Animal Models for Functional Gastrointestinal Disorders

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Functional gastrointestinal disorders (FGID), such as functional dyspepsia (FD) and irritable bowel syndrome (IBS) are characterized by chronic abdominal symptoms in the absence of an organic, metabolic or systemic cause that readily explains these complaints. Their pathophysiology is still not fully elucidated and animal models have been of great value to improve the understanding of the complex biological mechanisms. Over the last decades, many animal models have been developed to further unravel FGID pathophysiology and test drug efficacy. In the first part of this review, we focus on stress-related models, starting with the different perinatal stress models, including the stress of the dam, followed by a discussion on neonatal stress such as the maternal separation model. We also describe the most commonly used stress models in adult animals which brought valuable insights on the brain-gut axis in stress-related disorders. In the second part, we focus more on models studying peripheral, i.e., gastrointestinal, mechanisms, either induced by an infection or another inflammatory trigger. In this section, we also introduce more recent models developed around food-related metabolic disorders or food hypersensitivity and allergy. Finally, we introduce models mimicking FGID as a secondary effect of medical interventions and spontaneous models sharing characteristics of GI and anxiety-related disorders. The latter are powerful models for brain-gut axis dysfunction and bring new insights about FGID and their comorbidities such as anxiety and depression.

Keywords: functional gastrointestinal disorders, animal models, stress, irritable bowel syndrome, functional dyspepsia, intestinal permeability, visceral pain, mast cells

INTRODUCTION

Functional gastrointestinal disorders (FGID), such as functional dyspepsia (FD) and irritable bowel syndrome (IBS) are highly prevalent, occurring in 10–30% of the general population depending on the criteria used, and represent an important part of the workload of gastroenterology and primary care clinical practice. Those syndromes are characterized by chronic abdominal symptoms in the absence of an organic, metabolic or systemic cause that readily explains these complaints. Based on the Rome IV criteria, FD is defined as bothersome postprandial fullness, early satiation, epigastric pain and/or epigastric burning (1) while IBS is characterized by recurrent abdominal pain or discomfort in association with altered defecation patterns (2, 3). The etiology and pathophysiology remain incompletely understood, which is reflected in the paucity and limited effectiveness of the available treatment options. Moreover, in the last years, FGIDs have been conceptualized as disorders of brain-gut interaction, highlighting the bidirectional interplay between central and peripheral mechanisms and opening new possibilities for anxiety and depression animal models to study FGID.

Over the last 20 years, many animal models, which originally focused on one particular pathophysiological factor, have been developed for FGID. These models have contributed a lot to the understanding of the pathophysiological mechanisms behind the symptoms and were also used to test and validate therapeutic targets and potential therapies (4). However, many aspects of functional disorders remain poorly understood and unsolved in these unidimensional models. An animal model is considered suitable for a disorder, when the etiology of the disease is as close as possible to what is known in humans. Several conditions must be fulfilled, among them (1) the construct validity, i.e., the experimental conditions used to produce the animal model should replicate the cause of the disease in human, (2) the face validity, i.e., the symptoms observed in the animal should replicate the clinical features observed in the patients and (3) the predictive validity where the response to drugs in the animal models can predict reliably the potential response in the human counterpart. It is clear that unidimensional models for FGID do not fulfill these criteria. To overcome the weaknesses of the classic models, more recently, several new models have been introduced, either combining several of the putative factors involved in FGID pathophysiology or using new insights such as the impact of nutrition to try and represent the large panel of symptoms and complex interactions between the pathophysiological factors found in FGID (4).

In this review, we will summarize the state of the art concerning the most relevant and most commonly used pre-clinical models that have been developed for FGID and highlight their contribution to the understanding of FGID pathophysiology. Most of the published studies use rodent models, due to the possibility of genetic manipulations and the quick turnover of the models through fast reproduction and a large number of pups in one litter, in comparison to larger animal models such as pig models. Therefore, this review will largely focus on rodent models.

