0031-3998/07/6203-0240
PEDIATRIC RESEARCH

Vol. 62, No. 3, 2007
Printed in U.S.A.

REVIEW ARTICLE

New Insights in the Etiology and Pathophysiology of Irritable Bowel Syndrome: Contribution of Neonatal Stress Models

FREDERICK BARREAU, LAURENT FERRIER, JEAN FIORAMONTI, AND LIONEL BUENO

INSERM U843 [F.B.], Inflammation intestinale chez l'enfant, Université Paris 7, Hôpital Robert Debré, F-75019 Paris, France; INRA [L.F., J.F., L.B.], Neuro-Gastroenterology and Nutrition Unit, F-31931 Toulouse, France

ABSTRACT: Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders, characterized by abdominal pain and disturbed defecation that cannot be explained by structural abnormalities. Although IBS symptoms (visceral pain, increased gut permeability, motility alterations) are clearly established, the etiology of this pathology is loosely understood. Nevertheless, clinical studies have reported that some early abuse (physical and psychological) is often associated with IBS development. Thus, loss and separation in the family during childhood may contribute to the IBS development. The recent development of animal models has pointed out the importance of early traumatic experiences in favoring the occurrence of IBS in adult life. Among these different models, neonatal maternal deprivation (NMD), neonatal colonic irritation (inflammatory stimuli), and neonatal colonic pain (rectal distension) have been described to mimic some cardinal features of IBS. The purpose of this review is 3-fold. First, to present the different neonatal stress models. Second, to review the literature on the influence of these early traumatic experiences on the gastrointestinal tract disturbances observed in adult life. Finally, we will also present the mediators and mechanisms involved in gut dysfunction triggered by NMD and probably in IBS. **(*Pediatr Res* 62: 240–245, 2007)**

IBS is a disorder characterized by chronic abdominal pain and discomfort associated with alterations in bowel habits in absence of any detectable organic disease (1). Although IBS is a heterogeneous disorder in terms of etiology and pathophysiology, alterations in bowel habits are likely related to alterations in autonomic regulation of gut motility, whereas symptoms of abdominal pain and discomfort are thought to involve changes in perception of visceral events resulting from visceral hyperalgesia or allodynia (2,3).

Progress in the development of effective therapies has been hampered due to the lack of relevant animal models mimicking the key features of IBS. Many preclinical investigations have been performed with models of acute inflammatory insults to the gut (glycerol, mustard oil, acetic acid, zymosan), thereby mimicking more IBD than IBS, which is characterized by the lack of apparent inflammatory changes within the gut mucosa. Recently, several potential IBS models have been proposed, all of which mimicking some aspects of the human syndrome (4–6). They include an early life colon irritation (4), an adult stress-induced hypersensitivity to colonic distension (7), and an adult postinfective state in rodents (8,9). However, all of these models only reproduced some aspects of the pathophysiological features of the human syndrome.

Despite the incomplete knowledge about the pathophysiology of IBS, different types of stressors appear to play key roles in the development of IBS as well as in the modulation and maintenance of the disease throughout life. Indeed, early traumatic experiences such as childhood neglect, abuse, loss of a parent, and life-threatening situations during childhood have been shown to increase the risk of IBS development (10–12). Consequently, many investigators have attempted to develop animal models of stressful experience in childhood and, postnatal handling, early separation and daily periodic NMD have been proposed. The pioneering work of Levine *et al.* (13–15) has shown that even quite subtle alterations in the experience of rats during the early postnatal period can have long lasting consequences in terms of behavior, emotionality and stress responsiveness. Multiple variations of the NMD procedures exist with regard to duration (1–24 h) and number (1–14 d during the first 3 postnatal weeks) of the separation episodes. A primary feature of the NMD phenotype has been an enduring dysregulation of HPA axis reactivity to stress (16). Indeed, in the developing rodent, a period around postnatal d 2–14 is characterized by a limited, or even absent, adrenal response to stress (17), and has been defined as the stress hyporesponsive period (SHRP). Numerous experiments have demonstrated that maternal factors are critical for the regulation of the pup's HPA axis and the maintenance of SHRP (15). NMD abolishes this physiologic inhibitory state and induces a long-lasting hyperresponsiveness of the HPA axis response (18). In addition, rats periodically deprived during the early postnatal period show weak learning ability, memory capacity, associated with lower levels of acetylcholinesterase activity within the CNS (19), and a decreased

Received October 18, 2006; accepted March 9, 2007.
Correspondence: Frédérick Barreau, M.D., INSERM U843, Université Paris 7, Hopital Robert Debré, Paris, France; e-mail: barreau@rdebre.inserm.fr
This work was supported by the Institut National de la Recherche Agronomique (INRA).

