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Early Life Stress and the Development of the Infant Gut Microbiota: Implications for Mental Health and Neurocognitive Development

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Abstract

Purpose of Review We review the state of the literature examining associations between early life stress (ELS), gut microbiota, and neurocognitive development and mental health in animals and humans. We identify gaps in current models and areas for future research.

Recent Findings ELS is associated with changes in gut microbiota, which correspond to changes in affective and cognitive functioning in both animals and humans. Some of these ELS-induced psychological changes can be remedied by supplementation with probiotics in early life, suggesting a potential area for intervention for ELS-exposed children. Prenatal stress exposure is rarely studied in humans in relation to gut microbiota, but animal work has suggested important associations between prenatal stress and fetal programming that should be tested in humans.

Summary The gut microbiota plays an important role in the association between ELS, neurocognitive development, and mental health. More work is needed to fully understand these associations in humans.

Keywords Gut microbiota · Neurocognitive development · Early life stress · Mental illness

Introduction

Early life stress (ELS) is well-understood to have important implications for neurodevelopment and mental health. Several studies have documented that children exposed to ELS show differences in brain development [1, 2] and have increased risk for mental illness throughout the lifespan [3••, 4, 5]. Much work over the last several years has sought to elucidate the mechanisms behind these associations, with the goal of identifying pathways for unobtrusive, scalable interventions to promote healthy neurocognitive development in children exposed to ELS and ease the burden of mental illness. One mechanism gaining increasing attention is the system of trillions of microorganisms living within the gastrointestinal tract, known as the gut microbiota.

Scientific and clinical interest in the development of the gut microbiota has expanded rapidly in the last 10 years. Research in both animals and humans has provided some insight into processes that influence the development of the gut microbiota, as well as how this system influences the maturation of neural and psychological systems related to cognition and mental health. The gut microbiota has been implicated in a wide range of outcomes, including immune functioning [6], mental health [3••], metabolic programming [7], and neurodevelopment [8••,9]. With a surging interdisciplinary focus on the developmental origins of health and disease (DOHAD) [10], research examining how the development of the gut microbiota in particular can both promote and inhibit healthy development is more critical than ever.

The Development of the Gut Microbiota

There is a general consensus that the first 2 to 3 years of life represent a critical period for the colonization of the gut

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microbiota [11, 12]. There is some debate as to when colonization of this complex system begins, with some suggesting that infants are born "sterile," devoid of any bacteria [13], while others suggest that fetal colonization begins in the womb during the prenatal period [14]. However, it is clear that the most rapid population of the microbiota occurs during and after birth. There are a number of environmental and experiential influences that are known to affect early species colonization, including method of delivery (vaginal delivery vs. cesarean section) [15–18]), exposure to and duration of breastfeeding [18, 19], postnatal nutrition [15, 18, 20], exposure to animals (pets and pests in the home environment) [19], and ELS [3, 21•, 22••], among others. The gut microbiota is thought to be fully colonized by age three [11, 12], but the relative prevalence and diversity of bacterial strains can continue to evolve after early childhood in response to changes in lifestyle, particularly diet [20].

The Gut Microbiota and Neurocognitive Development

Research in both animals and humans has provided foundational evidence for the influence of the gut microbiota on neurocognitive development, as part of a system known as the microbiota-gut-brain axis, illustrated in Fig. 1. Experimental research in animals has provided much of our existing knowledge as to how the gut microbiota influences both structural and functional brain development and cognitive outcomes. Much of this work has focused on brain development in germ-free mice or in experimental manipulations of the gut microbiota of mice. Such models have demonstrated that mice raised under germ-free conditions exhibit altered patterns of synaptic plasticity and neurotransmitters in the striatum during adulthood compared with specific pathogen-free (SPF) mice with typical gut microbiota. These altered patterns of neural functioning correspond with increased motor activity and non-normative anxiety behaviors, including increased risk-taking [9]. However, mice born germ-free but exposed to gut microbiota early in development show behavioral and biological characteristics more similar to SPF mice, suggesting that the microbiota is critical for typical brain development in early life [9]. Similarly, experimental manipulations of the gut microbiota in germ-free or antibiotic-treated mice have resulted in differences in fear learning behavior, caused by changes in synaptic structure and reduced activity in the medial prefrontal cortex [23]. These findings have been replicated in developmental studies examining the effects of probiotic supplementation in rats with ELS-induced differences in the development of fear learning systems. These studies suggest that probiotic supplementation early in life protects against the cognitive effects of ELS [21•, 24–26] and that restoration of the gut microbiota early in development reverses some of the cognitive effects of ELS.

