The Homeostasis of the Gut-Brain Axis in Human and Animal Models of Alzheimers

Anvi Sinha

Abstract

The Gut-Brain Axis (GBA) is a bidirectional channel through which the enteric nervous system in the abdomen and the central nervous system in the cranium can communicate. The gut microbiome strongly influences the GBA and is composed of a variety of bacterial strains (e.g. E. col, B. bifidum) which are influenced by a variety of factors during growth and development. In this review, I will summarize studies focused on the influences of psychostimulants, early life adversity, and diet on human and animal microbiota as well as their influence on neurodegeneration in the brain. Drugs, stress, and diet are known to increase inflammation and disrupt the blood-brain barrier (BBB). In turn, activation of pro-inflammatory cytokines lead to neuroinflammation in the brain which have been shown to increase the susceptibility to Alzheimer's disease (AD) and overall cognitive impairment.

Introduction

The gut microbiome refers to the vast community of microorganisms residing in the gastrointestinal tract. Comprising bacteria, fungi, viruses, and other microorganisms, the gut microbiome exerts a profound influence on human health and plays a crucial role in numerous physiological processes. Recent studies have uncovered the bidirectional communication pathway between the gut and the brain, known as the gut-brain axis [1]. This intricate system enables constant signaling and information exchange between the gut microbiome and the central nervous system (CNS) through neural, endocrine, and immune pathways.

Mounting evidence suggests that alterations in the composition and function of the gut microbiome can impact brain health and contribute to neurodegenerative diseases such as Alzheimer's disease (AD). AD is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioral changes. It is the most common form of dementia, affecting millions of people worldwide [2]. Despite extensive research, the exact mechanisms underlying AD pathogenesis remain elusive. Recent scientific advancements have shed light on the potential role of the gut microbiome and the gut-brain axis in the development and progression of AD. Understanding the complex interplay between the gut microbiome and the brain has emerged as a fascinating frontier in Alzheimer's research. The gut microbiome produces an array of metabolites, including short-chain fatty acids, neurotransmitters, and immunomodulatory molecules, which can directly influence CNS function and neuroinflammation [3]. Additionally, the gut microbiome interacts with the intestinal barrier, modulates the immune system, and influences systemic inflammation, all of which have been implicated in AD pathogenesis.

Emerging studies have highlighted specific microbial imbalances in individuals with the disease, caused by a variety of environmental factors including early life stress [4], poorly balanced diets, and substance abuse [5]. These alterations are associated with increased neuroinflammation, oxidative stress, amyloid-beta (Aβ) plaque deposition, and tau hyperphosphorylation—hallmarks of AD pathology. Furthermore, animal models and preclinical investigations have demonstrated that manipulating the gut microbiome can influence cognitive function, amyloid deposition, and neuroinflammation. Understanding the complex relationship between the gut microbiome, the gut-brain axis, and AD holds tremendous potential for

developing novel diagnostic tools and therapeutic interventions. It may open doors for innovative therapeutic strategies, such as microbiota-based interventions, dietary interventions, or modulation of the gut-brain axis, to restore microbiome homeostasis and alleviate AD-related symptoms [6].

In this review, I will discuss the various factors that contribute to the degeneration of the gut microbiome, and the consequently affected mechanisms in the gut-brain axis. I will look at recent findings from human and animal studies, highlight potential mechanisms underlying the gut-brain connection in AD pathogenesis, and explore promising avenues for therapeutic interventions. By delving into the interplay between the gut microbiome and the brain, we hope to contribute to the growing body of knowledge and inspire further research aimed at unraveling the mysteries of Alzheimer's disease.

Psychostimulant Function in the Brain

Substance use disorder encompasses the detrimental effects of psychoactive drugs on the body and brain, and it is categorized into three main classes: hallucinogens, depressants, and stimulants. Among these, psychostimulants have gained particular attention due to their association with significant morbidity and profound impact on emotions. This class includes substances such as amphetamine, cocaine, 3,4-methylenedioxymethamphetamine (MDMA), caffeine, as well as other prescribed stimulants. At low doses, psychostimulants induce heightened alertness, enhanced cognitive function, improved mental attention, and sociability. Conversely, fatigue, sexual stimulation, and appetite are reduced because of excessive psychostimulant use. As the dose increases to moderate levels, euphoria and cognitive impairment start to emerge. Finally, high doses of psychostimulants can lead to tremors, agitation, psychosis, rapid muscle breakdown, and, with repeated use, substance use disorder[7].

Psychostimulants encompass a diverse range of chemical classes, including coca alkaloids (cocaine, benzoylecgonine), substituted phenethylamines, phenylpropanolamine, and aminoaryloxazolines. Cocaine, a benzoylecgonine derivative, while amphetamine is the prototype structure for synthetic psychostimulants and possesses both clinical anorexic and stimulant properties. Methamphetamine, a synthesized derivative of amphetamine, exhibits heightened wakefulness and stronger euphoric effects that can lead to rapid abuse, addiction, and other psychiatric consequences [8].