Abbreviations: 5HT, serotonin; 5HT1A/1B/2A/2B, serotonin receptor type 1A, 1B, 2A, 2B; ACTH, adreno cortico trophic hormone; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BB-DP, biobreeding diabetes prone; BDNF, brain-derived neurotrophic factor; CCL2, chemokine ligand 2; CEACAM6, CEA cell adhesion molecule 6; CNS, central nervous system; COX-2, cyclo-oxygenase 2; CGRP, calcitonin gene-related peptide; CRF, corticotropin-releasing factor; CRFR, corticotropin-releasing factor receptor; DSS, dextran sodium sulfate; ELS, early life stress; FD, functional dyspepsia; FGID, functional gastrointestinal disorders; FODMAP, Fermentable Oligo-, Di-, Mono-saccharides And Polyols; FSL, Findler sensitive line; GC-C/cGMP, guanylate cyclase C/cyclic guanylin monophosphate; GI, gastro intestinal; GR, Glucocorticoid receptor; HPA, hypothalamus-pituitary-adrenal; HSHF, high fat high sugar; IFN, interferon; IgE, immune globulin E; IL, interleukin; IBS, irritable bowel syndrom; IBD, inflammatory bowel disease; KO, knock out; MS, maternal separation; MPO, myeloperoxidase activity; MUC2, Mucin 2; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; P2X, purinergic receptor; PAR2, Protease-activated receptor 2; PI, post infectious; PND, post-natal day; POI, post-operative ileus; SCFA, short chain fatty acids; SERT, serotonin transporter; SHR, spontaneously hypertensive rat; SIS, social isolation stress; TFF3, rail fold factor 3; TLR, toll like receptor; TNBS, Trinitrobenzene sulfonic acid; TNF, tumor necrosis factor; TRH, thyrotropin-releasing hormone; TRPV1, transient receptor potentiate vanilloid 1; VNS, vagal nerve stimulation; WAS, water avoidance stress; WKY, wistar kyoto; ZO-1, Zona occludens 1.

PATHOPHYSIOLOGY OF FGID

Functional gastrointestinal disorders have long been regarded as purely psychosomatic conditions. In the last decade, however, evidence for a low-grade mucosal inflammation, dominated by mast cells, eosinophils, and T-lymphocytes (5–8), as well as impaired epithelial barrier function (9) and neuronal hyperexcitability (10) leading to visceral hypersensitivity (11) and dysmotility has accumulated in FGID, challenging the traditional paradigm of a purely functional disorder. Mast cells represent a crucial link in colonic neuro-immune interaction as they communicate with both the intrinsic; i.e., enteric, and extrinsic nervous system in the gut and release mediators such as tryptase and nerve growth factor, which are involved in visceral hypersensitivity and mucosal permeability in FGID patients (12). A commonly cited hypothesis is based on the concept that luminal antigens, originating from food components, microbiota or other noxious substances such as bile and acid can induce a mast cell and eosinophil predominant immune activation through a failing mucosal barrier which has been found in FD and IBS (13, 14). Nevertheless, it is still unclear whether the mucosal barrier function has any causal role in the pathogenesis of immune activation or whether it is a consequence of the inflammatory response or an unimportant epiphenomenon without a role in disease pathogenesis. Another potentially important player, the microbiota, have been studied intensively in the last years (15). While alterations in colonic and fecal microbiota have been described by several groups in IBS (16), disruption in microbial homeostasis in FD is still largely uncharted territory. Only a limited number of studies have reported alterations in gastric (17) and duodenal microbiota composition in FD (15).

As mentioned above, FGIDs are currently understood as disorders of the brain-gut axis, i.e., the neurohumoral communication system between the brain and the gastrointestinal tract, leading to gastrointestinal hypersensitivity and dysmotility (18). However, central alterations have been mostly studied in the context of visceral hypersensitivity, and the anterior cingulate gyrus, the prefrontal cortex, and the insular cortex have been found to be abnormally activated in IBS patients with visceral hypersensitivity (19). Other structures such as the amygdala or hippocampus have an altered functionality in FGID patients (20). Furthermore, these brain areas are also strongly implicated in psychiatric disorders such as depression and anxiety, two co-morbidities highly represented in FGID patients and associated with visceral hypersensitivity (11, 21, 22).