In addition to the development of fear learning systems, the gut microbiota has been implicated in recognition memory and cognitive flexibility in rodents. Specifically, poor diet-induced changes in the microbiota of adult rats have been shown to cause impairments in recognition memory [27, 28] and memory flexibility [29]. These studies together suggest that the gut microbiota plays a critical role in brain development across the lifespan and that early life may be a particularly sensitive period for the effects of the gut microbiota on cognition and neurodevelopment.

In humans, research examining associations between the gut microbiota and neurocognitive development is only just beginning to take off. One common way to study the gut microbiota is to use a measure called alpha diversity. Alpha diversity measures the number of different kinds of microbiota present in the gut and the relative abundance of each one. In a study by Carlson and colleagues, the authors reported that higher alpha diversity at 1 year of age was associated with poorer language outcomes at 2 years of age in a sample of 89 typically developing infants [30••]. Using measures from a subset of this sample ($n = 39$), Gao and colleagues found that increased alpha diversity in the gut microbiota at 1 year of age was related to decreased resting state functional connectivity between the amygdala and thalamus, as well as between the anterior cingulate cortex and right anterior insula [8••]. These brain regions are implicated in threat detection, emotion processing, and the development of anxiety disorders [31, 32]. In this same study, the authors found positive associations between alpha diversity of the gut microbiota and functional connectivity between the sensorimotor network and the inferior parietal lobe, regions of the brain with implications for language development [33]. Critically, higher functional connectivity between the sensorimotor network and the inferior parietal lobe at 1 year of age predicted poorer language outcomes at 2 years of age [8••]. These two studies together are suggestive of associations between the microbiota and neural changes underlying cognitive development. However, these findings are different from what is typically seen in adult studies, where higher bacterial diversity is associated with more positive outcomes [34]. These discrepancies highlight the importance of developmental studies in broadening our understanding of the early microbiota-gut-brain axis [35].

Additional evidence for the relation between the gut microbiota and neurocognitive development in humans comes from studies examining links between antibiotic or probiotic exposure and neurocognitive development. In a sample of preterm infants, Firestein and colleagues found that exposure to antibiotics in the perinatal period was related to higher EEG delta power at term age (a risk factor for ADHD) and higher attention problems at 4–5 years of age [36]. Researchers in this study hypothesized that these antibiotic effects may be