Abbreviations: CGRP, calcitonin gene-related peptide; **HPA,** hypothalamic pituitary adrenal; **IBS,** irritable bowel syndrome; **NGF,** nerve growth factor; **NMD,** neonatal maternal deprivation

immediate exploratory locomotor response to novelty (20). In agreement with these neuroendocrine changes, deprived rats also exhibit an increased anxiety-like behavior, anhedonia, an enhanced preference for ethanol, and impairment of male sexual behavior (16,21–24). However, it is well known that the effects of maternal separation depend upon such variables as duration of each episode of separation, number of episodes experienced, and timing of episodes during development. Nevertheless, among the different variables of NMD models, one procedure where newborn rats are daily removed from the mother for several hours during the first 2 wk of life (22,24–27) has been largely used to describe the repercussions of NMD on the gastrointestinal tract, and to develop an experimental model to investigate the etiology and pathophysiology of IBS (28–31).

NEONATAL NOXIOUS STIMULI

The early neonatal period is a time of great plasticity for both somatic and visceral sensory systems. This neuronal plasticity often contributes to adaptive or maladaptive function of the mammalian nervous system, and possibly to the development of chronic pain. The nociceptive neuronal circuits are both formed during embryonic and postnatal times when painful stimuli are absent or limited. During this critical period, particularly before the maturation of the descending inhibitory systems (32,33), pain can lead to prolonged structural and functional alterations in nociceptive pathways that can be maintained in adult life (4,34–36).

The first model of neonatal noxious somatic stimuli has been proposed by Anand and co-workers (37,38). In this model, the noxious stimulus was applied daily from day of birth to postnatal d 7. The paws of rat pups were stimulated once, twice, or four times a day at hourly intervals with a needle inserted rapidly through the paw. This experimental model may lead to an altered development of the nociceptive system characterized by decreased pain thresholds and hypersensitivity (37,38). Another model of early pain experience (laparotomy) on the day of birth has also highlighted the occurrence of long-lasting changes in pain behavior, probably mediated by alterations in the HPA-axis and antinociceptive circuitry (39).

Considering the gut, the first model of neonatal noxious stimuli has been developed by Al-Chaer *et al.* (4,40). In this model, colon injury was applied daily using colorectal distension during postnatal development between d 8 and 21. The distension was applied using an angioplasty balloon, inserted rectally into the colon in the awake neonate. Colon injury in neonates results in chronic visceral hypersensitivity, with characteristics of allodynia and hyperalgesia in adult rats, associated with peripheral sensitization, as well as central sensitization in the absence of identifiable peripheral pathology (4,40). Finally, the importance of neonatal noxious stimuli in the development of human functional intestinal disorders in later life has been recently pointed out by Anand *et al.* Indeed, noxious stimulation caused by gastric suction at birth may promote the development of long-term visceral hypersensitivity and cognitive hypervigilance, leading to an increased prevalence of functional intestinal disorders in later life (41). Although each neonatal noxious model mimics one feature of human syndrome and also evidence the importance of these traumatic experiences during neonatal period, all of these experimental models fail individually to reproduce all of the IBS features (Table 1).

NEONATAL INFLAMMATORY STIMULI

Developmental plasticity in physiologic systems is an important mechanism through which organisms can adapt their physiologic responses to better meet environmental demands. Although such alterations may prove some benefit for immediate survival, they may also result in a permanent alteration of physiologic responses to environmental challenges, and alter predisposition to pathology later in life (42–44). Thus, some groups have investigated the impacts of different inflammatory stimuli [lipopolysaccharide (LPS) administration, colonic inflammation] applied during early stages of postnatal development, on adult physiology.

Exposure of neonatal rats to a low dose of *Salmonella enteritidis* endotoxin (i.p. route) during the first week of life resulted in long-term changes in HPA axis activity (45). Moreover, this neonatal endotoxin exposure induced long-lasting effects on immune regulation, including increased sensitivity of lymphocytes to stress-induced suppression of proliferation and a protection from adjuvant-induced arthritis (45). Neonatal (d 3 and 5) LPS exposure alters behavioral and clinical course of periodontal disease in adult rats (46). In addition, it has been reported that a single LPS exposure during development (d 14) impacts upon adult pain and sensory processing, leading to a decrease in nociceptive thresholds and increases in responses to painful and even innocuous stimuli in adulthood (47).

Neonatal colonic inflammation, induced by mustard oil between postnatal d 8 and 21 results in chronic visceral hypersensitivity associated with central neural sensitization in the absence of identifiable peripheral pathology in adult rats (4). Taken together, these models highlight the emphasis of the neonatal inflammatory stimuli in the development of some aspects of the human IBS in later life. However, none of these models mimics all of the IBS features (Table 1).

Table 1. *Neonatal stress models and irritable bowel syndrome features*

Neonatal stress model	Visceral hyperalgesia	Motility alterations	Alterations of gut permeability and bacterial translocation	Somatic pain alterations	Depression and/or anxiety	HPA dysfunctions	Reference
NMD	Yes	Yes	Yes	Yes	Yes	Yes	(15,24,29–31)
Noxious stimuli	Yes	ND	ND	Yes	Yes	Yes	(4,37–40)
Inflammatory stimuli	Yes	ND	ND	Yes	Yes	Yes	(4,45–47)

ND, not determined.