STRESS-RELATED MODELS OF FGID

Stress is biologically defined as a physiological response to a stimulus that allows organisms to adapt to their environment (23). However, when stress becomes chronic or occurs whilst important development processes are ongoing, the consequences can be harmful and lead to a predisposition for several diseases, including cardiovascular diseases (24), metabolic disorders (25, 26), depression (27), neurodegenerative diseases (28), drug abuse

TABLE 1 | Effect of stress on the Gastrointestinal tract and brain in animal models.

Stress models	Stomach	Small intestine	Colon	Central nervous system
Prenatal stress			Hypersensitivity (66) Dysbiosis (67) Decreased innervation & increase colonic secretory response to adrenaline (68)	Overactivation of the HPA axis in adulthood with female predominance Epigenetic changes BDNF (SC-female predominance) (67)
Maternal separation	Susceptibility to erosion (83) Delayed gastric emptying (84)		Hyperpermeability during separation At weaning, fecal dysbiosis (74) & hyperpermeability (75) Adult hyperpermeability, mostly transcellular pathway associated with abnormal cholinergic regulation (81) Hypersensitivity (76, 78) Immune cells infiltration (MC Eo) (77) Hypersensitivity of neurons from myenteric plexus to IL6 modulation of secretory and motility functions (79) Increased number of enterochromaffin cells & Increased expression SERT (92)	Decreased activity of glucocorticoid negative feedback (85) increased ACTH response to stressors (86) Increased serotonin concentration in frontal cortex Increased expression 5HT1A/1B/2A in parietal cortex & hippocampus Increased activation of 5HT neuron in raphe magnus and spinal cord (60, 89) Increased sympathetic activity and decreased of parasympathetic activity (94) Increased release of glutamate & AMPA/NMDA receptors involved in remodeling of synapses in hippocampus (95, 96)
Limited bedding			At weaning, fecal dysbiosis (female predominance) & hyperpermeability (69, 74) In adulthood hypersensitivity (male predominance) (102–104)	Decreased social/exploratory behavior & impaired learning and memory (102, 103). Decreased dendritic branching hippocampus Altered thalamo-cortico-amygdala pathway Increased connectivity in locus coeruleus (females) (102) Acts on neuronal development of dentate gyrus (105)
Odor shock conditioning			Hyperpermeability and hypersensitivity through estrogen and GC-C/cGMP pathway; female predominance (104)	Increased expression CRF & GR receptors in central nucleus amygdala (109)
Water Avoidance Stress	Impaired gastric accommodation at D2 via peripheral 5HT2B signaling (119) Increased postprandial gastric contractions at D2 (120)	Hyperpermeability at D1 (112, 113) Structural changes mucus layer at D4 (115) Dysbiosis at D8 (117, 118)	Hyperpermeability at D1 (112, 113) Hypersensitivity at D3 (114) Structural changes mucus layer at D4 (115) Dysbiosis at D10 (117, 118)	Increased glial, neuronal activation in hypothalamus and amygdala & synaptogenesis in the hippocampus (121, 123)
Restrain stress			Increased fecal output CRFR1-dependent manner (37)	Neuronal activation over several brain structures and nuclei including the supraoptic nucleus, locus coeruleus, the ventrolateral medulla, the medial division of the central amygdaloid nucleus, nucleus of the solitary tract and even the dorsal nucleus of the vagus nerve, structures involved in food intake and stress response. Neurons also expressing phoexinin and/or nesfatin (127, 128)
Partial restraint stress	Delayed gastric emptying through sympathetic activation (36, 137) Increased active ghrelin concentration (137, 139, 140)		Hypersensitivity (134–136) Hyperpermeability through re-organization of the cytoskeleton (134) Changes in mucosal morphology and decrease of glial cells in the submucosa plexus Increased immune cells infiltration (MC Eo) (135)	Overactivation of the insular cortex related with colonic hypersensitivity (19)

(Continued)

