)	Collected in sterile containers	-20.0 °C	16S ribosomal RNA gene	QIAmp DNA Stool Mini Kit (Qiagen, Milan, Italy)	V2-4-8 and V3-6, 7-9 regions	Ion Torrent Personal Genome Machine system	BLAST against MicroSEQ ID database, unaligned reads against GreenGenes database	-
Lubomski <i>et al.</i> (2022) ^a	Collected into sterile pots	-80.0 °C	16S ribosomal RNA gene	MP Biomedicals FastDNA™ SPIN Kit for Faeces (MP Biomedicals, Santa Ana, California, USA)	V3-V4 regions (341F/805R)	MiSeq, Illumina, San Diego, California, USA	Silva (v.138) database	PCoA with Bray-Curtis dissimilarity, unweighted and weighted UniFrac distance

<i>Animal studies</i>								
Haney <i>et al.</i> (2018) ^b	Collected circa 2 fecal pellets after defecation in temporary, autoclaved cages	-80.0 °C	16S ribosomal RNA gene	Qiagen DNeasy Blood and Tissue kit (Hilden, Germany)	V4 region (U515F/806R)	MiSeq, Illumina, San Diego, California, USA	BLAST against SILVA database	PERMANOVA with Bray-Curtis and unranked Jaccard distances
Phillips Campbell <i>et al.</i> (2016) ^c	Surgically obtained 3 fecal pellets from the distal colon	-80.0 °C	16S ribosomal RNA gene	PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, California, USA)	V3-V4 regions (341F/785R)	MiSeq, Illumina, San Diego, California, USA	QIIME 1.8.0 against GreenGenes database 13.8	PCoA with weighted and unweighted UniFrac
Seewoo <i>et al.</i> (2022)	Collected 2-6 fecal pellets during behavioral testing sessions	-80.0 °C	16S ribosomal RNA gene	Qiagen DNeasy PowerSoil Pro Kit ^d	V3-V4 regions (341F/modified 806R)	MiSeq, Illumina, San Diego, California, USA	Bayesian LCA-based taxonomic classification method against NCBI 16S ribosomal RNA database	PCoA with weighted and unweighted UniFrac

^aOne sample from a patient receiving LCIG obtained two weeks after device initiation was removed from analyses due to insufficient generation of high-quality reads. Two DBS and four LCIG participants were unable to provide a sample two weeks prior to DBS/LCIG initiation

^bDNA could not be extracted from one sample at day 0 (WT mouse) and two samples at day 8 (one WT and one SOD1^{dl} mice) due to insufficient generation of high-quality reads

^cFecal samples were collected from 27 animals (6/9 sham PO + sham VNS, 6/12 PO + sham VNS, 8/10 PO + right-VNS, 7/9 PO + left-VNS)

^dInformation obtained from correspondence with the authors

LCA = latent class analysis, PCoA = principle coordinate analysis, NR = not reported

Table S4. Risk of bias assessment in the randomized controlled trial as assessed with the RoB2 tool. Judgements include low (+), moderate (-), serious (x), critical (xx) or unknown (?) risk of bias.







Domain	Ferrulli <i>et al.</i> (2021)
1. Risk of bias arising from the randomization process	
2. Risk of bias due to deviations from the intended interventions	
3. Missing outcome data	
4. Risk of bias in measurement of the outcome	
5. Risk of bias in selection of the reported result	
Overall risk of bias judgement	

Table S5. Risk of bias assessment in non-randomized study as assessed with the ROBINS-I tool. Judgements include low (+), moderate (-), serious (x), critical (xx) or unknown (?) risk of bias.









Domain	Lubomski <i>et al.</i> (2022)
1. Bias due to confounding	
2. Bias in selection of participants into the study	
3. Bias in classification of interventions	
4. Bias due to deviations from intended interventions	
5. Bias due to missing data	
6. Bias in measurement of outcomes	
7. Bias in selection of the reported result	
Overall risk of bias judgement	

Table S6. Risk of bias assessment in case reports as assessed with the JBI Critical Appraisal Checklist for Case Reports. Judgements include low (+), high (x) or unknown (?) risk of bias.

















Checklist item	Kanayama <i>et al.</i> (2019)	Artifon <i>et al.</i> (2020)
1. Were patient's demographic characteristics clearly described?		
2. Was the patient's history clearly described and presented as a timeline?		
3. Was the current clinical condition of the patient on presentation clearly described?		
4. Were diagnostic tests or assessment methods and the results clearly described?		
5. Was the intervention(s) or treatment procedure(s) clearly described?		
6. Was the post-intervention clinical condition clearly described?		
7. Were adverse events (harms) or unanticipated events identified and described?		
8. Does the case report provide takeaway lessons?		