NEONATAL MATERNAL DEPRIVATION

There is a growing body of evidence that indicates that all different aspects of mother–infant interactions play an important role in the development of the newborn. Studies, mainly performed in developing rodents, have demonstrated that the mother behavior influences various physiologic parameters in the infant as heart rate, sleep/wake cycles, and growth hormone production (48). Specific physiologic changes in the infant occurring slowly over a relatively protracted period of separation can be tightly linked to specific features of mother-infant interaction. Thus, 24 h of NMD decreases mean cardiac frequency (49). This decrease may result from the long duration of mother's milk deprivation, rather than a lack of other aspects of maternal care such as growth hormone secretion induced by tactile stimulation from the mother.

Although less investigated than emotional behavior or stress reactivity, several studies have established that early traumatic experience alters gastrointestinal homeostasis in the adult. The first studies have reported that early maternal deprivation increases gastric ulcer risk, and that early weaning predisposes rats to exacerbated stress-induced ulcer formation (50–52). More recently, some laboratories have developed NMD models, using different rat strains, which have highlighted the consequences of NMD on intestinal physiology in adults (28–31,53,54). In all these models, rat pups were daily exposed to a 180-min period of NMD during the stress-hyporesponsive phase (Table 2).

Gut mucosa immune status. NMD is described to modify the gut immune status in young and adult rats (31,55). Macroscopic observation of the colon of adult deprived rats reveals the presence of both focal hyperemia and mesenteric adherences (31). These macroscopic alterations are associated with several immune modifications. In both 14-d-old and 12-wk-old rats, colonic mucosa of deprived rats presents a greater number of mast cells than in controls (55). An increase of polymorphonuclear neutrophils number is also observed in both colonic and jejunal mucosa of adult maternally deprived rats (31,53). Moreover, expression of mRNA encoding for cytokines as interferon (IFN)-γ, IL-1β, IL-2, IL-4, and IL-10 in the colon is higher in deprived than in control rats. Thus, NMD induces an increase of both Th-1 and Th-2 cytokine profiles associated with a low-grade systemic inflammatory state in colon (31). In addition, it has been shown that NMD in rats facilitates primary infection by *Nippostrongylus brasiliensis* and enhances the inflammatory response of the jejunum, but does not induce severe breakdown of immunity to *N. brasiliensis* (53). Deprived rats exhibited an increase number of jejunal worms and fecal eggs and larvae on d 7 after primary infection associated with an increased MPO activity in jejunal mucosa. These data support that NMD promotes long-term alterations of the intestine immune status and reduces intestinal defense toward a nematode infection.

Visceral sensitivity and motility. Coutinho *et al.* (30) have shown that deprived rats exhibit visceral hyperalgesia and cutaneous hypoalgesia under baseline conditions, as well as visceral hyperalgesia and enhanced colonic motility in response to an acute psychological stressor in later life. Thus, exposure to a novel environment or acute stress resulted in a significantly enhanced fecal pellet output, used as an index of colonic motility, in deprived rats compared with nondeprived rats. More recently, using the same neonatal stress protocol it has been reported that deprived rats also develop acute and delayed stress-induced visceral hyperalgesia, associated with an enhanced stress-induced increase in colonic motility and increased anxiety-like behavior (56).

NMD provokes a greater visceral hypersensitivity to rectal distension in female than in male adult (28). This visceral hyperalgesia is also greater when all pups are removed from home cage than when only half of littermates is removed (28). This indicates that stress of the mother plays a role in the long-term effects of NMD. However, acute restraint stress induces hyperalgesia in control females only, and this effect was similar when either half of littermates or all pups were removed. These results indicate that long-term visceral hyperalgesia depends upon the type of NMD and that females are more sensitive than males (28).

Taken together, these data make evident that early traumatic experiences induce long-term changes of gut sensitivity under basal and stressful situations.

Intestinal epithelial permeability. A first series of experiments performed by Perdue *et al.* (54) has demonstrated that NMD provokes an immediate increase in passage of macromolecule through the colonic mucosa associated with higher amounts of bacteria adhering to and penetrating into the colonic epithelium. However, these colonic alterations rapidly

Table 2. *Long-term consequences of different NMD models on gut functions*

Rat strain	Period of separation	Age at experimentation date	Gastrointestinal disturbances	Reference
Long-Evans	PND 2–14	2 mo	Visceral hyperalgesia in basal condition and after stress Cutaneous hypoalgesia after stress	(30,56)
Sprague-Dawley	PND 4–21	2 mo	Vulnerability of colonic mucosa to acute stress (permeability, ion secretion)	(29)
Sprague-Dawley	PND 4–21	19–30 d	Elevated ion secretion Increased colonic permeability Bacterial translocation	(54)
Wistar	PND 1–14	3 mo	Susceptibility to stress-induced visceral pain	(28)
Wistar	PND 2–14	3 mo	Colonic hyperalgesia Elevated gut and colonic paracellular permeability Bacterial translocation Susceptibility to TNBS and parasite-induced inflammation	(31,53,55,63)

return to normal values (54). In addition, a mild stress increase short-circuit current, conductance, and transepithelial transport of macromolecules in maternally deprived rats, while having minimal effects in controls (29), evidencing that neonatally deprived rats develop persistent mucosal barrier dysfunction characterized by an impaired host defense to luminal bacteria and enhance gut mucosa vulnerability to stress.