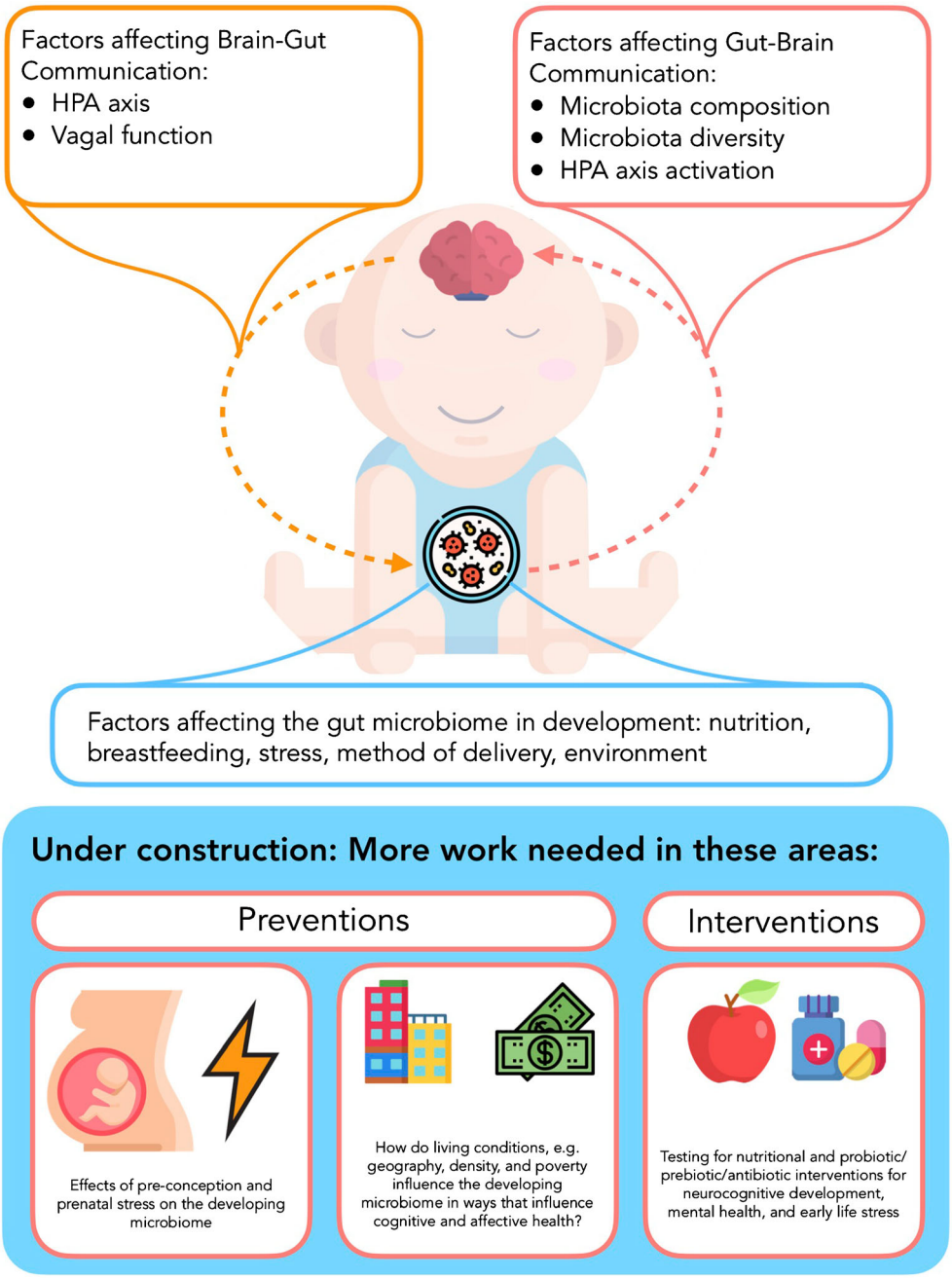

Fig. 1 Research in both animals and humans has provided foundational evidence for the influence of the gut microbiota on neurocognitive development, as part of a system known as the microbiota-gut-brain axis

attributed to differences in *Bifidobacterium*, a common earlier colonizer in the infant gut microbiota, as many antibiotics administered to infants (such as ampicillin) are known to preferentially deplete species of *Bifidobacterium*. Similarly, in a randomized control trial by Pärtty and colleagues, 75 typically developing, full-term infants were randomized to receive either *Lactobacillus* supplementation or a placebo for the first 6 months of life and followed up again when children were 13 years old to assess for neurodevelopmental disorders [37]. *Lactobacillus* is another common early colonizer in the infant gut microbiota and is known to promote healthy immune functioning and reduce the permeability of the gut wall [38]. Seventeen percent of children in the placebo group, compared with no children in the *Lactobacillus* group, had received a diagnosis of either autism spectrum disorder (ASD) or ADHD at follow-up. Additionally, the children who went on to develop a neurodevelopmental disorder showed fewer species of both *Lactobacillus* and *Bifidobacterium* in the gut at 6 months of age than in those who did not [37]. Importantly, while the authors did not include measures of nutrition in their analyses, they did examine duration of breastfeeding and exposure to antibiotics in early life, both of which are known to influence

the kind of bacteria present in the gut. They found no differences on either of these measures between the placebo group and the probiotics group or the group that went on to develop ADHD/ASD and those that did not. Taken together, these studies suggest that abundance of both *Bifidobacterium* and *Lactobacillus* in the infant gut microbiota may promote healthy neurocognitive development and that depletion of these bacteria may increase risk for neurodevelopmental disorders.

