Early-life stress origins of gastrointestinal disease: animal models, intestinal pathophysiology, and translational implications

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Pohl CS, Medland JE, Moeser AJ. Early-life stress origins of gastrointestinal disease: animal models, intestinal pathophysiology, and translational implications. Am J Physiol Gastrointest Liver Physiol 309: G927–G941, 2015. First published October 8, 2015; doi:10.1152/ajpgi.00206.2015.—Early-life stress and adversity are major risk factors for the adult onset and severity of important gastrointestinal (GI) diseases, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), in adulthood (2, 31, 67, 165). Despite the established link between early-life stress and GI disease later in life, the pathophysiological mechanisms remain poorly understood. A number of animal models of early-life stress/adversity have been employed to study the GI pathophysiology and health outcomes associated with early-life stress. This review presents the major GI pathophysiological and clinical findings in established rodent models and newer large-animal models of early-life stress. A primary focus of this review is on how the pathophysiology and clinical findings from animal models of early-life stress translate to specific human GI disease conditions. Species- and model-related factors that likely play significant roles in predicting the translational value of the models to humans are emphasized. In addition, this review provides insight into the relevant postnatal GI developmental tracks that are altered by a stressful early-life environment, ultimately leading to a new trajectory toward GI dysfunction and disease susceptibility later in life.

Stress: Definition and Role of Development of the Hypothalamic-Pituitary-Adrenal Axis

Definition. “Stress is defined as a state in which homeostasis is actually threatened or perceived to be so [and] homeostasis is re-established by a complex repertoire of behavioral and physiological adaptive responses of the organism” (48). The neonate and infant are exposed to tremendous, stressful changes in homeostasis at birth and weaning, and both immunologic and hypothalamic system adaptation and plasticity are necessary for survival. Importantly, the outcome of reestablished homeostasis has major implications for long-term health. The organism returns to original homeostasis (or eustasis), or the adaptive response creates a new homeostasis. The new homeostatic parameters can be inappropriate (allostasis) or beneficial (hyperstasis) (48). Adaptive responses to these early-life challenges likely dictate health later in life (88) and are central to long-term GI disease susceptibility. A number of early-life stressors, including psychosocial (maternal deprivation, loss of caregiver, and physical and emotional abuse) and immunologic (allergy, infectious, or metabolic/nutritional) stress, have been implicated as risk factors of GI disease onset later in life. While these stressors are diverse, they fit the definition of stress, in that they threaten the host’s homeostasis. If these stressors occur during a time of significant developmental plasticity, it is likely that new adaptations can reshape brain and gut function for the individual’s lifetime.

Role and development of the hypothalamic-pituitary-adrenal axis. The hypothalamic-pituitary-adrenal (HPA) axis is central to controlling homeostatic adaptations to stress. Psychological or physical stressors, such as inflammation, are integrated in the central nervous system (CNS), resulting in a cascade of positive- and negative-feedback responses mediated by hypothalamic and pituitary release of the stress hormones corticotropin-releasing factor (CRF) and adrenocorticotropic hormone (ACTH), respectively. ACTH stimulates the cortical adrenal gland to release glucocorticoids, which have many downstream
physiological effects that help the host quickly adjust to the environmental changes that initially induced hypothalamic release of CRF (48, 49).

During infancy, childhood, and early adolescence, the HPA axis is mostly hyporesponsive, with low basal glucocorticoid secretion until near puberty (106, 112, 151, 180). In humans and rodents, severe environmental stressors in the absence of parental care disrupt HPA axis maturation, and these early-life stressors, characterized by inappropriate release of stress hormones such as cortisol during subsequent stressful conditions, are associated with development of adult psychological and GI disease, such as IBS (63, 75, 166, 178). During the neonatal stage, the adrenal gland is highly sensitive to small quantities of ACTH, while the negative glucocorticoid receptor-feedback system is poorly developed (55, 177); thus HPA axis activation induced by early-life stress results in high and prolonged glucocorticoid production. Elevated glucocorticoids are detrimental to HPA axis and glucocorticoid receptor development; thus, prepubescent individuals exposed to early-life stress exhibit exaggerated CNS/behavioral and physiological responses to subsequent stressful episodes later in life (3, 44, 119). These phenomena support the hypothesis that early-life stress stimulates the HPA axis during a period when the neuroendocrine system is intended to be hyporesponsive. Inappropriate stimulation of the HPA axis during the intended hyporesponsive period results in altered development and life-long allostasis.

In health and disease, the HPA axis is known to play a role in regulation of gut function; thus the HPA axis has been established as an important component of the brain-gut axis (73, 178). Concurrent with development of the HPA axis early in life, the gut undergoes a similar extensive maturation period. Given the bidirectional communications between the HPA axis and the gut, aberrations in gut development due to environmental stress may directly induce HPA axis dysfunction. Conversely, HPA axis allostasis may contribute to inappropriate development of the gut. Interruptions in the development of either system are well associated with risk of developing GI disease.

Developmental Biology of the Postnatal Intestine

To understand how early-life stress might influence long-term GI development and disease susceptibility, it is important to consider the major intestinal developmental changes and adaptations across models of different animal species in the immediate postnatal period. In all mammals, the early postnatal period is marked by major developmental changes in preparation for long-term survival. Some major systems that undergo extensive development and programming during this time are the enteric, immune, and nervous systems, epithelial barrier function, and microbiota colonization and composition (124). While the early-life developmental changes in these systems allow the host to survive and thrive in the extraterrestrial environment, perturbations in normal developmental processes by stress during these periods of plasticity can lead to a deviation in long-term function of the GI system and an increase in disease susceptibility (Fig. 1).

