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Supplementary information

The gut microbiota–brain axis in behaviour and brain disorders

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Supplementary Table 1. Approaches and Tools for Investigating the Gut microbiota-brain axis

Technique/tool	Principle	Refs. to Microbiota Research	Other Refs.
16S microbiome profiling	Sequencing of the 16S rRNA gene isolated from gut samples (either tissue or fecal samples) is used to provide the general composition of the bacteria in the gut from a "bird's eye" perspective (i.e., roughly at the genus level and higher taxonomy). This information is a useful starting point for gut microbiota investigations.	[1–5]	
Shotgun metagenomic sequencing	Provides a comprehensive description of gut microbial communities at the species level as well as the gene catalog carried by those members through sequencing of DNA isolated from the gut microbiota. Can be used to define the "functional potential" of a polymicrobial community (e.g., their metabolic complexity, presence of classical virulence genes, etc.).	[6–8]	
Shotgun metatranscriptomic sequencing	Provides survey of gut microbiome transcriptional activity, which acts as a proxy for bacterial physiology. This RNA sequencing approach should be used in conjunction with metagenomic (DNA) sequencing to provide context to expression patterns, and is has been applied in chronic diseases, such as inflammatory bowel disease.		[9]
Metabolomics	Provides survey of molecules, nutrient sources, and other metabolic products that are available in different body compartments, including the gut, brain, and circulatory systems.	[10,11]	
***In vitro* bacterial culturing**	Culturing of bacteria *in vitro* allows for fine control of the bacteria's environment, which can be used to determine	[12,13]	

	bacterial metabolism (including the degradation of psychiatric drugs) and cooperative growth using signaling molecules.		
Germ-free and antibiotic treated mouse models	Provides ability to test necessity of gut microbiota in mediating biological effects, as removal of the gut microbiota can be tested to either enhance or suppress phenotypes. Germ-free animals must be bred and maintained under completely sterile conditions, but antibiotic administration has been used to study depletion of gut bacteria from animals born with a complete microbiota.	[14–18]	
Fecal microbiota transfers in mice (not clinical FMT)	The gut microbiota of mammals (e.g., humans or mice, etc.) can be transferred to germ-free or antibiotic treated rodents through gavage with slurries from donor fecal samples. This can be used to test if the gut microbiota is sufficient to transfer a phenotype from one mouse to another or from a human to a recipient mouse.	[1,2,10,19]	
Monocolonization	The colonization of a germ-free mouse with a single bacterial species to determine their sufficiency in modulating a phenotype. Can be expanded to include gene knockouts of specific pathways in genetically tractable organisms.	[11,20]	
Probiotic administration	Bacteria can be administered to rodent models through gavage or, in some cases, through drinking water to suppress adverse neuropsychiatric phenotypes. Stable colonization of probiotics is difficult to attain given the nature of colonization resistance by the gut microbiota, so probiotics may require chronic administration to have an appreciable effect.	[21,22]	
Gene knockouts (KO) and transgenic	Useful for studying genes and genetic pathways implicated disease or other outcomes. KO and transgenic animals can	[11,17,18,23]	

animals	be used for modelling human diseases associated to genetic risks.		
Cre and/or Lox-P recombinase animals	Site, cell type and time-specific recombination system widely used for conditional gene-targeting, a bioengineering tool repurposed from bacteriophage recombination systems.	**23,24**	
CRISPR/Cas9 system	Cas9 has been used to induce cell-type (e.g., neurons) genome-editing and generation of stable gene knockouts, a bioengineering tool adapted from bacterial "immune systems" to phage infection. Useful to model disorders associated to genetic risks, including ASD.		**25**
Pharmacological agents	Drugs for studying neurocircuitry and neurotransmitter involvement. Can have limited site and time specificity (e.g., 5-HT receptor agonists, neurotransmitter receptor antagonists, or sodium channel blockers).	**19,23,26**	
Vagotomy	The surgical severance of the vagus nerve that disrupts signaling from various peripheral organs to the brain. Different types of vagotomy can be used depending on the goal (e.g., truncal vagotomy, selective vagotomy).	**21,27,28**	
Optogenetics	Enables genetically engineered cells to express membrane bound light-sensitive proteins (opsins) that can affect neuronal activity, inhibition, or modulation of intracellular signaling in transgenic animals (e.g., Cre-recombinase animals). Activity turned on or off by light.	**N/A**	**29**
Chemogenetics	Engineered proteins are used to reach neuronal temporal and spatial specificity in transgenic animals. Designer receptors exclusively activated by designer drugs (DREADDs) are the most common tool used to define neuronal population activity.	**30**	**31**
Electrophysiology	Technique that explores electrical activity from action potentials in neuronal cells *in vitro* and *in vivo* (live	**10,22,23**	

	behaving animals).		
Golgi staining, Dyes and Tracing Proteins	Tracing tools that can be deliver to nervous system cells (e.g., non-specific membrane tracing; Fluorogold and Retrobeads; dextran amines).	[32]	
Immediate Early Genes (IEG) readouts	Allows a broad overview of recently activated neurons during defined windows of time and in response to a specific stimulus.	[33]	
Functional Magnetic Resonance (fMRI)	Whole brain imaging in a live subject for functional connectivity measurements. Poor temporal resolution. Not cell-type specific.	[34,35]	
Electron microscopy	Allows ultrastructure cell morphology and visualization of fine synapsis connectivity.	[36,37]	

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