Cocaine and amphetamines increase dopamine levels in both the CNS and the periphery through inhibiting or reversing the dopamine transporter (DAT) [9]. Interestingly, various populations of leukocytes, such as B cells, T cells, and monocytes, express different subtypes of dopamine receptors. When these receptors are stimulated by dopamine, it can influence their production of cytokines and other inflammatory mediators [10]. Emerging evidence suggests that cocaine itself might activate PRRs (Pattern Recognition Receptors) and induce an independent inflammatory response, distinct from its influence on dopamine, through several other mechanisms [11].

Animal Models of Stimulant Use Disorder (SUD)

Neuroinflammation resulting from psychostimulant use has been observed in various animal models[12]. Glial cells are implicated in the activation process, with methamphetamine inducing dose-dependent microglial activation throughout the brain [13]. Studies have shown that inhibiting microglial activation using minocycline or the toll-like receptor 4 (TLR-4)

antagonist ibudilast reduces the rewarding effects of stimulants in mice [14]. However, the precise mechanism underlying microglial activation by psychostimulants remains unclear, and ongoing research aims to elucidate this aspect [12].

Recent studies on rats suggest that cocaine, in particular, may bind to the TLR4 receptor, which is expressed on microglia in the central nervous system [15]. In silico and in vitro modeling conducted by Northcutt et al. (2015) [16] demonstrated that cocaine binds to the TLR4 receptor in mice. Subsequent studies indicated that signaling through TLR4 is essential for cocaine induced dopamine release, conditioned place preference (CPP), and self-administration of cocaine). Other studies have revealed that TLR4 activity in the ventral tegmental area (VTA) affects the reinstatement of cocaine seeking via IL-1 signaling [17]. However, Tanda et al. (2016) [18] disputed the effects of TLR4 antagonists on dopamine release, suggesting non specific effects on behavior. The role of TLR4-mediated signaling in substance use models remains an area of ongoing research, holding potential significance in addiction neurobiology.

Research on SUD models has highlighted the significance of cytokine and chemokine signaling, impacting brain plasticity and behavior [19]. Recent work by Calipari et al. (2018) identified upregulated granulocyte colony-stimulating factor (G-CSF) after prolonged cocaine exposure, with G-CSF treatment enhancing neuronal activation in the nucleus accumbens (NAc) and prefrontal cortex post-acute cocaine injection. G-CSF heightened cocaine-related behaviors like conditioned place preference (CPP), locomotor sensitization, and self-administration. In contrast, Lewitus et al. (2016) [20] observed increased microglial production of TNF-α in the NAc following cocaine, impacting dopamine D1 receptor-containing neurons. TNF-α knockout mice showed heightened cocaine sensitization, while TNF-α inhibition reduced it. However, Northcutt et al. (2015) suggested that IL-1β via TLR4 signaling enhanced cocaine-induced dopamine release and behavioral responses. The conflicting outcomes arise from differing emphases in research. Lewitus investigated locomotor sensitization and glutamatergic plasticity in the nucleus accumbens (NAc), whereas Northcutt concentrated on cocaine's rewards and dopamine release from VTA neurons. This suggests that these similar pathways might exert distinct influences on drug responses, highlighting the need for further research on these molecular mechanisms.

Additionally, chemokine signaling has implications for psychostimulant use disorders. Introducing chemokine monocyte chemoattractant protein 1 (MCP-1) into rat midbrains elevates locomotor activity and striatal dopamine release [21], whereas knocking out the MCP-1 receptor CCR2 diminishes cocaine-induced locomotor sensitization and ERK signaling activation in the striatum [22]. Additionally, prolonged cocaine exposure elevates stromal cell-derived factor 1 (SDF-1) levels in both humans and mice (Araos et al., 2015), and infusing SDF-1 protein into the intraventricular region or VTA enhances cocaine-induced locomotion [23].

Substance Use Disorder in Humans

Numerous studies have investigated the impact of cocaine on the peripheral expression
of inflammatory mediators. Some studies have reported that acute cocaine use alters cytokine expression in serum or isolated peripheral leukocytes [24]. Abstinent cocaine users have been found to exhibit decreased serum levels of MCP-1 and several pro-inflammatory cytokines, including IL-6, IL-17, and TNF-α. However, there have been conflicting findings, with some studies indicating higher levels of IL-6 and decreased levels of the

anti-inflammatory cytokine IL-10 in active cocaine users [25].

Patients with SUDs exhibited increased serum expression of pro-inflammatory markers in response to drug cues or unpleasant images, suggesting a pro-inflammatory response to certain environmental stimuli [26]. These changes in peripheral immune function may potentially contribute to the development or persistence of psychostimulant use disorders. While peripheral inflammation may not directly correlate with central inflammatory processes, peripheral monocytes and T cells have demonstrated significant effects on the brain and behavior and can potentially cross the blood-brain barrier [27]. Postmortem examinations of the midbrain in cocaine addicts have shown an increase in activated microglia and activated macrophages, along with a decrease in dopamine cell bodies [28]. PET studies using tracers that bind to activated glial cells have indicated increased microglial activation in subjects with methamphetamine use disorder, with a negative correlation between the duration of abstinence and microglial activity [29]. However, another PET study in patients with long-term cocaine use disorder did not find differences in microglial binding between controls and patients with cocaine use disorder [30]. These findings suggest the involvement of peripheral and central inflammatory mechanisms in psychostimulant use disorders, but further research is needed to fully understand these processes.