![Fig. 1. Proposed paradigm of early-life stress and gastrointestinal (GI) disease development. Evidence from rodent and porcine models and human data demonstrate that early-life stress is a major risk factor in GI disease development and severity later in life. Early-life psychosocial stressors occur during high developmental plasticity (green) and initiate a trajectory toward increased GI disease susceptibility (red line) later in life. Animal models, such as neonatal maternal separation (NMS) and colonic irritation in rodents, maternal separation (MS) in nonhuman primates, and early-weaning stress (EWS) in pigs, can be used in the study of early-life stressors at times of intestinal development (comparable to human perinatal and childhood intestinal development) and enhanced susceptibility to development of GI dysfunction later in life (adulthood). Common mechanisms of early-life stress-induced disease between animal models (boxes at right) are increased intestinal permeability, altered microbiota, increased enteric nervous system activity, heightened mast cell numbers and activation, corticotropin-releasing factor (CRF), cholinergic nervous system [choline acetyltransferase (ChAT)], substance P (SP), and serotonin (5HT). Collectively, these mechanisms can result in clinical signs of GI disease, including abdominal pain, diarrhea, constipation, and increased susceptibility to enteric infections.](image-url)
Postnatal development of the enteric nervous system. The enteric nervous system (ENS) exerts regulatory control over numerous GI functions, including motility, visceral sensation, secretion, and absorption, and immune and epithelial barrier function (77, 79, 122). Therefore, alterations in ENS function can lead to profound clinical GI symptoms, which represent a central pathophysiological process in stress-related GI disorders. Although the ENS can operate independently of CNS input, the ENS is essential in integration of signaling between the gut, the CNS, and the brain. Major developmental processes and adaptations that exhibit a high degree of plasticity take place in the ENS during postnatal life. Given the plasticity of this system, stressful or harmful stimuli in early postnatal ENS development have the potential to alter normal ENS development and life-long function. Key ENS postnatal processes include the formation of functional neural circuits, gangliogenesis, differentiation of neuron phenotypes, and neuron cell death (111, 154). After neurogenesis and gangliogenesis, the ENS undergoes a normal decline in the number of neurons via apoptosis, as demonstrated in laboratory animals and humans (7, 80, 155, 186). Although the mechanisms of “ENS pruning” in the postnatal gut are incompletely defined, it is thought that these mechanisms might include loss of appropriate survival factors (36, 41), possibly including nerve growth factor (NGF) and glial-derived neurotrophic factor (74, 109, 179, 182). In addition, throughout the postnatal period, the neurochemical composition of the ENS changes significantly. Of particular importance are alterations in cholinergic innervation to the gut, where the proportion of neurons expressing acetylcholine, the major excitatory neurotransmitter in the GI tract, increases dramatically (58), often doubling and accounting for ~44% of all neurons in the submucosal plexus and 62% of all neurons in the myenteric plexus by maturity (78, 93). ENS neurite outgrowth is another important postnatal event (77). In summary, the ENS undergoes significant development and maturation during the early postnatal period and exhibits a high degree of plasticity. Therefore, an understanding of how early-life stress influences normal ENS development could be critical to the understanding of early-life stress-induced GI disease.

Postnatal development of the GI immune system. In childhood, birth and weaning represent major challenges for early-life host immunity. At birth, the host must adapt to microbial colonization of the lungs, intestine, and skin, as well as consumption of milk antigen, all of which have the potential to induce massive inflammation. Similarly, at weaning, the host must cope with the psychological stress of maternal separation (MS) or deprivation while also adapting to a sudden exposure to food antigens and changes in microbial community without the support of maternal immunity. Massive change in the gut transcriptional profile at birth and weaning indicates the adaptive effort of the host during disruptions in homeostasis (147). Ontologically, the early-life immune system has been described as suppressed, yet active (52, 76). It is hypothesized that the perinatal period is a “window of opportunity” for tolerogenic induction and that excessive inflammatory interruption during this period may lead to maladaptive responses with long-term health consequences (14, 150). Research over the last 20 years has described multiple layers of exogenous and endogenous immunosuppressive mediators that modulate infant immunology to promote an active, yet tolerogenic, immune system. Exogenously, maternal milk provides immune-supportive factors such as secretory IgA, maternal leukocytes, and milk glycans, all of which modulate and neutralize intestinal microbes. Additionally, breast milk provides massive amounts of anti-inflammatory cytokines and peptides, which negatively regulate neonatal Toll-like receptor and inflammatory cytokine expression (134). Endogenously, several pathways are involved in inhibition of the innate immune system, which, in turn, leads to polarization toward a tolerogenic lymphocyte population early in life (14, 52, 65, 76). However, the neonatal immune system is not inherently unresponsive or defective. Presence of the commensal microbes induces neonatal immune activity, represented by development of secondary lymphoid organs in mice, pigs, and humans during the postnatal period (14, 19, 150). Clinically, neonates can respond to vaccination, although the response is weak. Additionally, various types of leukocytes from the neonate can be stimulated to induce inflammation. Finally, in early and abruptly weaned pigs, antibodies to new feedstuffs can be detected (14, 52, 65). These observations highlight the inflammatory capability of the neonate and reinforce the idea that there may be consequences to immune overstimulation during this quiescent period.

At weaning, the immunosuppressive dominance gives way to a spike in inflammation. Mast cell degranulation and proliferation, intraepithelial lymphocyte proliferation, mucosal inflammatory cytokine induction, and T-cell stimulation coordinate the homeostatic adjustment to weaning (52, 142). Host inflammatory and metabolic pathways are also upregulated with weaning to cope with a dynamic microbiota and an introduction to novel food antigens, factors that are likely controlled by Toll-like receptor and IL-1 pathways (29, 147). Weaning can be abrupt or gradual, can occur at different stages of development, and is associated with an inflammatory response; thus the time and/or developmental stage at which weaning occurs can interrupt the tolerogenic period. This becomes particularly important in stressful situations such as early weaning, when premature immune stimulation during the tolerogenic window of opportunity can have serious health implications later in life.