Other studies have reported that repeated NMD induce long-term alterations of gut paracellular permeability in adult rats (31,53,55). Male Wistar pups separated during postnatal d 2–14 exhibit at 12 wk of age, an increase in both jejunal and colonic paracellular permeability, associated with bacterial translocation (31). Similarly, weaning caused marked disturbances in intestinal barrier function, as demonstrated by significant reductions in transepithelial electrical resistance and increases in intestinal permeability to mannitol compared with unweaned control pigs (57).

Pathways involved in the genesis of visceral and permeability alterations. Among previous studies, some of mediators and cell type have been characterized in the genesis of visceral sensitivity and gut permeability alterations induced by NMD. Thus, NGF and CRF (29,54,55) and mast cells (55) participate in triggering and maintaining gut dysfunction provoked by NMD. However, other cell types such as polymorphonuclear neutrophils and neurons and other mediators such as cytokines (58,59) and proteases (60) might be also involved in the gut alterations induced by NMD. Polymorphonuclear neutrophils and nerve fibers are described in some models of pain to act in concert with mast cells to generate noxious stimuli (61,62). In addition, mediators released from mast cells such as cytokines [IFN-γ, tumor necrosis factor (TNF)-α and IL-4] or proteases (rat mast cell protease II) promote visceral pain and/or increase in gut paracellular permeability.

CRF is largely known to be involved in stress-induced gut disturbances in adult animals, and its role in gut dysfunctions induced by NMD has been described recently. Indeed, treating (i.p. route) animals with either nonselective CRF-R1/2 (α-helical CRF 9-41) or selective CRF-R1 (NBI-35965, CP-154, 526 and SSR-125543) receptor antagonists abolishes the effects of NMD on gut permeability, bacterial translocation, and visceral hypersensitivity (29,54,56,63,64). It is established that CRF-R1 mediates stress-induced gut disturbances in adult animals (64–66); these results confirm that CRF-R1 is likewise involved in NMD-induced gut dysfunctions in newborn rat. However, since the literature is controversial regarding the ability of CRF antagonists to cross the blood-brain barrier, it is unclear whether CRF mediated NMD-induced gut alterations through pathways involving central and/or peripheral mechanisms. In addition, the origin of CRF is still under debate. Although CRF secretion is mainly localized in the CNS, a colonic CRF production has been detected in the mucosal cells in the neighborhood of the base of the colonic crypts (67,68). In agreement, we have shown that both preproCRF and mature CRF are present within the colonic mucosa, and that NMD increases preproCRF expression in colonic mucosal, while it does not modify colonic mucosal CRF content in adult rats (63). These results also reinforce the likely peripheral involvement of CRF in NMD-induced gut disturbances.

NGF is able to trigger noxious stimuli (62) and NGF levels within the CNS have been found increased in rats submitted to NMD (69). Recently, it has also been shown that NGF participates in the genesis of gut dysfunction triggered by NMD (55). Indeed, NGF neutralization by specific antibodies (i.p. route), given before each neonatal stress session, suppressed the effects of NMD on visceral hypersensitivity to rectal distension and gut permeability (55,63). However, little is known about central and/or peripheral mechanisms involved in the NGF induced gut dysfunction in deprived rat. Nevertheless, since NGF depletion (autoimmunization) produces thermal hypoalgesia, associated with high serum titers of anti-NGF IgG, and an absence of anti-NGF IgG in the cerebrospinal fluid (70), one can hypothesize that antibodies directed against NGF do not cross the blood-brain barrier and that thermal hypoalgesia is the consequence of peripheral NGF neutralization. Accordingly, gut dysfunctions induced by NMD are likely mainly triggered by a peripheral mechanism. In agreement with this hypothesis, we have observed an enhanced colonic mRNA and protein expression of NGF in deprived rats that can be related to these gut alterations (55).

These data raise the question of the interplay between CRF and NGF, and several studies have brought investigators to focus on mast cells as a pivotal effecter of NMD. During the last decades, studies from various groups have highlighted the importance of mast cells in stress-related changes in intestinal barrier function (65), *i.e.* mucin or ion secretion, water secretion, para- and transcellular permeability, and visceral pain. Thus, in adult rats, repeated stress has been shown to increase the number of colonic mucosal mast cells, which play an important role in stress-induced increase in gut paracellular permeability and visceral hyperalgesia, as demonstrated by using mast cell deficient Ws/Ws rats (71), or mast cells stabilizers (7,72). It is noteworthy that the use of a mast cell membrane stabilizer abolishes both visceral hypersensitivity and increased permeability induced by NMD in adult rats (55). Moreover, CRF is a well-known mast cell secretagogue, and mast cells synthesize, store, and release NGF (73). Thus, the close link between CRF, mast cells, and NGF has been recently established in animals submitted to NMD (63), since it has been reported that CRF, through CRF-R1 receptor, promotes NGF release from mast cells, inducing a downstream elevation of gut permeability (Fig. 1) (63).