TABLE 1 | Continued

Stress models	Stomach	Small intestine	Colon	Central nervous system
Crowding stress		Hyperpermeability In WKY rat, activated mast cells (146)	Increased mast cells density (145) In WKY rat activated mast cells, MPO level & transient hyperpermeability, alteration of mitochondrial activity (146)	Anxiety-like and depression-like symptoms Early transient changes (Day 3) in the nitrergic expression in the PFC, hippocampus, hypothalamus (147)
Social isolation			Alterations in the IL-18 pathway and MUC2/TFF3 expression (149)	Anxiety-like and depression-like symptoms Reduced BDNF levels & increased reactivity of the HPA axis (148) Changes in the nitrergic expression in the PFC, hippocampus, hypothalamus (147)
Abdominal surgery	Impaired gastric emptying Low ghrelin concentration (249, 251, 252)	Impaired motility through the activation of the inhibitory reflex pathway increased cytokines expression (TNFa, IL1α, IL6, IL1β, CCL2) (241) infiltration resident macrophages (245)	Impaired motility, delayed transit increased cytokines expression in muscular layers (240) (241) Increased permeability non-related to TLR2/4 (244)	Activation of nuclei (supraoptic nucleus, locus coeruleus, paraventricular nucleus of the hypothalamus & rostral raphe pallidus) expressing nucleobindin2/nefastin complex involved in the decrease of food intake and GI transit (253)

5HT, serotonin; 5HT1A/1B/2A/2B, serotonin receptor type 1A, 1B, 2A, 2B; ACTH, adreno cortico trophic hormone; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; CCL2, chemokine ligand 2; CRH, corticotropin-releasing hormone; CRHR1 corticotropin-releasing hormone receptor type 1; D1/2/3/4/8/10, day 1/2/3/4/8/10; Eo, eosinophils; GC-C/cGMP, guanylate cyclase C/cyclic guanylin monophosphate; GI, Gastrointestinal; GR, Glucocorticoid receptor; HPA, hypothalamus-pituitary-adrenal; IL, interleukin; MC, mast cell; MPO, myeloperoxidase activity; MUC2, Mucin 2; NMDA, N-methyl-D-aspartate; PFC, prefrontal cortex; SC, spinal cord; SERT, serotonin transporter; TFF3, rail fold factor 3; WKY, wistar kyoto.

(29), etc. Over the last decade, the incidence of the stress-related disease has increased, especially in societies where socio-economic pressure plays a crucial role in daily life (30). Physical and psychological stress have been documented intensively as decisive factors in the clinical course of several disorders including FGID (31). The hypothalamus-pituitary-adrenal gland (HPA) axis is the endocrine effector of the stress response, with a central role for corticotropin-releasing hormone (CRH), secreted in the hypothalamus, but also locally in the GI tract (32). By binding to its receptors, CRH stimulates the production and the release of glucocorticoids including cortisol in humans and corticosterone in rodents (33), key regulators of the physiological adaptation to stress (34). In normal conditions, the HPA axis is under rigorous regulation, at both the neuronal and hormonal level, since the glucocorticoid and mineralocorticoid receptors are part of a negative feedback loop which protects organisms against the harmful effect of prolonged exposure to stressors. Nonetheless, a combination of repeated environmental stressors may lead to a maladaptive response resulting in altered brain structure and function (35), predisposing to disease. CRH receptors are found to be expressed in both the GI tract and the central nervous system (CNS) suggesting a crucial role for this factor in the stress-induced disruption of gut homeostasis (36) including transit (37–39), visceral sensitivity (40–42), intestinal permeability (36, 43, 44) and gastric inflammation (45).

Psychological stress and anxiety, often reported by FGID patients, influence the onset of symptoms and predict the clinical outcome (46). Recently, data from our group identified

a crucial role for CRH and mast cells in this response, translating previous rodent studies to the human situation (47). Intriguingly, also in inflammatory bowel disease (IBD), longitudinal studies of patients in clinical remission have indicated that stress increases the risk of disease relapse, although the underlying mechanism remains elusive (48). It is still unclear whether stress induces inflammatory changes or whether it is a modulator of symptom perception independent of gut inflammation. Recent studies found that stress influenced the composition of the microbiota, associated with mood disorders and alterations in neurotransmitter pathways (49). Several stress-related animal models have been developed to elucidate the role of stress in the observed changes in the altered sensorimotor function of the gastrointestinal tract in patients with FGID.