Postnatal development of the intestinal epithelial barrier. The intestinal epithelium undergoes rapid maturation during the postnatal period. While some postnatal epithelial changes are thought to be genetically “hard-wired,” many are driven by environmental, microbial, and endocrine cues. One of the earliest and most critical epithelial changes in the postnatal period is establishment of intestinal epithelial barrier function. Intestinal epithelial barrier function refers to the ability of the epithelium to form a selectively permeable barrier, regulated predominantly by tight junction proteins and mucus, which prevent the vast amounts of luminal antigens, pathogens, and toxins from gaining entry into the underlying tissues and systemic circulation. Impairment of the epithelial barrier results in exposure of luminal constituents to the underlying immune, circulatory, and nervous systems, inciting local neuroinflammatory events and systemic inflammation. Disturbance of this epithelial barrier, characterized by heightened intestinal permeability, or “leaky gut,” is a hallmark in the pathogenesis of major GI diseases, including IBD, IBS, celiac disease, and food allergy/intolerance (38, 100, 128). The postnatal development of intestinal barrier properties has been
investigated in multiple animal species and humans. At birth, the neonatal intestinal barrier is highly permeable; it matures during the postnatal period, as indicated by a progressive decline in permeability with age; however, species-specific variations exist. In full-term human infants, Weaver et al. (183) showed that GI permeability (measured as urinary lactulose-to-mannitol ratios) remained stable during the first 4 days of postnatal life. Catassi et al. (40) demonstrated that GI permeability, also measured as urinary lactulose-to-mannitol ratio, declined significantly, by ~3.7-fold, between 1 and 7 days of age, indicating a rapid postnatal decline in GI permeability (40). They also showed that breast feeding accelerated the decline in GI permeability (40). GI permeability was markedly higher in preterm than full-term infants and then declined rapidly with age (175, 183). In mice, GI permeability (measured by in vivo FITC-4-kDa dextran permeability) is high at birth and declines with age; however, the most pronounced reductions in GI permeability occur later in mice (at 2–3 wk of age) than in humans (139). Similarly, in neonatal rabbits, small intestine permeability was high at birth and then declined progressively into adulthood (>120 days) (171). In full-term piglets, GI permeability [measured by in vivo lactulose-to-mannitol ratio (sugar absorption tests)] remained stable after birth, with little change between birth and 10 days of age (98). However, utilizing ex vivo jejunal preparations on Ussing chambers, De Quelen et al. (56) reported that intestinal permeability increased between 0 and 14 days of age and declined thereafter. Together, these findings indicate that while many species exhibit a postnatal maturational decline in intestinal permeability, the time course is very different. The implications for these species and, therefore, model differences relative to human clinical relevance are discussed later in this review. In addition to the developmental aspects of intestinal epithelial permeability, other key barrier and innate epithelial cell changes, including marked changes in the expression and repertoire of antimicrobial peptides, pattern recognition receptors, and immune signaling pathways (144), nutrient transporters (110, 168), and crypt-epithelial regenerative complexes (57, 94), also occur in the postnatal period. Furthermore, similar to the other GI system changes described above, postnatal epithelial development is modulated and shaped extensively by dietary, microbial, neuroendocrine, and environmental influences and differs by species.

Postnatal establishment of the enteric microbiota. The microbiota exerts a large influence on GI function and health throughout life, but its composition is determined largely during the postnatal period (71, 90, 132). While most of the available literature suggests that colonization of the GI tract occurs at birth, with first exposure in the vaginal canal (90, 181), there is also evidence to suggest that colonization occurs in utero (101). Given that this is the founding group of bacteria, any abnormal stress or inflammatory state of the mother can influence the microbiota of the offspring (141, 181). Breast milk has a profound impact on the microbiota and further aids in colonization, with a higher proportion of Bifidobacteria in breast-fed than formula-fed individuals (71, 181, 189). At weaning, the microbiota is subject to great change with the transition from breast milk to solid food at weaning and may play a role in diseases such as IBD (6, 54, 188). One of the key roles of the microbiota in the neonate is establishment of oral tolerance to commensal microorganisms and food (104, 162). Additional roles of the microbiota in the developing mucosal immune system include development of gut-associated lymphoid tissue and intestinal lymphocytes and antimicrobial peptide secretion into the lumen (102). Neonatal colonization is also required for normal neurological development, including development of the HPA axis (62, 133, 161), which further highlights this period as a critical window in development.

In summary, tremendous and complex developmental changes occur in the GI tract during early postnatal life. During this time, enteric neuronal, immune, epithelial, and microbial signaling act in concert to prepare the host for adaptation to, and survival during, the immediate and long-term postnatal environment. In addition, the enteric systems exhibit a high degree of plasticity; thus disturbances in the normal developmental windows, such as early-life stress/adversity, can lead to long-lasting changes in intestinal function and disease susceptibility.

Animal Models of Early-Life Stress-Induced GI Disease

Several animal models have been employed to investigate the impact and mechanisms of early-life stress on GI disease. While all these models (see below) are centered on disruption of the early-life environment with psychosocial stress or chemical injury, they differ in terms of stressor types, species variations, and clinical and pathophysiological outcomes. We describe the established animal models of early-life stress used for GI disease investigations, with a focus on model and species differences, pathophysiologic findings from each model, and translatability to human GI disease.