Psychostimulant effects on the Gut-Brain Axis

Recent research is exploring the gut-immune-brain axis (often referred to as the gut-immune-brain axis [31]), focusing on how the gut microbiome influences addictive disorders. While most studies have centered on affective disorders, neurodevelopmental disorders, and neurodegenerative diseases [32], there is a growing body of evidence indicating that changes in the gut microbiome may impact addictive behaviors. For example, in a study by Kiraly et al. in 2016 [33], researchers observed that depleting the gut microbiome in mice resulted in increased sensitivity to low doses of cocaine, altered gene expression in the nucleus accumbens, and changes in crucial pathways related to addiction.

While research on the interaction between the gut microbiome and psychostimulants is still limited, translational studies suggest that the gut microbiome can influence responses to drugs of abuse, and conversely, psychostimulant treatment can alter the composition of the microbiome. A study involving rats injected with methamphetamine every other day reported modest increases in bacterial diversity, minor shifts in bacterial families, and a decrease in the short-chain fatty acid propionate in the caecal content. Short-chain fatty acids (SCFAs) produced by the gut microbiota are linked to the integrity of the blood-brain barrier and play a crucial role in microbiota and cocaine's behavioral effects [33]. They enhance the integrity of tight junctions in the blood-brain barrier, maintaining its selective permeability. SCFAs also have anti-inflammatory and neuroprotective properties, helping prevent disruptions in blood-brain barrier function.

Furthermore, in a study conducted by Volpe et al. in 2014 [35], individuals with cocaine use disorder, both with and without HIV, were compared to healthy controls. The findings revealed that cocaine use disorder was associated with significant changes in the gut microbiome. Specifically, non-HIV cocaine users exhibited a notable increase in the presence of the Bacteroidetes phylum. Additionally, there was a strong indication of higher levels of bacterial DNA in the serum of cocaine users, indicating a potential increase in bacterial translocation from the gut, which could contribute to inflammation within the body.

The Manifestation of Psychostimulant Use in Alzheimers

The depletion of the gut microbiome by excessive use of psychostimulants can be modeled by germ-free (GF) animals raised in a sterile, gnotobiotic environment, preventing the postnatal colonization of their gastrointestinal (GI) tract. Studies comparing GF mice (ones that lack an intestinal microbiota) with conventionally reared mice (ones that possess a normal gut microbiota) have revealed important insights into the role of the microbiota-gut-brain axis in cognitive function. GF mice exhibited deficits in non-spatial and working memory tasks, such as the novel object recognition test and spontaneous alternation in the T-maze. Furthermore, a decrease in brain derived neurotrophic factor (BDNF) expression in the hippocampus was observed among the GF mice [36]. BDNF is a critical neurotrophin for synaptic plasticity and cognitive function, with its reduced levels associated with higher amyloid-beta burden in AD patients [37]. Notably, Neufeld and colleagues discovered a sex-dependent modulation of BDNF expression: while female GF mice showed upregulated BDNF mRNA expression in the hippocampus, a significant decrease was noted in their male counterparts [38]. In conclusion, these findings highlight the intricate relationship between BDNF, cognitive function, and sex-specific responses in GF mice.

Furthermore, GF mice demonstrated increased adult hippocampal neurogenesis, which is known to play a vital role in cognitive processes [39]. These mice also displayed microglial immaturity and defects in microglial proportions, resulting in impaired innate immune responses that may contribute to the pathogenesis of neurological diseases, including AD [40]. Prior research by Sudo et al. found decreased expression of N-methyl-D-aspartate (NMDA) receptor 2A (NR2A) mRNA in the cortex and hippocampus of GF mice compared to specific pathogen-free mice [41]. Similarly, a more recent study by Neufeld identified a downregulation of the NMDA receptor NR2B subunit mRNA in the central amygdala of GF mice [38]. The NMDA receptor is crucial for synaptic plasticity and cognitive function, and increased activation of this receptor may be significant in Aβ-dependent synaptic dysfunction seen in AD [42]. Germ-free studies have proven valuable in shedding light on the underlying mechanisms of the microbiota-gut-brain axis and its implications for cognitive function and the pathogenesis of neurological disorders, including AD.

In a large-scale nationwide cohort study conducted in Taiwan, researchers analyzed data from 17,075 patients with amphetamine-related disorders (ARD) and 51,225 individuals in the control group without ARD. Among the ARD cohort, 1,751 individuals developed dementia, compared to 2,147 cases in the control group (883.10 vs. 342.83 per 100,000 person-years) [43]. Both amphetamine use disorder and amphetamine-induced psychotic disorders were found to be linked to an increased risk of developing overall dementia, Alzheimer's dementia, vascular dementia, and other dementia subtypes. This study provides substantial evidence indicating a strong association between amphetamine related disorders and the risk of dementia, encompassing various dementia types.