Though only speculative at this stage (more research is required), it is interesting to consider potential mechanisms underlying the aforementioned effects. One possible mechanism for these findings is that higher abundance of *Lactobacillus* is known to stabilize the gut wall [38] and prevent colonization by proinflammatory bacteria [39, 40], thus preventing beneficial bacteria inside the gut from escaping and protecting the gut from colonization by harmful microorganisms. Certain strains of bacteria in the gut are thought to activate production of proinflammatory cytokines, which in turn activate parts of the vagus nerve. The vagus nerve alerts the central nervous system about this systemic inflammation, which in turn initiates changes in behavior and neural functioning [41–43], which could ultimately affect neurocognitive development. However, as no studies have examined all stages of this mechanistic pathway together, especially in humans, many questions remain about the processes which underlie microbiota associations with cognitive function. Moreover, in humans, very few studies have examined microbiota associations with brain function, even via correlation [3••, 8••, 30, 44], leaving much more foundational work to be done establishing those basic links in humans. As such, more studies are needed with direct assessments of microbiota composition and diversity, as well as specific measures of vagal function, inflammation, and neural activity to build a full picture of the complex mechanisms underlying these neurocognitive effects. Moreover, studies that move beyond alpha diversity to incorporate the wide array of analytic approaches and questions that can be used in, and asked of, the microbiota are needed [45].

The Gut Microbiota and Mental Health

Perhaps more well-documented than associations with neurocognitive development are relations between the gut microbiota and mental health in both animals and humans. Causal evidence for the microbiota mental health link comes from germ-free rodent models. Rodent studies have shown that germ-free adults exhibit abnormal anxiety behaviors and impaired social skills [46, 47]. In one study, germ-free (GF) mice were compared with a group of GF mice who were colonized at birth with a typical mouse microbiota (CAB), and to a group who was colonized in the neonatal period with four species of *Bifidobacterium* (BFD), meant to mimic the human infant gut microbiota [47]. The GF mice showed abnormally low levels of anxiety behaviors compared with the CAB mice and BFD mice. The anxiety behaviors of the BFD group fell between that of the GF and CAB mice, suggesting only a partial rescue of the behaviors of the GF animals following neonatal BFD colonization [47]. These findings have been replicated [9, 48] and suggest that early life is a *critical period* for development of the microbiota and subsequent stress/brain health, but that changes to the microbiota and supplementation with specific bacteria/bacterial strains very early in development may reverse some of the behavioral and mental health costs of early bacterial disruptions.

In humans, work in this area is less well-developed but provides some insight into how the gut microbiota may influence concurrent and future symptoms of mental illness. Associations between changes in the gut microbiota and mood disorders are reasonably well-established in adults [49–52]. Additionally, diagnoses of functional gastrointestinal disorders (irritable bowel syndrome, Crohn's disease, etc.) are higher in individuals with mood disorders than in the general population [53], suggesting that these symptoms may be connected. However, not much work has been done to understand the developmental origins of these microbiota differences nor how early these associations between gastrointestinal distress and mental health symptoms emerge. In a sample of 344 children (ages 3–18) either raised with their biological parents or who had been internationally adopted from orphanages and institutions (post-institutionalized), Callaghan and colleagues found strong associations between gastrointestinal distress and both concurrent and future anxiety symptoms [3••]. In a subpopulation of the larger study, they also found that group membership (post-institutionalized vs. not) was associated with differences in microbiota diversity and that specific microbial taxa (*Bacteroides* and an unidentified genus in the family *Lachnospiraceae*) were associated with unique patterns of functional reactivity to emotional stimuli in the medial prefrontal regions, the post-central gyrus, and the anterior cingulate cortex, regions implicated in emotional functioning [54, 55]. While this evidence is indirect, it is promising in suggesting that there is an association between mental health and gut bacteria (or at least the functioning of the microbiota-gut-brain axis as a whole) in developing humans. Moreover, direct associations have been made between neurodevelopmental disorders such as attention-deficit hyperactivity disorder (ADHD), autism, and the gut microbiota [56, 57]. Nonetheless, more research is needed to understand if direct associations exist between symptoms of anxiety, depression, and the gut microbiota in developing human populations, and beyond those associations, to test whether manipulations of the microbiota (by supplementing with beneficial bacteria or promoting more specific nutritional guidelines) may have the same effects at reducing mental illness symptoms in developing humans as it has in

adult humans [58, 59] and in the animal literature [21•, 24, 25, 60]. If support for these treatments is found, it would have important implications for the prevention and treatment of mental disorders early in development, when most mental health problems first emerge [61].