Models of Early-Life Stress-Induced GI Disease in Rodents

Neonatal MS. The most commonly utilized animal model of early-life stress is the neonatal MS (NMS) model in rodents. There are several different types of NMS, including short handling, where the pups are handled for 15 min/day during the postnatal period, and long MS, where the pups are separated from the dam for 3 h/day. Here we focus mainly on the long-NMS model, as few studies have used the short-NMS model to address GI outcomes. Nonetheless, unlike long NMS, short NMS (handling) has been shown to be protective (decreased anxiety responses) (120, 125) and does not result in long-term GI dysfunction (136). In long NMS, rat or mouse pups are separated from their dam daily for 3-h periods: rat pups between 2 and 14 days of age or between 4 and 20 days of age (25, 84) and mouse pups between 1 and 14 days of age or between 1 and 18 days of age (12, 114). While the majority of NMS research has been carried out in mice, much of the NMS research concerning GI function has been performed in rats. The NMS model is based on disruption of development of the HPA axis by induction of stress and HPA axis activation during the hyporesponsive period (4–14 days of age in rodents) (135, 143), as discussed previously in this review. Among the most-studied GI-related effects of NMS in rodents are long-term changes in GI motor (e.g., motility) and sensory (e.g., visceral hypersensitivity) function. Adult rodents that were exposed to NMS exhibited delayed gastric emptying and...
accelerated colonic transit (13, 33). Increased fecal pellet output (an indirect measure of increased motility) was observed in NMS rats that were subjected to psychological stressors later in life (27, 51, 156), indicating heightened or exaggerated motility responses to adversity. Fecal output and altered colonic transit, while not identical, are comparable to symptoms of altered bowel frequency, such as diarrhea or constipation, in IBS in humans (66, 69). The effects of NMS on visceral sensitivity, a surrogate marker for abdominal pain, have also been well documented. Because abdominal pain is a key symptom of stress-related GI disorders such as IBS (4), study of the effects of NMS on visceral sensation is highly relevant to human GI disorders. A number of investigations (50, 51, 60, 89, 152) have demonstrated lasting visceral hypersensitivity, as determined by increased sensitivity (lower threshold) to colonic distension, in rats subjected to NMS. There have been several reports of the differences between males and females exposed to NMS, and the most prominent differences were in HPA function, where females appear to have a more reactive HPA response (61). Rostoczy et al. (152) demonstrated greater visceral hypersensitivity in females than males in response to two MS protocols: removal of all pups from their home cage or separation of half of the pups from their littermates. The increased visceral hypersensitivity in female rodents is consistent with the paradigm of human IBS, where disease is more prevalent in females. However, there is a paucity of data concerning the mechanistic differences in the GI system between male and female rats exposed to NMS.

Further investigations into the mechanisms of NMS-induced visceral hypersensitivity in rodents showed that mast cell degranulation, CRF receptor activation, transient receptor potential cation channel (TRP) subfamily V member 1, and increased intestinal permeability were central mechanisms in the visceral hypersensitivity observed in this model (156, 172). These mechanisms appear to be similar to those proposed in human IBS pathophysiology; thus, mast cells, CRF receptors, and voltage-gated sodium channels are currently targets for drug development for human GI disorders associated with abdominal pain (4, 44).

In addition to motility and visceral hypersensitivity changes induced by NMS, changes in neurochemical phenotype of enteric neurons have been reported. Gareau et al. (84) demonstrated that NMS resulted in increased numbers of cholinergic (choline acetyltransferase-positive) enteric nerves in 20-day-old weanling pups. They also reported that ex vivo application of the muscarinic receptor antagonist atropine reduced the elevated permeability of intestinal tissues from NMS rats (84), indicating a functional role of cholinergic signaling in NMS-induced permeability changes. The role of cholinergic function in the early-life stress GI disorders has not been further investigated in rodents but could be a major target in human GI disease. Serotonin, a major neurotransmitter involved in motility, secretion, and visceral hypersensitivity, was elevated in the colon of NMS rats following water avoidance stress or colonic distension (27, 149). Increased serotonergic signaling in the NMS model has important implications in human IBS, in which dysregulated serotonin signaling is thought to play a significant role in GI symptoms (37). NMS rats were also shown to exhibit increased intestinal mucosal nerve fiber density and synaptogenesis in the GI tract, which was prevented by administration of an antibody against NGF (25). These findings are consistent with those reported by Dothel et al. (64), who showed increased nerve fiber outgrowth and NGF in adult IBS biopsies.

Another consistent GI pathology observed in the NMS model is persistent elevation of GI permeability. Numerous investigations utilizing the NMS model demonstrated transcellular and/or paracellular permeability defects in adult rats that had been subjected to NMS (22, 84, 85, 114, 159). Increased intestinal permeability in this model is highly relevant to human GI disorders, as increased intestinal permeability is a well-established pathophysiology in diseases including IBS, IBD, and food allergy (39) and is linked with abdominal pain, inflammation, and disease susceptibility. The mechanism by which early-life stress induces persistent defects in intestinal permeability in humans and animals remains to be fully elucidated; however, studies utilizing the NMS model have shown that activation of multiple signaling pathways is involved. CRF receptor signaling pathways have been shown to mediate NMS-induced intestinal permeability, as peripheral administration of CRF receptor antagonists reduced intestinal permeability in NMS rats (84, 85, 159). In addition, as described previously, enteric muscarinic receptor blockade also inhibited intestinal permeability in NMS rats, suggesting an interplay between enteric cholinergic nerves and CRF receptors in regulating NMS-induced intestinal permeability.

There have been several investigations of the impact of NMS on subsequent GI inflammatory and/or psychological stress responses later in life. Barreau et al. (21) showed that rats exposed to NMS were more susceptible to infection by *Nippostrongylus brasiliensis*. In another study, Barreau et al. (22) demonstrated increased colonic myeloperoxidase, numbers of mast cells, and expression of cytokines in rats that had been subjected to NMS. Furthermore, administration of 2,4,6-trinitrobenzenesulfonic acid induced higher inflammatory and intestinal permeability responses in NMS rats than in normal-reared controls (22). Similarly, adult rats and mice that had been subjected to NMS exhibited a worsening of colitis induced by dextran sulfate sodium (129, 176). Lennon et al. (114) demonstrated that while NMS had no effects on colonic cytokine levels and colitis histological scores in adult wild-type mice, adult IL-10−/− mice exposed to NMS exhibited an early onset and increased severity of colonic cytokine levels (increased IL-12 p40 and IFN-γ mRNA) and colitis scores and intestinal permeability. They also showed that NMS and IL-10 deficiency contributed to increased intestinal permeability in the absence of marked inflammation, suggesting that both IL-10 deficiency and NMS were required to induce the onset of severe colitis. This is consistent with a “two-hit” (NMS and IL-10 deficiency) theory that has been associated with human IBD, as well as other GI diseases. Findings from the NMS model and colitis susceptibility are consistent with human evidence of early-life adversity leading to increased chance of developing IBD in humans (1, 2, 70). MS animals also show greater reactivity to psychological stressors, such as water avoidance or restraint stress, later in life. In response to these acute stressors, animals subjected to NMS display increased visceral hypersensitivity (27, 51, 152, 156, 172, 174), increased intestinal short-circuit current (Isc) and macromolecular permeability (159), increased numbers of mast cells (172), increased motility (27, 156), and increased fecal output (51). While human data for responses of IBS patients to acute stressors are...
limited, increased cortisol, GI symptoms, and skin conductance in response to an acute stress test have been shown (105). Together, these studies show that NMS predisposes rodents to inflammatory and psychological stressors, leading to exacerbated intestinal injury.