Early Life Stress on Brain Function and Gut Microbiome

During the initial years of life, the developing brain is extremely susceptible to the influence of environmental factors. The social ecology of childhood encompasses a range of both positive and negative experiences that form a framework for adolescents to achieve age-specific developmental milestones [44]. The experiences encountered during this critical period can make permanent changes to the structure and functioning of the brain through epigenetic modifications, such as DNA methylation/demethylation and chromatin modifications,

and heighten the vulnerability to mental illnesses later in life [45]. Frequent low grade stressors (such as insecurity and inattention), large life changes, and traumatic experiences (abuse/neglect) disrupt the ecology and result in harmful effects on children's health extending into adulthood [46].

When parents provide appropriate and sufficient care during the early years, it has a positive impact on the offspring's brain development, but inadequate parental caregiving can pose a risk for mental illness in the offspring during adulthood [47]. Early life adversity (ELA) refers to adverse experiences such as neglect, physical and emotional abuse that occur in the early stages of life [48]. Extensive research, encompassing studies involving humans and experimental animal models, has revealed a strong association between ELA and various issues, including conduct disorders, impaired cognitive development, and a heightened risk of dementia, Alzheimer's disease (AD), and other related neurodegenerative conditions.

The impact of adverse childhood experiences (ACEs) is a growing concern for public health [49]. Vincent Felitti designed the ACEs survey, which yielded that a notable 63.5% of adults have reported experiencing at least one ACE, and 12% reported enduring four or more such events [50]. Subsequent investigations, encompassing children as well, have unveiled even graver rates, ranging from 67% to a staggering 98% [51]. This issue is particularly critical for preschool children, who remain exceptionally susceptible to child abuse, neglect, and domestic violence [52]. Their constrained ability to express these traumas behaviorally and verbally poses a significant challenge to reporting, and ACEs from early childhood are often concealed [53]. The U.S. Children's Bureau documented that in 2018 alone, a distressing 678,000 children fell victim to abuse and neglect. Among these harrowing cases, 60.8% involved neglect, 10.7% were linked to physical abuse, 7.0% were sexual abuse, and an alarming 15.5% endured the anguish of two or more forms of abuse [54]. This vividly underscores the urgency of addressing and mitigating the escalating prevalence of ACEs to safeguard the well-being of the most vulnerable members of society. ACE exposure has profound effects on child development, with increased risk across various aspects of life, including cognitive development, quality of life, social functioning, economic prospects, psychiatric well-being, and physical health outcomes [55]. Despite recent efforts to address the public health challenges posed by ELA, current understanding of this issue is still limited and should be further explored .

Animal Models of Early Life Stress

Various animal models have been established to replicate the long-term effects of early life adversity (ELA) seen in humans [56]. These animal models allow researchers to conduct studies under controlled conditions, compensating for the limitations in human research and ethical restrictions. They involve exposing the subjects to different forms of stress (separation, resource scarcity, restraint stress, social defeat stress) and manipulating the amount and quality of parental care during the early postnatal period.

The maternal separation or deprivation procedure is where mother-pup interactions are
altered during the early postnatal period. In maternal separation, pups are separated from their mother for a specific period each day (2–5 hours) over several days to induce acute, predictable stress levels [57]. Maternal deprivation involves a more prolonged separation, usually one 24-hour
session [58]. These procedures have been associated with long-term behavioral

abnormalities and impaired cognitive performance in the exposed pups [59]. Chronic early life stress situations involve subjecting the pups to multiple prolonged periods. Pups may be exposed to a few types of stressors, including forced swimming, physical restriction, placement on an elevated platform, and foot shock exposure during early postnatal days (PND), leading to significant physical and psychological stress. An early foot shock paradigm has been developed to mimic early trauma or abuse experiences [60]. In an early foot shock paradigm procedure, the pups are placed in a closed, dark, electric shock apparatus during early postnatal time windows and subjected to continuous electric foot shocks to mimic early abuse experiences [61].

Offspring receiving higher levels of maternal caregiving display elevated neurotrophic factors and improved spatial learning and memory [62]. Researchers revealed poor cognitive performance in adult rodents with a history of maternal separation or deprivation [63]. Interestingly, cognitive deficits resulting from maternal deprivation appear to be more pronounced in female animals on postnatal day 40 (PND 40), suggesting an age-dependent and hormone-related susceptibility to cognitive impairment in females [62]. In mice subjected to maternal deprivation, cognitive impairment becomes more evident with age, as observed in middle-aged mice (1.4 years old) in a visual-discrimination task. Reduced levels of brain-derived neurotrophic factor (BDNF) and synapse-related proteins, such as postsynaptic density 95 (PSD95) and synaptophysin [64], along with fewer mature neurons, have been detected in the hippocampus and prefrontal cortex of animals experiencing maternal separation or deprivation, providing further evidence of poor maternal care's impact on brain development [65]. Studies have revealed that animals exposed to sporadic maternal care exhibit progressive cognitive deficits in adulthood, accompanied by impaired hippocampal long-term potentiation (a molecular basis of learning and memory), dendritic atrophy, and synaptic degeneration [66]. Mice exposed to LBN from postnatal day 2 to day 9 showed reduced survival of newborn neurons in the hippocampus, leading to altered cognitive performance [67].