Neonatal maternal deprivation, a pathophysiological model for IBS. Recently, clinical studies on biopsies from IBS patients have brought an important advance in the understanding of the mechanisms involved in visceral pain, and strengthened the observations in animal models (74,75). In IBS biopsies, mast cell density is increased in the intestinal mucosa, and mast cells appear closer to nerve fibers than in controls (74,75). Indeed, in the ileum and rectosigmoid mucosa of IBS patients, the density of neuron specific enolase, substance P, and serotonin positively stained nerve fibers was increased and appeared in clusters, surrounding an increased number of mast cells (74). Moreover, colonic mast cell infiltration and mediator release in proximity to mucosal innervation may contribute to abdominal pain perception in IBS patients (75). The severity and frequency of perceived abdominal painful

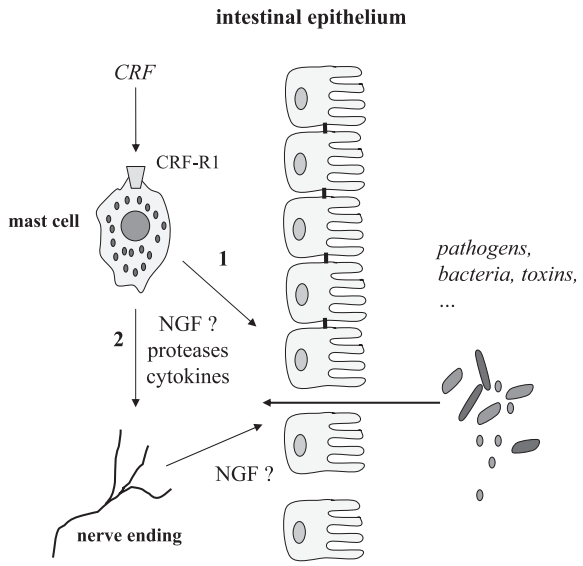

Figure 1. Putative mechanisms involved in NMD-induced visceral hyperalgesia and increase in gut paracellular permeability. The interaction between CRF and mast cell CRF-R1 receptors provokes NGF and other mediators release. The resulting activation and release of mediators such as NGF, proteases or cytokines may (*1*) directly target the intestinal epithelial cells or (*2*) sensitize nerve ending to promote visceral hyperalgesia and activate enterocyte to increase the paracellular permeability.

sensations are also correlated with the presence of activated mast cells (tryptase release) in proximity of nerve endings in the gut wall (75). In addition, clinical studies using sodium cromoglycate as a mast cell stabilizer have reported the primary role of mast cells in IBS. Thus, mast cell–mediated food intolerance has been evidenced as a major factor in the pathogenesis of IBS, and sodium cromoglycate treatment for one month resulted in a significant improvement of intestinal IBS symptoms (76–78).

In agreement with these clinical data, we have reported that NMD promotes long-term changes of colonic mast cell phenotype and nerve terminal distribution, characterized by an increase of nerve endings density in close proximity of mast cells within the colonic wall (unpublished data). In addition, the amount of mediators (RMCP II) released is also increased within the colonic mucosa of deprived rats (unpublished data). Since mast cells mediators (tryptase, cytokines) are known to alter enteric nervous system physiology and to promote visceral hypersensitivity (60,79), the higher release of such mediators concomitant with the closer proximity of colonic innervation also supported that these anatomical modifications may contribute to the disturbed sensory-motor function (28,30).

In conclusion, the studies reviewed herein indicate that various types of early traumatic events have long-term consequences on gastrointestinal functions. These studies have brought important advances in the understanding of mechanisms probably involved in the development of IBS. Among the different early traumatic experiences applied during the neonatal period, NMD results in permanent visceromotor and somatic alterations associated with neurochemical changes, altered HPA responsiveness to stressors, and an increased risk of developing depression-like behaviors, thereby mimicking all the main features of IBS in humans (Table 1). Consequently, NMD may constitute a valuable experimental model to investigate the pathophysiology of IBS and to identify novel pharmacological targets.