As described previously, the microbiota undergoes significant development in early in life and has emerged as a key player in a number of GI diseases in humans. A few investigations have shown that NMS in rodents alters the composition of the microbiota (20, 81, 83). Specifically, decreased populations of Lactobacillus species (83) and increased total numbers of adherent bacteria (83), with specific increases in Clostridia, Enterococcus, and Escherichia coli species (20), have been reported. Additionally, administration of probiotics reduced transcellular intestinal permeability and ion transport in NMS rodents (83).

**Limited-nesting stress.** In the limited-nesting stress model of early-life stress in rats or mice, the dam is given insufficient nesting/bedding material on postnatal days 2–9 (92, 145). The goal of this model is to disrupt normal maternal care, thus mimicking child abuse and neglect (89). Relatively few studies have measured GI-related outcomes with this model; however, pups exposed to limited nesting display visceral hypersensitivity (92, 145). Therefore, the limited-nesting stress model is relevant to GI disease-associated early-life stress and abdominal pain (e.g., IBS) (89).

**Odor-attachment learning.** Odor-attachment learning is a model of early-life stress specifically designed to mimic an abusive caregiver (89). At 8–12 days of age, rat pups are conditioned with different types of odor-conditioning: paired conditioning, where an electric shock is paired with a final odor stimulus, and unpaired conditioning, where an electric shock occurs 2 min after the final odor stimulus, which resulted in no association between the shock and the odor (42, 170). Unpaired odor shock results in long-term visceral hypersensitivity (170), which is particularly pronounced in females (42). Therefore, odor conditioning is a valid model for studying the effects of unpredictable neonatal stressors on long-term GI function and pain.

**Neonatal colonic inflammation.** Neonatal colonic irritation models involve administration of a chemical irritant or antigen (e.g., LPS, mustard oil, or acetic acid) to the animal at ~5–10 days of age (89). These protocols inflict significant injury to the colon and are particularly effective for modeling the effects of neonatal GI infection and trauma. Several neonatal colonic inflammation studies reported induction of visceral hypersensitivity (5, 47, 187). Additionally, neonatal colonic inflammation and visceral hypersensitivity appear to be associated with TRP subfamily V member 1 (187) and TRP subfamily A member 1 (47). Exposure of neonates to LPS resulted in increased mast cell degranulation and circulating IL-1β in response to a subsequent irritant (formalin) later in life (191).

**Neonatal repeated colonic distension.** Repeated colonic distension, another model of neonatal GI injury, is typically performed at 8, 10, and 12 days of age in rat pups (5, 43, 116, 117). Similar to neonatal colonic inflammation, repeated colonic distension also produces visceral hypersensitivity (5). Neonatal exposure to repeated mechanical irritation of the colon has been reported to result in increased stool liquidity, increased mucosal permeability (conductance), and increased potassium chloride-induced contractions (43). Additionally, rats displayed decreased lumbosacral activation thresholds and increased amplitude of lumbosacral response (117).

**Models of Early-Life Stress-Induced GI Disease in Large Mammals**

**Porcine early-weaning stress model.** A large-animal model that has emerged as a valid model to study early-life stress-induced GI disease is the porcine early-weaning stress (EWS) model. The EWS model is based on interruption of the natural weaning process, with an abrupt and stressful early weaning. Weaning in mammals is defined as the transition from maternal breast milk to solid food or infant formula (96). In nature, weaning is a prolonged, gradual process as the nursing animal or infant transitions to food and social independence. In the EWS model, piglets are removed from their dam at an early age (15–18 days of age) compared with a gradual weaning over 3 mo, as in nature (14). Weaned piglets are moved to a nursery environment that encompasses a number of additional stressors, including psychosocial (e.g., maternal and sibling separation, transport and transition to a new environment, fighting, and establishment of a new social hierarchy) and immunological (e.g., exposure to new dietary antigens and pathogens) stressors. Moreover, animals are exposed to weaning stressors during a period of significant postnatal GI development, as mentioned previously. Given the complex stressful events associated with early weaning, EWS provides a novel model of adverse early-life events, such as loss of caregiver, abandonment, or early, abrupt transition to formula or solid food. Furthermore, early weaning is a routine practice in animal agriculture systems; therefore, studying the mechanisms of EWS provides an opportunity to understand disease mechanisms and improve the health and well-being of agricultural animals.

Under normal, unstressed housing conditions, EWS pigs are generally healthy and exhibit growth rates similar to those of late-weaned (weaning age >23 days) control pigs. In response to weaning, EWS and late-weaned control pigs exhibit marked elevations in serum CRF and cortisol (131), indicating that, regardless of the age of the animals at weaning, the perceived stress is comparable. However, baseline GI pathophysioloogy and stress reactivity are markedly different between EWS pigs and controls. EWS pigs exhibit a chronic, relapsing functional diarrhea that persists into adulthood (unpublished observations). Compared with rodent models, the chronic relapsing diarrhea is a unique clinical feature in the porcine EWS model and is relevant and translatable to human chronic-stress diarrheal conditions such as diarrhea-predominant IBS. When faced with a later-life enteric pathogenic challenge (enterotoxigenic E. coli), EWS pigs exhibit a more rapid onset and severity of diarrhea (126). In comparison, in humans, early or premature (<4–6 mo of age) weaning in infants was shown to be a risk factor for developing subsequent gastroenteritis (68, 113). Therefore, the EWS pig could be a valid model of specific weaning-related GI disorders in humans. The mechanisms of diarrhea that are observed in the EWS model are not completely understood but are likely due to heightened secretory mechanisms. Increased intestinal $I_{sc}$, a measure of net electronegic ion transport, has been observed in the EWS pig intestine compared with weaned or late-weaned controls (130, 158). The increased $I_{sc}$ tone in the EWS intestine was...
inhibited by pharmacological blockade of CRF receptors, mast cell degranulation, and enteric nerves (130, 131, 158), suggesting an interplay between the ENS and mast cells in this model.