Chronic exposure to unavoidable plantar electroshock during the early post-weaning period in rodents leads to impaired spatial memory in adulthood, evident through poor performance in the Y-maze or Morris water maze [68]. Rats exposed to a single platform and acute swimming stress during adolescence also exhibit inferior cognitive performance in adulthood, highlighting the potential long-lasting effects of even brief stress experiences early in life on cognitive health [69]. Altogether, findings from animal models provide valuable insights into the interplay between ELA and later cognitive impairment.

Early Life Adversity in Humans

ELA encompassing instances of physical, emotional, or sexual abuse, neglect, and other unfavorable environmental conditions in the early stage of life [70]. Twenty years ago, a retrospective investigation made the first identification of robust connections between adverse childhood experiences and a heightened susceptibility to major diseases [52], resulting in an escalation in concerns on this topic. Conducting invasive research on humans poses challenges, many human-based studies have proven that unfavorable encounters during this vulnerable developmental phase can escalate the likelihood of various adult-onset conditions - not only psychiatric disorders and cardiovascular ailments but also diabetes mellitus and neurodegenerative diseases [71].

Tools like the Adverse Childhood Experiences (ACEs) questionnaire are utilized in public

health initiatives to assess, comprehend, and prevent health outcomes associated with childhood trauma [72]. However, it's essential to consider other preventable sources of early life stress beyond ACEs, such as food and housing insecurity, bullying, discrimination, inattentive parenting, or family separations. Unfortunately, clinicians do not routinely screen for trauma or assess a child's social ecology, partly because there is a lack of validated, objective metrics that can be measured over time.

Vanaelst et al. conducted a systematic review of various inventories that assess the occurrence of adverse childhood events [49]. These inventories were derived from existing stress questionnaires and modified to inquire about significant life events, chronic environmental stressors (such as family, school, relationships, and health), and other stressors related to childhood experiences [73]. The concept of cumulative risk was initially proposed by Holmes and Rahe in their Social Readjustment Rating Scale [74]. Later, this approach was adapted to study child adversities by Rutter [75] and then used in other research studies [76]. The cumulative risk approach is based on the idea that dealing with challenges in one area of life is
generally more manageable than facing challenges in multiple areas simultaneously. It is straightforward to use and understand, it shows robust statistical associations that engage non academic stakeholders [52], it takes into account the co-occurrence of various childhood adversities [77], and it helps to identify individuals who are at the highest risk for experiencing negative outcomes [78].

Felitti, along with Robert Anda and their team, conducted the ACEs Study, surveying 9,508 adults to explore ten adverse experiences [79]. The study revealed significant associations between adverse childhood experiences (ACEs) and various negative health outcomes. Compared to individuals with no ACEs, those exposed to four or more ACEs faced 4- to 12-fold higher risks for drug abuse, alcoholism, depression, and suicide, as well as 2- to 4-fold increased risks for smoking, poor health, multiple sexual partners, sexually transmitted diseases, and 1.4- to 1.6-fold increased risks for physical inactivity and obesity [80]. Furthermore, ACEs displayed linear relationships with heart disease, cancer, lung disease, fractures, liver disease, and multiple other health outcomes. These findings spurred further research and influenced social policy to address the rising prevalence of ACEs, especially in pediatric age groups [73].

Research exploring the connection between early life adversity (ELA) and neurological consequences has gained significant attention due to the positive association between adverse childhood experiences and poor health outcomes later in life [52]. Numerous clinical studies have investigated ELA as a potential risk factor for cognitive impairment, focusing on child neglect, physical abuse, and parental separation. The parent-child coregulation, which involves mutual influence and coordination of emotional, behavioral, and physiological states, plays a critical role in the healthy development of children, impacting various domains, including emotional and cognitive functioning [81]. Longitudinal studies have demonstrated that secure infant-caregiver attachment predicts adult competence in areas such as educational attainment, occupational success, and social functioning [82]. A Helsinki birth cohort study revealed that men separated from their parents during World War II scored lower in cognitive reasoning tasks both at age 20 and later at age 70 compared to non separated subjects [83]. Moreover, the adverse effects of ELA extend to various cognitive outcomes, including general cognition and working memory [63]. Poly-victimization, experiencing multiple forms of victimization during a specific period, further amplifies the detrimental effects of ELA,

particularly in cases of physical/emotional abuse, harsh parenting, and domestic violence.

The Romanian orphanage studies support the lasting effects of childhood neglect and deprivation on cognitive and emotional development [84]. Individuals raised in institutions with severe deprivation exhibited lower executive functioning and a higher risk of psychopathology compared to their non-institutionalized peers. Early childhood deprivation was also associated with structural brain changes in adulthood, with adoptees experiencing smaller total-brain volumes, lower intelligence quotient, and increased attention deficit/hyperactivity disorder symptoms. Cross-sectional studies utilizing scales with high internal consistency, validity, and test-retest reliability have shown that increased ELA exposure is linked to compromised cognitive flexibility, processing speed, and working memory [85]. Moreover, these negative effects may be exacerbated in individuals with depression, as evidenced by smaller orbitofrontal cortex and hippocampal volumes compared to never-depressed individuals. The existing human studies provide evidence that exposure to ELA is a risk factor for developing cognitive impairment later in life. These findings underscore the importance of addressing early life adversity and its potential long-term consequences on cognitive health.