REFERENCES

1. Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA 1999 Functional bowel disorders and functional abdominal pain. Gut 45:II43–II47
2. Mayer EA 2000 The neurobiology of stress and gastrointestinal disease. Gut 47:861–869
3. Mayer EA, Gebhart GF 1994 Basic and clinical aspects of visceral hyperalgesia. Gastroenterology 107:271–293
4. Al-Chaer ED, Kawasaki M, Pasricha PJ 2000 A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. Gastroenterology 119:1276–1285
5. Collins SM, McHugh K, Jacobson K, Khan I, Riddell R, Murase K, Weingarten HP 1996 Previous inflammation alters the response of the rat colon to stress. Gastroenterology 111:1509–1515
6. Vallance BA, Croitoru K, Collins SM 1998 T lymphocyte-dependent and -independent intestinal smooth muscle dysfunction in the T. spiralis-infected mouse. Am J Physiol 275:G1157–G1165
7. Gue M, Del Rio-Lacheze C, Eutamene H, Theodorou V, Fioramonti J, Bueno L 1997 Stress-induced visceral hypersensitivity to rectal distension in rats: role of CRF and mast cells. Neurogastroenterol Motil 9:271–279
8. McLean PG, Picard C, Garcia-Villar R, More J, Fioramonti J, Bueno L 1997 Effects of nematode infection on sensitivity to intestinal distension: role of tachykinin NK2 receptors. Eur J Pharmacol 337:279–282
9. Bercik P, Wang L, Verdu EF, Mao YK, Blennerhassett P, Khan WI, Kean I, Tougas G, Collins SM 2004 Visceral hyperalgesia and intestinal dysmotility in a mouse model of postinfective gut dysfunction. Gastroenterology 127:179–187
10. Hill O 1970 Some psychiatric non-sequelae of childhood bereavement. Br J Psychiatry 116:679–680
11. Hill OW, Price JS 1967 Childhood bereavement and adult depression. Br J Psychiatry 113:743–751
12. Lowman BC, Drossman DA, Cramer EM, McKee DC 1987 Recollection of childhood events in adults with irritable bowel syndrome. J Clin Gastroenterol 9:324–330
13. Levine S 1967 Maternal and environmental influences on the adrenocortical response to stress in weanling rats. Science 156:258–260
14. Smotherman WP, Brown CP, Levine S 1977 Maternal responsiveness following differential pup treatment and mother-pup interactions. Horm Behav 8:242–253
15. Levine S 2001 Primary social relationships influence the development of the hypothalamic–pituitary–adrenal axis in the rat. Physiol Behav 73:255–260
16. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM 2000 Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog Brain Res 122:81–103
17. Rosenfeld P, Gutierrez YA, Martin AM, Mallett HA, Alleva E, Levine S 1991 Maternal regulation of the adrenocortical response in preweanling rats. Physiol Behav 50:661–671
18. Levine S 2000 Influence of psychological variables on the activity of the hypothalamic-pituitary-adrenal axis. Eur J Pharmacol 405:149–160
19. Dorner G, Tonjes R, Hecht K, Hinz G, Poppe I, Poppei M, Tsamaloukas A 1981 Pyridostigmine administration in newborn rats prevents permanent mental ill-effects produced by maternal deprivation. Endokrinologie 77:101–104
20. Matthews K, Wilkinson LS, Robbins TW 1996 Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood. Physiol Behav 59:99–107
21. Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ 2000 The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 22:219–229
22. Wigger A, Neumann ID 1999 Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. Physiol Behav 66:293–302
23. Rhees RW, Lephart ED, Eliason D 2001 Effects of maternal separation during early postnatal development on male sexual behavior and female reproductive function. Behav Brain Res 123:1–10
24. Huot RL, Thrivikraman KV, Meaney MJ, Plotsky PM 2001 Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology (Berl) 158:366–373
25. Plotsky PM, Meaney MJ 1993 Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res Mol Brain Res 18:195–200
26. Boccia ML, Pedersen CA 2001 Brief vs. long maternal separations in infancy: contrasting relationships with adult maternal behavior and lactation levels of aggression and anxiety. Psychoneuroendocrinology 26:657–672
27. Kalinichev M, Easterling KW, Holtzman SG 2000 Periodic postpartum separation from the offspring results in long-lasting changes in anxiety-related behaviors and sensitivity to morphine in Long-Evans mother rats. Psychopharmacology (Berl) 152:431–439
28. Roszkoczy A, Fioramonti J, Jarmay K, Barreau F, Wittmann T, Bueno L 2003 Influence of sex and experimental protocol on the effect of maternal deprivation on rectal sensitivity to distension in the adult rat. Neurogastroenterol Motil 15:679–686

