Table S1. Summary of Key Evidence Supporting Microbiome–Gut–Brain Axis Regulation During Adolescence

Evidence category	Experimental approach/model	Major findings relevant to adolescent neurodevelopment	Proposed mechanisms linking microbiota to brain function	Representative references
Microbiota regulation of brain development and behavior	Germ-free (GF) animals, microbial colonization studies, and microbiota manipulation models	Absence or disruption of microbiota alters stress responsivity, anxiety-like behaviors, neurodevelopmental trajectories, and expression of neuroplasticity-related genes. Restoration of microbial communities during developmental windows can partially normalize behavioral phenotypes.	HPA-axis regulation, altered neurotransmitter signaling, immune maturation, neurotrophic factors including BDNF, and microbial metabolite signaling	Sudo et al., 2004; Diaz Heijtz et al., 2011; Crumeyrolle-Arias et al., 2014; Cryan et al., 2019
Adolescent microbiome as a critical developmental window	Developmental microbiome profiling across life stages; adolescent animal and human studies	Adolescence represents a period of simultaneous microbial maturation, pubertal hormone changes, immune remodeling, and neural circuit refinement, suggesting increased sensitivity to microbiome perturbations.	Interaction between microbial metabolites, synaptic maturation, immune signaling, endocrine regulation, and adolescent brain plasticity	Borre et al., 2014; Flannery et al., 2019; Carson et al., 2023; Cryan, 2025

Microbiome disruption and neurobehavioral outcomes	Antibiotic-induced microbiome depletion and adolescent microbiota perturbation models	Microbiome depletion during adolescence produces persistent behavioral alterations, including anxiety-like behaviors, cognitive changes, and altered stress responses.	Microglial activation, neuroinflammation, altered hippocampal neurogenesis, neurotransmitter metabolism, and immune-mediated signaling	Möhle et al., 2016; Lach et al., 2020; Cook and Prinz, 2022; Fishbein et al., 2023
Microbial regulation of neurotrophic pathways	Microbiota manipulation combined with molecular analysis of brain tissue	Alterations in gut microbial communities modify central BDNF expression and neuronal signaling pathways associated with cognition, emotional regulation, and synaptic plasticity.	BDNF signaling, hippocampal plasticity, serotonin pathways, immune modulation, and microbial-derived metabolites	Bercik et al., 2011; Dash et al., 2022; Cryan et al., 2019
Microbial metabolites as mediators of brain function	Metabolomics, microbial functional profiling, SCFA analysis, and dietary interventions	Microbial-derived metabolites including short-chain fatty acids (SCFAs), bile acids, and tryptophan metabolites influence neuronal development, mood regulation, and inflammatory pathways.	Epigenetic regulation, blood–brain barrier modulation, immune regulation, enteroendocrine signaling, and neurotransmitter synthesis	Cryan et al., 2019; Needham et al., 2020; Cheng et al., 2024; Tang et al., 2022
Dietary modulation of microbiome–	Dietary interventions, omega-3	Nutritional interventions modify microbial composition and can prevent or attenuate	Increased beneficial microbial taxa, SCFA production, reduced	Provensi et al., 2019; Robertson et al., 2017; Horn et

brain communication	supplementation, fiber/prebiotic approaches, and stress models	stress-associated cognitive and behavioral changes during adolescence.	inflammation, improved neurogenesis, and altered stress signaling	al., 2022; Bogachuk et al., 2024
Microbiome, stress, and early-life adversity	Early-life stress models, psychosocial stress cohorts, inflammatory profiling	Early adversity alters microbiome composition and interacts with stress systems to increase vulnerability to psychiatric outcomes.	HPA-axis activation, inflammatory signaling, immune dysregulation, altered microbial metabolites, and impaired neuroplasticity	Beurel and Nemeroff, 2024; Koss and Gunnar, 2018; Warren et al., 2024
Microbiome regulation of reward and addiction-related pathways	Behavioral models of reward, dopamine pathway analysis, substance-use related microbiome studies	Microbiome alterations influence reward sensitivity, dopamine signaling, and vulnerability to substance-use-related behaviors, processes particularly relevant during adolescent brain maturation.	Dopaminergic signaling, vagal communication, microbial metabolites, mesolimbic pathway regulation, and neuroimmune interactions	Hamamah et al., 2022; Barbu et al., 2025; Browning et al., 2024
Sex differences, puberty, and microbiome–hormone interactions	Sex-stratified microbiome analyses, hormone manipulation studies, pubertal development studies	Sex steroids shape microbial composition, while microbial communities influence hormone metabolism, contributing to sexually dimorphic	Estrobolome activity, androgen-associated microbial patterns, immune modulation, bile acid metabolism, and hormone-	Sisk and Zehr, 2005; Elderman et al., 2018; Kaliannan et al., 2018; Vemuri et al., 2019; Shobeiri et al., 2022

		neurodevelopmental outcomes.	dependent microbial signaling	
Human adolescent microbiome studies and translational evidence	Longitudinal cohorts, 16S rRNA sequencing, shotgun metagenomics, metabolomics, behavioral assessments	Human studies demonstrate developmental changes in microbial diversity and associations between microbiome composition, mental health, metabolism, and neurodevelopmental outcomes.	Microbial ecology, diet-dependent metabolites, immune pathways, endocrine regulation, and host–microbe interactions	Hollister et al., 2015; Laue et al., 2022; Mancabelli et al., 2024; Mohr et al., 2024
Microbiome–immune–brain communication	Immune profiling, microglial analysis, inflammatory pathway assessment	Microbial signals regulate immune development and microglial function, linking intestinal ecology with neurodevelopment and disease susceptibility.	Microglial maturation, cytokine signaling, intestinal permeability, immune tolerance, and neuroinflammation	Cook and Prinz, 2022; Park and Im, 2022; Morais et al., 2021
Clinical and therapeutic approaches targeting the microbiome	Probiotic, psychobiotic, synbiotic, and microbiota-targeted intervention studies	Modulation of microbial communities represents a potential strategy to influence stress, anxiety, depression, and cognitive outcomes, although adolescent-specific trials remain limited.	Restoration of microbial diversity, SCFA production, serotonin pathways, immune regulation, and gut barrier function	Heidarzadeh-Rad et al., 2020; Merkouris et al., 2024; Kumar et al., 2023