Early Life Stress effect on the Gut-Brain Axis

As indicated previously, an abundance of recent research indicates that the gut microbiota has a significant impact on brain function, forming bidirectional interactions known as the brain-gut microbiome axis [86]. The immune system, the vagus nerve, the enteric nervous system, and microbial-derived intermediates have been identified as mechanisms for these interactions [87], which play a crucial role in neuroimmune signaling. Disruptions in the normal gut microbiota can affect CNS neurotransmission [88].

Stress has been found to alter gut microbiota and disrupt intestinal barrier integrity [89], and researchers are now focusing on how early life adversity (ELA) affects the brain-gut-microbiome axis. ELA has been associated with altered systemic immune responses, increased visceral sensation, and changes in the fecal microbiota in young animals [90]. Studies have also shown that ELA-induced visceral hypersensitivity is partially mediated by alterations in specific gut bacteria [91]. Sex-dependent gut dysbiosis has been observed in mice exposed to multi-hit ELA, with distinct changes in the abundance of certain bacterial genera in male and female mice [92]. These alterations in gut microbiome resemble those observed in early AD. Additionally, ELA exposed animals exhibited elevated pro-inflammatory cytokines in their colons [93]. Many findings suggest that the gut microbiota plays a crucial role in brain function and cognitive health, and disruptions in the gut-brain communication from stress and early life experiences contribute to cognitive impairment.

Early Life Stress Impact on the Microbiome of Animal Models

In rodents, early life stress (ELS) has been shown to impact the gut microbiome, with lasting effects into adulthood [94]. An early study involved infant macaques from their mothers for one week, resulting in reduced fecal Lactobacillus levels and increased stress-related behaviors in the macaques [95]. Another study in rats found that ELS-exposed adults had changes in gut microbiota and higher levels of corticosterone, TNF-α, and IFN-γ compared to non-ELS rats
[90]. In mice, ELS increased levels of Bifidobacterium bifidum, Lactobacillus, Clostridium leptum, and Clostridium coccoides, and these effects were mitigated by adrenalectomy [96]. A significant rat study showed that ELS reduced the Firmicutes:Bacteroidetes ratio in the adult

gut and increased taxa associated with inflammation, such as Akkermansia, Flexibacter, and Prevotella [97].

Mice subjected to social disruption stress for two hours daily over six days experienced a reduction in gut microbial diversity and richness [98]. The social disruption stressor involved an aggressive male mouse being placed into the home cage of the resident mice. Immediately following the stressor, levels of gut *Bacteroides* were lower and *Parabacteroides* were higher compared with non-stressed controls. At fifteen hours post-stressor, levels of bacteria in the genus *Roseburia* were increased compared with controls, along with levels of proinflammatory cytokines, including interleukin-6 (IL-6) and MCP-1. Later studies in rodents have found similar stress-induced alterations in the gut microbiota. For instance, exposure to a single two-hour social disruption stressor altered gut microbial community composition, particularly reducing abundance of the genus *Lactobacillus* [99]. This social disruption stressor increased cytokine production in mice, but only in mice with intact microbiota, not in germ-free animals, similarly suggesting that gut microbiota may moderate stress-induced inflammation [100].

Recent research suggests that ELS may have different impacts on males and females. Mice exposed to various forms of ELS showed sex-dependent differences in gut microbiota, behavior, and gene expression in the prefrontal cortex [92]. ELS affected the abundance of specific taxa in males, including Lachnospiraceae and Porphyromonadaceae families, unclassified Firmicutes, and Bacteroides, Lactobacillus, and Alloprevotella genera. In females, ELS impacted Lactobacillus and Mucispirillum genera. Another study found increased fecal bacteria of the Bacteroides genus and decreased bacteria of the Lachnospiraceae family in both sexes of rats. However, specific differences were observed in each sex, including changes in relative abundance of certain genera and variations in cytokine levels [93]. Restoring the gut barrier of ELS-exposed rat pups through pharmacological inhibition of myosin light chain kinase normalized relative abundance of several taxa in adulthood and normalized behaviors and corticosterone levels [101].

Additionally, genotype may play a role in vulnerability to the effects of ELS. In rats, the impact of ELS on gut microbiota was influenced by the serotonin transporter (5-HTT) genotype, with diminished 5-HTT expression exacerbating the shift towards an inflammatory profile [102]. This was characterized by higher abundance of taxa such as Desulfovibrio, Mucispirillum, and Fusobacterium.

Early Life Stress on the Human Gut Brain Axis

In the clinical laboratory, acute stressors offer a standardized method for measuring the physiological response to mild stress in humans. One commonly used stressor is the Trier Social Stress Test (TSST), which involves public speaking and reliably increases cortisol and proinflammatory cytokine levels in adults. Only one published study has explored the link between the gut and chronic stress with acute laboratory stressors. A sample of healthy pregnant women underwent the TSST, and their cytokine and cortisol responses to the stressor were assessed. Then, stool samples were collected to assess gut microbial community composition.