29. Soderholm JD, Yates DA, Gareau MG, Yang PC, MacQueen G, Perdue MH 2002 Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. Am J Physiol Gastrointest Liver Physiol 283:G1257–G1263
30. Coutinho SV, Plotsky PM, Sablad M, Miller JC, Zhou H, Bayati AI, McRoberts JA, Mayer EA 2002 Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat. Am J Physiol Gastrointest Liver Physiol 282:G307–G316
31. Barreau F, Ferrier L, Fioramonti J, Bueno L 2004 Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. Gut 53:501–506
32. Boucher T, Jennings E, Fitzgerald M 1998 The onset of diffuse noxious inhibitory controls in postnatal rat pups: a C-Fos study. Neurosci Lett 257:9–12
33. Fitzgerald M, Koltzenburg M 1986 The functional development of descending inhibitory pathways in the dorsolateral funiculus of the newborn rat spinal cord. Brain Res 389:261–270
34. Fitzgerald M, Beggs S 2001 The neurobiology of pain: developmental aspects. Neuroscientist 7:246–257
35. Lidow MS, Song ZM, Ren K 2001 Long-term effects of short-lasting early local inflammatory insult. Neuroreport 12:399–403
36. Ruda MA, Ling QD, Hohmann AG, Peng YB, Tachibana T 2000 Altered nociceptive neuronal circuits after neonatal peripheral inflammation. Science 289:628–631
37. Anand KJ, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM 1999 Long-term behavioral effects of repetitive pain in neonatal rat pups. Physiol Behav 66:627–637
38. Anand KJ, Scalzo FM 2000 Can adverse neonatal experiences alter brain development and subsequent behavior? Biol Neonate 77:69–82
39. Sternberg WF, Scorr L, Smith LD, Ridgway CG, Stout M 2005 Long-term effects of neonatal surgery on adulthood pain behavior. Pain 113:347–353
40. Lin C, Al-Chaer ED 2003 Long-term sensitization of primary afferents in adult rats exposed to neonatal colon pain. Brain Res 971:73–82
41. Anand KJ, Runeson B, Jacobson B 2004 Gastric suction at birth associated with long-term risk for functional intestinal disorders in later life. J Pediatr 144:449–454
42. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB 1999 The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 160:1–12
43. McEwen BS, Stellar E 1993 Stress and the individual. Mechanisms leading to disease. Arch Intern Med 153:2093–2101
44. Munck A, Naray-Fejes-Toth A 1992 The ups and downs of glucocorticoid physiology. Permissive and suppressive effects revisited. Mol Cell Endocrinol 90:C1–C4
45. Shanks N, Windle RJ, Perks PA, Harbuz MS, Jessop DS, Ingram CD, Lightman SL 2000 Early-life exposure to endotoxin alters hypothalamic-pituitary-adrenal function and predisposition to inflammation. Proc Natl Acad Sci U S A 97:5645–5650
46. Breivik T, Stephan M, Brabant GE, Straub RH, Pabst R, von Horsten S 2002 Postnatal lipopolysaccharide-induced illness predisposes to periodontal disease in adulthood. Brain Behav Immun 16:421–438
47. Boisse L, Spencer SJ, Mouihate A, Vergnolle N, Pittman QJ 2005 Neonatal immune challenge alters nociception in the adult rat. Pain 119:133–141
48. Kuhn CM, Butler SR, Schanberg SM 1978 Selective depression of serum growth hormone during maternal deprivation in rat pups. Science 201:1034–1036
49. Hofer MA 1975 Survival and recovery of physiologic functions after early maternal separation in rats. Physiol Behav 15:475–480
50. Ackerman SH, Hofer MA, Weiner H 1978 Early maternal separation increases gastric ulcer risk in rats by producing a latent thermoregulatory disturbance. Science 201:373–376
51. Ackerman SH, Hofer MA, Weiner H 1978 Predisposition to gastric erosions in the rat: behavioral and nutritional effects of early maternal separation. Gastroenterology 75:649–654
52. Glavin GB, Pare WP 1985 Early weaning predisposes rats to exacerbated activity-stress ulcer formation. Physiol Behav 34:907–909
53. Barreau F, de Lahitte JD, Ferrier L, Frexinos J, Bueno L, Fioramonti J 2006 Neonatal maternal deprivation promotes *Nippostrongylus brasiliensis* infection in adult rats. Brain Behav Immun 20:254–260
54. Gareau MG, Jury J, Yang PC, MacQueen G, Perdue MH 2006 Neonatal maternal separation causes colonic dysfunction in rat pups including impaired host resistance. Pediatr Res 59:83–88
55. Barreau F, Cartier C, Ferrier L, Fioramonti J, Bueno L 2004 Nerve growth factor mediates alterations of colonic sensitivity and mucosal barrier induced by neonatal stress in rats. Gastroenterology 127:524–534
56. Schwetz I, McRoberts JA, Coutinho SV, Bradesi S, Gale G, Fanselow M, Million M, Ohning G, Tache Y, Plotsky PM, Mayer EA 2005 Corticotropin-releasing factor receptor 1 mediates acute and delayed stress-induced visceral hyperalgesia in maternally separated Long-Evans rats. Am J Physiol Gastrointest Liver Physiol 289:G704–G712