Compared with the rodent NMS model, EWS in pigs induces both immediate (within 24 h) and long-lasting increases in intestinal permeability (130, 131, 158). Intestinal permeability in EWS pigs is associated with disruption of the expression and localization of tight junction proteins, including occludin, claudins, and zonula occludens-1 (97, 140). Intestinal barrier defects in EWS pigs are most pronounced within 24 h postweaning but persist throughout life. Comparably, human infants weaned directly onto formula after parturition exhibited increased GI permeability compared with age-matched, breastfed infants, and this increased permeability persisted for ≥1 wk during the postnatal period (40, 184). Additionally, early weaning onto formula in infants has been correlated with increased risk for IBD and celiac disease (108, 164). The mechanisms for increased GI permeability and disease susceptibility in early-weaned infants are not known but could be similar to mechanisms defined in the porcine EWS model (e.g., CRF and mast cell-dependent pathways). Dietary factors (e.g., milk protein, fat, and growth factors), immunological stimuli (e.g., introduction to novel antigen), and psychological factors likely also play a role in intestinal permeability in EWS pigs and early-weaned infants. In the pig, diet (milk vs. cereal-based) (30) and dietary soy antigens (115) have been demonstrated to influence intestinal inflammation and function in the newly weaned pig and, therefore, could also play a role in intestinal permeability disturbances observed at weaning.

A predominant histopathological feature of the porcine EWS model is the presence of increased numbers of intestinal mast cells and their active degranulation status (131, 158). As mentioned previously, mast cells are critical stress effector cells within the brain-gut axis and have been shown to be increased in many stress-related and allergic GI disorders. For example, IBS is associated with increased numbers of mucosal mast cells, and activated mast cells have been correlated with clinical symptoms of abdominal pain and diarrhea in humans (16–18). Mast cells are capable of releasing numerous pre-stored granule mediators (e.g., histamine, TNF, and proteases) as well as synthesized mediators (e.g., cytokines, chemokines, and lipid-derived mediators). Released mast cell products can act on numerous cell types, triggering increases in secretion, permeability (epithelial and endothelial), immune cell recruitment, and enteric nerve depolarization. The functional significance of heightened mast cell activity in the porcine EWS model was demonstrated in studies by Smith et al. (158) and Moeser et al. (131), where intraperitoneal administration of the mast cell-stabilizing agent sodium cromolyn reduced intestinal permeability in EWS pigs, demonstrating that persistent mast cell activation is a central mechanism in intestinal permeability defects in the EWS model. Interestingly, despite the intestinal permeability defects and persistent mast cell activation in EWS pigs, histological lesions and inflammatory responses are limited. In contrast, NMS has been shown to increase baseline inflammation in rats (22). The lack of significant histological inflammation in pigs, along with the significant functional and clinical GI abnormalities, is consistent with biopsy findings in human IBS. The precise mechanisms by which mast cells are regulated by EWS remain to be elucidated; however, EWS- and mast cell-mediated intestinal permeability in the porcine EWS model was shown to involve activation of the intestinal CRF receptor system (130, 131, 137, 158). As with rodent models of early-life stress and the human GI diseases, the interplay between the ENS, the CRF system, and mast cells is clearly evident in the porcine EWS model. Together, these findings suggest that the ENS-CRF-mast cell axis represents a conserved pathophysiological response to early-life stress across different species and different types of early-life stressors.

In addition to providing a model of early-life stress/adversity, the porcine EWS model also provides a model to study the effects of weaning age and formula in infants on long-term GI development and health outcomes later in life. In summary, EWS in pigs and humans involves multiple stressors, including psychological, immunological, and dietary, which together induce GI injury during a highly developmental and plastic period in GI development (Fig. 1). Given the similar pathophysiology and clinical GI outcomes induced by EWS and early life adversity in pigs and humans, respectively, the porcine EWS model holds promise as a valuable translational model for human GI disease linked with adversity early in life.

**Models of Early-Life Stress-Induced GI Disease in Nonhuman Primates**

**MS in nonhuman primates.** The impact of early-life stress has also been studied in a nonhuman primate model of MS. Infant primates were peer-reared, separated from their mother at birth and placed in a peer group at ~1 mo of age, or mother-reared until ~7 mo of age (15, 72, 153). While several behavioral (decreased locomotion and increased stereotypical behaviors) and neuroendocrine (increased cortisol and serotonin transporter gene expression) changes were noted, few GI effects were characterized. There is, however, evidence that MS in rhesus macaques alters the development of the microbiota, with lower numbers of intestinal bacteria, specifically, a reduction of *Lactobacillus* species (15). Changes in the microbiota of NMS rodents or MS nonhuman primates are comparable to those observed in IBS and IBD (99, 121).

**Translational Considerations for Animal Models of Early-Life Stress-Induced GI Disease**

The early-life stress animal models discussed here, along with their major GI pathophysiological and clinical outcomes and strengths and limitations, are presented in Table 1. As demonstrated in Table 1 and discussed in the text, several animal models of early-life adversity span multiple species. While some common pathophysiology and mechanisms mimic clinical GI disease states in humans, there are inherent differences with regard to species and models that present advantages and/or limitations within the context of translatability to humans.