The IL-6 response was positively associated with the abundance of Bacteroides and negatively correlated with Clostridiales, Lachnospiraceae, Dialister, and Enterobacteriaceae. The tumor necrosis factor-alpha (TNF-α) response was positively associated with the abundance of Bacteroides, Prevotella, and Megasphaera and negatively correlated with Ruminococcaceae. The C-reactive protein (CRP) response was positively associated with the abundance of

Ruminococcaceae and Megasphaera, but serum cortisol response was positively associated with the abundance of Rikemllaceae and Dialister and negatively correlated with Bacteroides [103].

Several studies have investigated the connections between childhood adversities and the gut microbiome, both in real-time and in adulthood, revealing interesting associations. In a study focusing on infants in the neonatal intensive care unit (NICU) during their first six weeks of life, higher stress scores were linked to the presence and relative abundance of specific gut genera, namely Proteus and Veillonella [104]. In healthy five-to-seven-year-old children, the gut microbiome was associated with parent-child dysfunction, and the abundance of gut B. fragilis was linked to reduced family turmoil and improved behavioral outcomes [105].

Moving into adulthood, psychiatrically healthy women with a history of multiple childhood adversities displayed altered gut microbiota, particularly differential abundance of Prevotella, Erysipelotrichaceae, and Phascolarctobacterium [103]. Another study examined the impact of adversity in infancy, such as institutional or foster care, on gut microbiome composition in adolescence, revealing lasting effects on diversity [106]. In adults, those with a history of trauma and PTSD showed differences in the abundance of certain gut phyla. Moreover, childhood adversity was associated with specific gut metabolites, indicating a potential link between gut function and brain connectivity in ACE-exposed adults [107].

Overall, these findings from human and animal models highlight the intricate connections between early life stress, gut microbiota, and various physiological and behavioral responses, demonstrating the significance of considering sex and genetic factors in understanding the impact of ELS on gut-brain interactions.

Early Life Stress in Alzheimer's

Recent research has highlighted the role of gut microbiome abnormalities in the development of Alzheimer's disease (AD). Notably, a significant contrast has been observed in the gut microbiota between individuals with AD and those who are healthy [108]. Additionally, individuals with mild cognitive impairment display comparable gut microbiota changes to AD patients [109]. These findings suggest a potential link between the gut microbiome and AD pathogenesis.

Animal models of Early Experiences on Cognitive Impairment

The potential connection between early-life adversity (ELA) and Alzheimer's disease (AD) has been extensively studied in rodent models. In one study, APPswe/PS1dE9 mice exposed to limited bedding and nesting materials (LBN) exhibited aggravated Aβ plaque load at 10 months of age, accompanied by increased glial activation and inflammatory signals in the hippocampus [110]. LBN exposure also led to higher levels of Aβ40 and Aβ42 in the hippocampus and elevated the expression of β-site APP-cleaving enzyme 1 (BACE1), a critical enzyme involved in Aβ processing and production in this mouse model [111]. LBN exposure resulted in synaptic damage and exacerbated cognitive impairment.

Researchers have explored the effects of maternal separation on AD disease progression in various transgenic mouse models of AD. Hui et al. demonstrated that chronic maternal separation worsened cognitive deficits and led to increased Aβ plaque formation and neural damage in adult APPswe/PS1dE9 mice [112]. Another study by Tanaka and colleagues focused on vascular pathological changes following maternal separation. They observed narrowed vessels in the prefrontal cortex with decreased capillary pericyte coverage and

disruption of the blood-brain barrier (BBB) in both amyloid precursor protein (APP) wild-type and heterozygous APP mutant (AppNL−G−F/wt) mice, which resulted from microglial activation. Maternally separated AppNL−G−F/wt mice also exhibited exacerbated cognitive impairment at four months of age [113]. Results suggest that ELA can elevate the risk of developing AD-like pathology even in the absence of AD risk genes. The gut-brain axis is proposed as a common pathogenic mechanism through which ELA affects AD pathology. Analysis of the gut microbiota in transgenic AD animals and healthy controls using 16S rRNA gene sequencing revealed distinct compositions [14]. Intriguingly, germ-free 3×Tg AD mice, lacking gut microbiota, exhibited significant reductions in cerebral amyloid plaques and neurofibrillary tangles compared to mice with normal gut microbiota. Additionally, transplantation of microbiota from healthy animals alleviated amyloid burden and tau pathology [114], while gut microbiota from AD individuals worsened AD progression and impaired cognitive function in healthy animals.

In animal studies, young adults subjected to maternal separation showed altered systemic immune responses, increased visceral sensation, and changes in fecal microbiota [90]. Specific
gut microbial populations, like Butyricimonas, Butyricicoccus, and Corynebacterium, partially mediated the ELA-induced visceral hypersensitivity. Additionally, the gut dysbiosis observed in ELA-exposed mice mirrored gut microbiome changes seen in early AD [91]. Similar gut barrier disruption and inflammatory responses were observed in ELA-exposed piglets [115], and significant gut microbiome alterations have been found in humans with a history of ELA. These findings suggest that disruptions in the brain-gut-microbiome axis contribute to ELA-induced cognitive impairment, but the exact mechanisms and specific microbiota involved remain to be elucidated. In rats exposed to maternal separation, activation of microglia and increased pro-inflammatory factors were observed [116]. Activation of the stress system can also influence blood-brain barrier permeability, facilitating the flow of peripheral inflammatory factors into the brain, potentially contributing to chronic inflammation in ELA-related cognitive impairment or AD [117]. However, further research is required to comprehensively understand the mechanisms through which ELA triggers chronic systemic inflammation and neuroinflammation.