Table S7. Risk of bias assessment in animal studies as assessed with SYRCLE’s Risk of Bias tool. Judgements include low (+), high (x) or unknown (?) risk of bias. *Indicates that the information underlying the judgement that was retrieved through correspondence with the study’s authors. **Indicated that the information underlying the judgement was published in a prior publication¹.

Domain (type of bias)	Haney <i>et al.</i> (2018)	Phillips Campbell <i>et al.</i> (2016)	Seewoo <i>et al.</i> (2022)
1. Sequence generation (selection bias)			
2. Baseline characteristics (selection bias)			
3. Allocation concealment (selection bias)			
4. Random housing (performance bias)			
5. Blinding (performance bias)			
6. Random outcome assessment (detection bias)			
7. Blinding (detection bias)			
8. Incomplete outcome data (attrition bias)			
9. Selective outcome reporting (reporting bias)			
10. Other sources of bias (other)			

Table S8. Fecal microbiome profiles of disease state in the included studies

Study	α -diversity	β -diversity	Relative bacterial abundances	Additional findings
<i>Human studies</i>				
Kanayama <i>et al.</i> (2019)	NA	NA	NA	NA
Artifon <i>et al.</i> (2020)	NA	NA	NA	NA
Ferrulli <i>et al.</i> (2021)	NA	NA	NA	NA
Lubomski <i>et al.</i> (2022)	PD patients versus HCs No differences in Shannon and Simpson's indices at week 0	Separation between HCs and PD patients at week 0	PD patients versus HC Phylum level: ↑ <i>Verrucomicrobia</i> ↓ <i>Firmicutes</i> , <i>Bacteroidetes</i> Order level: ↑ <i>Verrucomicrobiales</i> , <i>Pasteurellales</i> Family level: ↑ <i>Verrucomicrobiaceae</i> , <i>Pasteurellaceae</i> ↓ <i>Veillonellaceae</i> Genus level: ↑ <i>Akkermansia</i> ↓ <i>Blautia</i> , <i>Faecalibacterium</i> , <i>Roseburia</i> , <i>Fusicatenibacter</i> , <i>Haemophilus</i> , <i>Gemmiger</i> , <i>Butyricoccus</i> , <i>Streptococcus</i>	NR
<i>Animal studies</i>				
Haney <i>et al.</i> (2018)	SOD1^{dl} mice versus age-matched WT controls No differences in Chao1 and Simpson indices one day before VNS surgery	No separation between SOD1 ^{dl} mice and age-matched WT controls one day before VNS surgery	NR	NR
Phillips Campbell <i>et al.</i> (2016)	PO + sham VNS vs sham PO No differences in number of observed OTUs, Phylogenetic diversity whole tree	No separation between animals receiving PO and sham VNS and animals receiving sham PO and sham VNS at day 43	PO + sham VNS vs sham PO Phylum level: ↑ Actinobacteria/Proteobacteria ratio Family level: ↑ <i>Lachnospiraceae</i> Genus level:	Functional metabolic pathway analysis <i>PO + sham VNS vs sham PO</i> ↓ bacterial genera expressing genes associated with ATP-binding cassette transport.

	and Chao1 index at day 43		↑ <i>Bacteroides</i> , <i>Lactobacillus</i> , <i>Christensenellaceae</i> , <i>Epulopiscium</i> , <i>SHA-98</i> , <i>Victivallaceae</i> ↓ <i>Dehalobacterium</i> , <i>Ruminococcus</i> , <i>Mogibacteriaceae</i> , <i>Desulfovibrio</i>	↑ bacterial genera expressing genes associated with amino sugar and nitrogen metabolism
Seewoo <i>et al.</i> (2022)	After 2 weeks of CRS No change number of observed OTUs, abundance-coverage estimator, Shannon entropy and inverse Simpson index	No separation between pre-CRS and post-CRS	After 2 weeks of CRS Class level: ↑ Deltaproteobacteria Order level: ↑ Desulvibrionales Genus level: ↑ <i>Anaerostipes</i> , <i>Frasingicoccus</i> (members of <i>Lachnospiraceae</i> family)	After 2 weeks of CRS No changes in KEGG pathways

ATP = adenosine triphosphate, CRS = chronic restraint stress, HC = healthy control, KEGG = Kyoto Encyclopedia of Genes and Genomes, NA = not applicable, NR = not reported, OTU = operational taxonomic unit, PD = Parkinson's Disease, PO = pressure overload, VNS = vagal nerve stimulation, WT = wild type.

SI References

1. Beaumont E, Wright GL, Southerland EM, et al. Vagus nerve stimulation mitigates intrinsic cardiac neuronal remodeling and cardiac hypertrophy induced by chronic pressure overload in guinea pig. *Am J Physiol - Hear Circ Physiol*. 2016;310(10):H1349-H1359. doi:10.1152/ajpheart.00939.2015