Regardless of the early-life stressor used in animal models of early-life adversity (e.g., NMS, EWS, or colonic irritation), it is evident that common pathophysiological, including increased intestinal permeability, altered ENS development, upregulation of the CRF system, and mast cell activation (Fig. 1), are conserved across species. While it is essential that valid animal models possess mechanisms similar to human disease, the nature of the stress and comparative genetic and biological differences across different animal models and species (compared with humans) is significant. Therefore, the species used
### Clinical signs
- Anxiety and depression-like behaviors
- ↑ Fecal pellet output in response to later-life stress

### Pathophysiology
- ↑ Baseline intestinal permeability
- ↑ Intestinal permeability in response to later-life psychosocial (WAS) stress
- Colonic hypermotility
- Visceral hypersensitivity
- ↑ Mast cell activation
- ↑ Central and enteric CRF system activity
- Microbiome alterations
- ↑ Bacterial attachment to intestinal epithelium
- ↑ Susceptibility to colitis
- ↑ Cholinergic nervous system activity

### Mechanisms
- CRF system
- Mast cells
- Nerve growth factor
- Cholinergic nervous system
- Serotonin

### Strengths
- Well established and characterized
- Murine genetic models (e.g., knockout, knockin, transgenic lines) and tools are readily available
- Model mimics some of the major pathophysiology related to human disease (visceral hypersensitivity)

### Table 1. Animal models of early-life stress-induced GI disease

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<tr>
<td>NMS</td>
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<td>EWS porcine model</td>
<td>MS macaques</td>
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<td>Protocol</td>
<td>Mice or rat pups are separated from the dam for 3 h/day during postnatal days 2–14 or 4–20 in rats and 1–14 or 1–18 in mice</td>
<td>Pups are conditioned with an odor and an electric shock (paired and unpaired) between postnatal days 8 and 12</td>
<td>Pups are subjected to colonic injury in the form of repeated colorectal distension or chemical exposure (e.g., mustard oil) between postnatal days 5 and 10 or 8 and 12, respectively</td>
<td>Early-weaned piglets are removed from the sow at 15–18 days age; control animals are removed at ≥24 days of age</td>
<td>Macaques are peer-reared, separated from their mother at birth and placed in peer group at ~1 mo of age, or mother-reared until ~7 mo of age</td>
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<tr>
<td>Clinical signs</td>
<td>● Anxiety and depression-like behaviors</td>
<td>● Depressive behaviors</td>
<td>• Stool liquidity</td>
<td>• Baseline chronic, relapsing diarrhea</td>
<td>● Locomotion</td>
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<td>Pathophysiology</td>
<td>● Visceral hypersensitivity</td>
<td>• Visceral hypersensitivity</td>
<td>• Visceral hypersensitivity</td>
<td>• Clinical severity (diarrhea) in response to later-life infectious challenge</td>
<td>• Stereotypical behaviors</td>
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<td></td>
<td>• Visceral hypersensitivity</td>
<td>• Females display more severe visceral hypersensitivity</td>
<td>• Mast cell degranulation and circulating IL-1β in response to a secondary irritant</td>
<td>• Baseline intestinal permeability</td>
<td>• Alterations in fecal microbiome MS macaques</td>
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<td></td>
<td>• Intestinal permeability in response to later-life psychosocial (WAS) stress</td>
<td>• Intestinal permeability</td>
<td>• Intestinal permeability in response to later-life infectious challenge</td>
<td>• Intestinal permeability in response to later-life psychosocial (mixing) stress</td>
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<tr>
<td></td>
<td>• Colonic hypermotility</td>
<td>• Motility (with KCl)</td>
<td>• Suppressed innate immune response to later-life infectious challenge</td>
<td>• Mast cell activation</td>
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<tr>
<td></td>
<td>• Visceral hypersensitivity</td>
<td>• Lumbosacral activation thresholds</td>
<td>• ↑ Mast cell activation</td>
<td>• Activation of peripheral and enteric CRF system</td>
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<tr>
<td></td>
<td>• ↑ Mast cell activation</td>
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<td>• ↑ ENS activity</td>
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<td></td>
<td>• ↑ Central and enteric CRF system activity</td>
<td></td>
<td>• Activation of peripheral and enteric CRF system</td>
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in the model could have a significant role in the translatability and eventual therapeutic efficacy in humans. For example, rodent models employ stressors such as intermittent MS, water avoidance stress, and restraint stress, which may not be directly relevant to the complexity of life stressors in humans. In the EWS model, piglets are subjected to significant psychosocial trauma as they are removed permanently from their mother and siblings and forced to adapt to a new environment and social hierarchy with unfamiliar pigs. Therefore, the design of the porcine EWS model may more closely resemble human conditions where children are forced to adapt to strenuous conditions without proper parental care.

In addition to the nature of the stress, species differences (genetic, anatomic, and clinical) present both strengths and limitations that should be considered in the selection of the model and the potential translational value. Rodent models have been, and will likely continue to be, critical to the understanding of GI diseases associated with stress. Some obvious strengths of laboratory rodent models are that techniques and reagents for genetic manipulation and molecular biology are readily available. Several genetic animal models and approaches (e.g., knockout, knockin, transgenic, and conditional knockdown) are powerful tools to study in vitro and in vivo contributions of specific mediators. However, genetic