57. Moeser AJ, Klok CV, Ryan KA, Wooten JG, Little D, Cook VL, Blikslager AT 2007 Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am J Physiol Gastrointest Liver Physiol 292:G173–G181
58. Ferrier L, Mazelin L, Cenac N, Desreumaux P, Janin A, Emilie D, Colombel JF, Garcia-Villar R, Fioramonti J, Bueno L 2003 Stress-induced disruption of colonic epithelial barrier: role of interferon-gamma and myosin light chain kinase in mice. Gastroenterology 125:795–804
59. Berin MC, Yang PC, Ciok L, Waserman S, Perdue MH 1999 Role for IL-4 in macromolecular transport across human intestinal epithelium. Am J Physiol 276:C1046–C1052
60. Coelho AM, Vergnolle N, Guiard B, Fioramonti J, Bueno L 2002 Proteinases and proteinase-activated receptor 2: a possible role to promote visceral hyperalgesia in rats. Gastroenterology 122:1035–1047
61. Rice AS, Farquhar-Smith WP, Nagy I 2002 Endocannabinoids and pain: spinal and peripheral analgesia in inflammation and neuropathy. Prostaglandins Leukot Essent Fatty Acids 66:243–256
62. Shu XQ, Mendell LM 1999 Neurotrophins and hyperalgesia. Proc Natl Acad Sci U S A 96:7693–7696
63. Barreau F, Cartier C, Leveque M, Ferrier L, Moriez R, Laroute V, Rosztoczy A, Fioramonti J, Bueno L 2007 Pathways involved in gut mucosal barrier dysfunction induced in adult rats by maternal deprivation: corticotrophin-releasing factor and nerve growth factor interplay. J Physiol 580:347–356
64. Million M, Grigoriadis DE, Sullivan S, Crowe PD, McRoberts JA, Zhou H, Saunders PR, Maillot C, Mayer EA, Tache Y 2003 A novel water-soluble selective CRF1 receptor antagonist, NBI 35965, blunts stress-induced visceral hyperalgesia and colonic motor function in rats. Brain Res 985:32–42
65. Soderholm JD, Perdue MH 2001 Stress and gastrointestinal tract. II. Stress and intestinal barrier function. Am J Physiol Gastrointest Liver Physiol 280:G7–G13
66. Greenwood-Van Meerveld B, Johnson AC, Cochrane S, Schulkin J, Myers DA 2005 Corticotropin-releasing factor 1 receptor-mediated mechanisms inhibit colonic hypersensitivity in rats. Neurogastroenterol Motil 17:415–422
67. Kawahito Y, Sano H, Kawata M, Yuri K, Mukai S, Yamamura Y, Kato H, Chrousos GP, Wilder RL, Kondo M 1994 Local secretion of corticotropin-releasing hormone by enterochromaffin cells in human colon. Gastroenterology 106:859–865
68. Kawahito Y, Sano H, Mukai S, Asai K, Kimura S, Yamamura Y, Kato H, Chrousos GP, Wilder RL, Kondo M 1995 Corticotropin releasing hormone in colonic mucosa in patients with ulcerative colitis. Gut 37:544–551
69. Cirulli F, Micera A, Alleva E, Aloe L 1998 Early maternal separation increases NGF expression in the developing rat hippocampus. Pharmacol Biochem Behav 59:853–858
70. Chudler EH, Anderson LC, Byers MR 1997 Nerve growth factor depletion by autoimmunization produces thermal hypoalgesia in adult rats. Brain Res 765:327–330
71. Santos J, Yang PC, Soderholm JD, Benjamin M, Perdue MH 2001 Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. Gut 48:630–636
72. Castagliuolo I, Wershil BK, Karalis K, Pasha A, Nikulasson ST, Pothoulakis C 1998 Colonic mucin release in response to immobilization stress is mast cell dependent. Am J Physiol 274:G1094–G1100
73. Leon A, Buriani A, Dal Toso R, Fabris M, Romanello S, Aloe L, Levi-Montalcini R 1994 Mast cells synthesize, store, and release nerve growth factor. Proc Natl Acad Sci U S A 91:3739–3743
74. Wang LH, Fang XC, Pan GZ 2004 Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. Gut 53:1096–1101
75. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R 2004 Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 126:693–702
76. Barau E, Dupont C 1990 Modifications of intestinal permeability during food provocation procedures in pediatric irritable bowel syndrome. J Pediatr Gastroenterol Nutr 11:72–77
77. Grazioli I, Melzi G, Balsamo V, Castellucci G, Castro M, Catassi C, Ratsch JM, Scotta S 1993 [Food intolerance and irritable bowel syndrome of childhood: clinical efficacy of oral sodium cromoglycate and elimination diet]. Minerva Pediatr 45:253–258
78. Stefanini GF, Saggioro A, Alvisi V, Angelini G, Capurso L, di Lorenzo G, Dobrilla G, Dodero M, Galimberti M, Gasbarrini G, Manghisi O, Marsigli L, Mazzacca G, Rigo L, Sacerdoti G, Scolozzi R, Surrenti C, Grazioli I, Melzi G 1995 Oral cromolyn sodium in comparison with elimination diet in the irritable bowel syndrome, diarrheic type. Multicenter study of 428 patients. Scand J Gastroenterol 30:535–541
79. Collins SM 1996 The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology 111:1683–1699