Contributions of Early Life Stress on Alzheimer's Pathology in Humans

Numerous studies consistently link early-life adversity (ELA) to a higher risk of developing Alzheimer's disease (AD) and other dementias. Norton et al. followed 4108 subjects aged 65 to 105 for 18 months, revealing a higher number of confirmed AD cases within 18 months in those who experienced parental death during childhood [118]. Similarly, an Australian study using the Childhood Trauma Questionnaire (CTQ) found that individuals with higher CTQ scores were more likely to receive an AD diagnosis based on National Institute of Aging/Alzheimer's Disease Association criteria [119]. Another longitudinal study of 2682 males investigated the association between childhood stress and late-life dementia and AD, observing a higher prevalence of AD among those who experienced various childhood stressors [120].

Japan, facing an increasing prevalence of dementia, has conducted several cohort studies to explore the interplay between ELA and dementia prevalence. Utilizing the Adverse Childhood Experience Questionnaire, which covers family violence, physical and psychological abuse, neglect, parental death, parental divorce, and parental mental illness, these studies reported an increased number of clinically confirmed dementia cases within a

3-year follow-up period in participants with three or more adverse childhood experiences [121]. Furthermore, individual-level social capital scores were found to be a variable influencing this vulnerability, with increased dementia risk observed primarily in participants with low social capital [122].

These findings underscore the substantial risk associated with various forms of early-life adversity (ELA) for developing dementia or Alzheimer's disease (AD), regardless of the diversity of ELA experiences. While the gut microbiome is considered a key factor in this connection, only a limited number of studies have explored its impact on human cognition compared to animal studies. One such study linked gut microbiota composition in both obese and non-obese individuals to cognitive performance, including speed, attention, and cognitive flexibility in a Trail Making Test. The study also revealed changes in neural activity in brain regions like the thalamus, hypothalamus, and amygdala, suggesting that obesity influences both gut microbiota composition and subsequent cognitive function [123].

Notably, a probiotic mixture containing B. longum and different Lactobacillus strains showed positive effects on cognitive function and metabolic status in Alzheimer's disease patients [124]. Patients diagnosed with fibromyalgia demonstrated cognitive improvements in impulsive choice and decision-making following a multispecies probiotic intervention [125], a group with an altered microbiome, as indicated by disrupted microbiota metabolites [126]. These collective findings suggest the potential efficacy of probiotics in enhancing cognitive function both in healthy individuals and clinical populations with conditions like Alzheimer's disease. Nevertheless, more research is needed to understand the mechanisms behind how specific strains or interventions can modulate cognition and the limitations that exist in this regard.

The accumulation of Aβ peptide and abnormal forms of tau protein are considered traditional indicators of AD, but they may not directly imply causality [127]. Beyond viruses, bacteria have also been associated with AD pathogenesis. Studies on GF APP-PS1 mice [128] revealed reduced Aβ pathology compared to conventional animals of the same background, supporting the potential role of the microbiota in Aβ biology and AD pathogenesis [129]. Additionally, Aβ has shown antimicrobial properties in murine AD models [130]. Many questions remain regarding the role of viruses and bacteria in AD pathogenesis. It seems evident that microorganisms are involved at crucial stages of the AD pathogenic cycle, and further research is needed to determine whether Aβ accumulation represents a malfunctioning immune response or acts as a disease driver [131].

Two studies have investigated the gut microbiota composition in individuals with Alzheimer's disease (AD) compared to controls. In the first study, which included 25 AD patients with mild dementia and 25 matched controls, researchers found that AD patients had reduced gut microbiota richness and diversity. Specific changes were observed in various taxa, including a decrease in Firmicutes, an increase in Bacteroidetes, and a decrease in Bifidobacterium. These alterations in the gut microbiota were strongly correlated with the pathological load of Aβ and phosphorylated tau species in a subgroup of patients who underwent lumbar puncture for AD markers. The second study also identified changes in microbiota composition in AD at different taxonomic levels, although there were some variations compared to the first study. Notably, the Firmicutes:Bacteroidetes ratio was different, which is of interest considering the well-established link between AD and type II diabetes mellitus [132][108].

Conclusion

Emerging research has illuminated a compelling link between the gut microbiome and Alzheimer's disease in both human and animal models. The interplay between gut bacteria and brain health underscores the potential of microbiome-targeted interventions in managing or preventing cognitive decline. The influence of psychostimulant use and early life stress on the gut microbiome reflects the intricate bidirectional relationship between mental health, environmental factors, and microbial composition. As we delve deeper into understanding these connections, new avenues for therapeutic strategies and interventions that target the gut-brain axis, like alterations in diets, are promising for addressing Alzheimer's disease and more neurological disorders.

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