<table>
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<td>Early-Life Stress Models in Rodents</td>
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<td>NMS</td>
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<td>Model mimics some of the major pathophysiology related to human disease</td>
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<td>Clinical features not directly comparable to human GI symptoms</td>
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<td>Clinical features not directly comparable to human GI symptoms</td>
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<td>Irrelevant stressors compared with human stressors</td>
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<td>Clinical features (increased pellet output) not directly comparable to human GI symptoms</td>
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<tr>
<td>Significant differences in murine GI development and nervous and immune systems compared with humans and large animals</td>
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<td>Relatively little GI data available outside visceral hypersensitivity</td>
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Features of each model, including methods, clinical features, pathophysiological hallmarks, and advantages and disadvantages, in relation to early-life adversity resulting in later-life gastrointestinal (GI) disease in humans. CNS, central nervous system; CRF, corticotropin-releasing factor; ENS, enteric nervous system; EWS, early-weaning stress; NMS, neonatal maternal separation (MS); TLR, Toll-like receptor.
manipulation of large-animal species such as the pig is now possible and becoming more common. Because of their small body size, rodent colonies are easy to manage, and the inbreeding of rodent genetic lines reduces animal-to-animal variation in biological responses. However, human populations are heterogeneous, and variation between individual animals better reflects human disease conditions and provides a framework for personalized medicine. On the basis of the EWS studies and years of research using the pig as a biomedical model, the pig also is valid model for studying stress-related GI disease in humans. Moreover, the pig possesses distinct species-specific translational advantages compared with rodent. It is well accepted that the GI anatomy, physiology, biochemistry, and evolution are more similar between humans and pigs than between humans and rodents (32, 91, 103, 185). At full term, there are common GI developmental pathways shared by all species, but the neonatal intestine is considered to be in advanced stages of development in the human and pig compared with rodents. In consideration of histological morphology and epithelial ontogeny, humans and pigs have fully developed villi and crypts in the small intestine at birth, whereas the rodent develops intestinal crypts in the postweaning period (59). In addition, as discussed above, the postnatal development of intestinal barrier function (decline in intestinal permeability) occurs largely in the first 7 days of life, compared with 14–21 days of life in mice. Therefore, the morphological ontogeny described above and the delayed decline in intestinal permeability in mice suggest that, during the first 7–14 days of life, the murine intestine is more comparable to a preterm human intestine. This could have significant implications for interpreting clinical relevance of murine models of early-life GI stress/injury that occurs between 1 and 14 days of age (NMS, colonic neonatal irritation, and limited nesting), as the GI injury is induced during a relatively underdeveloped state compared with humans. Ontogeny studies of the ENS suggest that, at birth, rodents, pigs, and humans have a semimature ENS, which undergoes significant postnatal modification (35, 80, 138, 186). However, the complexity of the human ENS is more similar to that of the porcine than the rodent intestine. For example, the human and porcine gut have additional interneuronal networks and plexi that are absent in the rodents (167). Furthermore, the colocalization of neurotransmitters to certain neuronal subtypes is more common between humans and pigs than between humans and rodents (32). Considering the neuronal nature of psychological stress, the interconnection between the brain and the gut, and the ENS as a clinical drug target, the added complexity of the porcine ENS may more similarly mimic human stress-induced GI syndromes and better predict therapeutic efficacy in humans. Similar to the ENS, the neonatal immune system is present, but rudimentary, in humans, pigs, and rodents. Considering the mechanistic role of the immune system in early-life stress, it is important to note that the frequency of preserved immunologically related genes between humans and pigs is ~80%, while the number of orthologous immunological genes between mice and humans is only 6% (123). Immunologically, mast cells are a major component of gut-mediated stress responses and play a major role in the weaning process in humans, pigs, and rodents. Mast cell degranulation at weaning is similar across these three mammalian species; however, the mediators released from mast cells are species-dependent. For example, humans and pigs have only 17–18 genes in the granzyme/mast cell tryptase/serine protease superfamily, while mice have up to 26 genes in this family (53). Release of these additional and different proteases in rodents compared with those in humans and pigs may contribute to different mechanisms of pathology and disease. Clinically, in the EWS model, pigs develop a functional, relapsing diarrhea, which has not been reported in rodents. This unique clinical presentation for an animal model of early-life stress mirrors stress-related diarrhea in humans and, thus, can serve as clinical readout, especially when evaluating therapies directed at diarrhea symptoms.

Neurobiological comparison between humans, pigs, and rodents reveals several differences between species; however, the HPA axis demonstrates a conserved neurological pathway for mediating homeostasis between the brain and the gut during stress across all species. The complexity of the human brain may be better modeled in the pig, as the human and the pig possess advanced gyrencephalic neuroanatomy (87). Consistent with their anatomy, several lines of behavioral evidence indicate that pigs are highly intelligent and capable of performing several cognitive tasks. For example, pigs have been shown to be able to pass the “mirror test,” indicating that they are self-aware animals, a property that only primates and a handful of other mammals are known to possess (86). Therefore, pigs likely have a more complex cognition of psychological stress and may have a more complicated brain-gut integration of stress than rodents.

Finally, inherent differences in diet separate humans and pigs from mice, because pigs and humans are naturally omnivores, whereas rodents are naturally granivorous. This important distinction explains differences in metabolism and digestive biochemistry between rodents and humans, and these differences in digestion could influence developmental mechanisms and subsequent clinical outcomes.

Summary

Animal models of early-life stress have provided key insight into the important brain-gut signaling pathways associated with long-term changes in GI function and disease susceptibility. The precise mechanisms linking early-life stress with GI disease remain to be elucidated. Without continued investigations and development of knowledge in this field, effective therapies for treating stress-induced diseases associated with early-life stress will remain limited. Between animal models, many common biological pathways are activated by early-life stress; however, inherent species-related differences could have major translational significance. Rodent models predominate in the literature and are expected to continue to be extremely valuable models for study of stress-related GI diseases in humans. However, large-animal models, such as the porcine EWS model, are gaining recognition in this field. Given the high degree of genetic, anatomic, and physiological homology between pigs and humans, large-animal models such as the pig are expected to offer unique advantages over murine models, particularly in translation from basic biological mechanisms to the predictability of therapeutic successes in humans.

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AUTHOR CONTRIBUTIONS
C.S.P., J.E.M., and A.J.M. developed the concept and designed the research; C.S.P., J.E.M., and A.J.M. prepared the figures; C.S.P., J.E.M., and A.J.M. drafted the manuscript; C.S.P., J.E.M., and A.J.M. edited and revised the manuscript; C.S.P., J.E.M., and A.J.M. approved the final version of the manuscript.

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