Early Life Stress and the Development of the Gut Microbiota

Early life stress is associated with a host of adverse physical and psychological outcomes in humans, including increased prevalence of psychopathology [4, 62] and differences in brain development [2]. Exposure to ELS is known to influence the developing gut microbiota [3••, 63]. In humans, bacteria in the gut indirectly influence the amount of cortisol the body releases in response to stress via the hypothalamic-pituitary-adrenal (HPA) axis, and these bacteria are critically important for the development of normative stress responses [64, 65]. While the exact mechanism of these associations has yet to be fully explored in humans, research from animal models suggests that elevated cortisol increases the permeability of the gut wall, thus allowing bacteria from the gut to translocate outside the gut wall and impacting both the diversity and the composition of the gut microbiota [66]. Certain bacteria in the gut promote the development of proinflammatory cytokines, which are known to then activate the HPA axis at many levels [64], thus creating a feedback loop between the HPA axis and the gut microbiota. This bidirectional pathway has important implications for the regulation of stress responses across the lifespan and, due to the importance of the first 2 years of life in the colonization of the gut microbiota, introduces the question of how ELS may play a role in the maturation of the gut microbiota and subsequent developmental outcomes.

Of particular interest in the study of stress and development is the role of prenatal stress in fetal programming and neurodevelopment. Emerging research in humans has suggested that elevated maternal stress during pregnancy is related to differences in neurocognitive development [67, 68] and increased risk for mental illness [69]. However, little is known about the mechanisms behind these associations, particularly as they relate to the gut microbiota. To date, there is very little research on the influence of prenatal stress on the developing infant gut microbiota and subsequent developmental outcomes in humans. However, work in animals suggests that a link does exist and provides an idea of the mechanism for pregnancy stress-induced microbiota changes in the mother, which then influence infant microbiota and neurodevelopment. Specifically, recent work in mice has found that the gut microbiota of the dam naturally changes over the course of pregnancy, and stress during pregnancy disrupts these natural changes by altering the composition of the dam's gut and vaginal microbiota [70, 71, 72•]. In these studies, stress in the early prenatal period was associated with a decreased abundance of *Lactobacillus* in the maternal vaginal microbiota, resulting in lower relative abundance of *Lactobacillus* in the gut microbiota of the pups [70, 71, 72•]. Those prenatal stress-induced changes were shown to influence fetal metabolic and brain programming, such that pups exposed to increased prenatal stress were at neurodevelopmental risk [70, 71, 72•]. Hence, animal studies reveal a clear mechanistic pathway for vertical transmission of prenatal stress effects on neurocognitive development via the microbiota, which needs to be replicated in humans. Indeed, although the prenatal stress data in humans are currently lacking, there is evidence from Hantsoo and colleagues that maternal childhood stress (i.e., *maternal* adverse childhood experiences; *m*ACEs) is associated with pregnancy-related changes in the gut microbiota and that these microbiota changes are associated with maternal inflammatory stress responses and elevated HPA axis reactivity during pregnancy [73••]. Hence, similar to the animal studies just discussed, it is likely that early stress influences on maternal microbiota have the potential to influence infant microbiota composition and subsequent neurocognitive development, though this will need to be explicitly tested. Importantly, in the Hantsoo and colleagues study, it was shown that for mothers who had experienced high ACES, diets high in polyunsaturated fatty acids protected against heightened immune response to stress during pregnancy, highlighting a clear pathway for clinical interventions to be investigated in future studies of the role of the microbiota in fetal programming effects on neurodevelopment [73••].

Beyond the prenatal period, there is also evidence supporting stress-induced changes to the microbiota which might underlie differing patterns of neurodevelopment. For example, animals exposed to maternal separation experience abnormal fear learning and fear extinction patterns, as well as more adult-like patterns of fear memory [21•, 24, 25, 60, 62, 74]. However, these effects seem to be prevented by concurrent supplementation with *Lactobacillus*, suggesting (indirectly) that the experience of maternal separation may have impacted the developing gut microbiota [21•, 25]. Studies directly assessing the effects of maternal separation on the developing gut microbiota have provided support for this hypothesis. By 4 weeks of age, maternally separated rats show distinct differences in the relative abundance of several species of bacteria in their guts compared with control animals, and these differences have shown to persist into adulthood [63]. Similar findings have been documented in other studies of maternally separated rats [75, 76] and monkeys [77]. Taken together, these findings suggest that stress-induced changes in the gut microbiota may account for some of the stress-induced changes in behavior and psychological functioning seen in humans through alterations in neural functioning, though more research is needed to more directly test these hypotheses.

In humans, as mentioned earlier, ELS is known to be a strong predictor of gastrointestinal problems in childhood and into adulthood [3••, 78]. These gastrointestinal symptoms could potentially be caused by stress-induced changes in the gut microbiota. Indeed, as mentioned earlier, in a small proof-of-concept study in post-institutionalized children (institutional care is a potent stressor for humans), Callaghan and colleagues found differences in the diversity of the gut microbiota compared with non-institutionalized children. These differences were associated with both concurrent and future anxiety and whole-brain reactivity to fearful faces [3••]. More work is needed in this area, however, to better understand the mechanisms linking ELS and microbiota development, with attention paid to specific stress physiology pathways.

The work that has been done examining stress physiology has provided some insight into the mechanisms linking ELS with microbiota development. Measures of physiological stress and gut microbiota diversity and composition have differed by different measures of stress. To date, only one study (that we are aware of) has examined specific measures of stress physiology in the association between ELS and the development of the gut microbiota in humans. This study, by Michels and colleagues, used measures of heart rate variability at baseline (pnn50), hair cortisol, and self-reports of negative and positive events to assess stress in a sample of 93 children between the ages of 8 and 16 [22••]. They found that higher stress, as measured by low pnn50 and more lifetime negative events, was associated with less diversity in the gut microbiota and lower relative abundance of certain phyla of bacteria, namely, *Firmicutes*. However, these associations did not emerge for hair cortisol. Additionally, they found that these effects were age-specific, only present in pre-adolescents but not in adolescents [22••]. These findings suggest that early childhood may be a window of increased sensitivity for the influence of stress on the development of the gut microbiota. Given that the first 2 to 3 years of life are suggested to be a critical period for the development of the gut microbiota, more prospective research in infants is needed to better understand the effects of stress experienced very early in development.

Conclusions, Future Directions, and Areas for Intervention

While there is a growing foundation of knowledge in this area, much more research is needed to fully understand the role of the microbiota-gut-brain axis in human development. Given recent shifts in the field of neurodevelopment toward examining prenatal influences on brain development, more work is needed in both humans and animals to describe the prenatal processes that influence the development of the gut microbiota and to examine the gut microbiota as a potential mediator between prenatal exposures (such as stress or nutrition) and subsequent neonatal brain development; this need is highlighted in Fig. 1. Given the findings in animal models, prospective studies in humans should examine how stress during pregnancy alters the maternal vaginal microbiota, as this is the primary source of early bacterial exposure for infants born vaginally and could be one potential mechanism linking prenatal stress exposure with neurodevelopment. Similarly, while there is a large body of research documenting changes in the microbiota in response to ELS, there is little work about the effects of moderate but prolonged elevated stress on the development of the microbiota and specific stress physiology pathways that may play a role in the development of the microbiota-gut-brain axis. This work could have strong implications for infants growing up in poverty, who are more likely to experience heightened prolonged environmental stress [1], and less likely to have access to nutritious foods [79], both of which are known to influence the developing microbiota [3••, 15, 18, 20].

Studies examining gut microbiota differences as a function of socioeconomic status within populations in the same geographic region are rare. Most of this work thus far has taken the form of comparison studies between populations in high-income versus low-income countries. These studies have identified cultural differences in diet, feeding patterns, and breastfeeding as important drivers of differences in gut microbiota composition [80, 81]. However, more work is needed to better understand how differences in socioeconomic status within given populations influence individual differences in microbiota development. Elucidating the role of the microbiota in neurodevelopment in contexts of heightened stress such as poverty may provide opportunities for precise, scalable, unobtrusive interventions and policies to promote healthy brain development and mental health for children in vulnerable populations.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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