In this section, we made a distinction between models of stress applied to adult animals and those involving stress around birth (pre- and post-natal models), i.e., early life stress (ELS). The stress models presented in this section are summarized in **Table 1**. Evidence from literature indicates that a similar stress paradigm has different effects depending on whether it occurs while the brain is still under development or when the neuronal circuits are already fully developed. Indeed, several studies in humans found that stressors in early life are more likely to result in psychiatric and functional disorders, including FGID (50, 51). Post-natal ELS models use this strategy to induce an increased corticosterone concentration in pups during a period in which they are normally only exposed to low corticosterone levels due to the continuous maternal care (52, 53). The two first weeks of

life in rodents (from PND2 til 14) correspond to an insensitive period to environmental stimuli for the HPA axis called stress hyporesponsiveness period (SHRP) (54). During this SHRP, the HPA axis is quiescent and circulating corticosterone, ACTH and CRH levels are very low (55). During this period, stimuli that normally induce corticosterone increase in adults, do not affect pups and the reduced adrenal sensitivity observed is illustrated by the fact that pups do not show a significant elevation of corticosterone concentration following injections of high doses of ACTH (56). The HPA axis maturation mechanisms have not been fully uncovered. Part of these involves enhanced negative feedback due to the low expression of transcortin at the pituitary level (57, 58) and also the decreased expression or transport of hypothalamic secretagogues (59) further supported by the fact that the glucocorticoid regulation of hypothalamic CRH gene expression is not mature during the SHRP (60). Moreover, the regulation of the hypothalamic expression of the arginine vasopressin gene - that occurs at very early stages (61)—which is involved in the ACTH stress response in young rats has a key role for the control of ACTH release from the pituitary (62). Furthermore, it is also the most critical period in the development of central structures such as the amygdala (63) and hippocampus (64). In those two structures, neurodevelopment is very active with neurogenesis, cell differentiation, and migration (65). The existence of such a hyporesponsiveness period suggests that high-stress level might be harmful to the normal development of the brain and could affect the maturation of behavior dependent on those brain systems that are normally developing at that time like the emotional learning systems (57).

Prenatal Stress

In the last decade, data coming from clinical psychiatry showed that women's health status before and during pregnancy is a determining factor for the development of psychiatric disorders, including schizophrenia and depression, socio-emotional problems or altered stress response of the children later in life (49). History of a poor socio-economic context, malnutrition, obesity, metabolic disease, depression, and anxiety in the mother, has been linked to the development of FGID in the child (51). Several paradigms have been used in rodent mothers, including repeated daily immobilization, exposure to noise, sleep deprivation or an alternation of unpredictable stressors to mimic the human situation (49). The effect of those maternal stressors on the development of psychiatric disorders in the offspring is well described, and their effect on the GI tract have been studied in a couple of them. Winston et al. demonstrated that unpredictable chronic stress, i.e., a random sequence of twice daily application of either water avoidance stress, cold restraint stress or forced swimming stress, applied from mid-gestation until delivery in pregnant Sprague Dawley rats induced colonic hypersensitivity in both male and female offspring. Besides, when the animals were re-exposed to the same pattern of stress as their dam at 8–12 weeks of age, they displayed an increased response compared to the offspring of non-stressed dams with a more pronounced effect in females (66). The observed effect in females correlated with epigenetic modifications of the brain-derived neurotrophic factor (BDNF)

gene in the dorsal horn of the lumbosacral spinal cord. Using the same stress model in mice, Jasnarevic et al. could point out a strong link between the stress-altered maternal microbiota and neurodevelopmental alterations and microbiota composition in the offspring (67). This important finding was further confirmed in another study that demonstrated long-lasting changes in the intestinal microbiota composition, associated with a deficiency in the innervation of the distal colon and an increased colonic secretory response to adrenergic stimulation and an exaggerated response of the HPA axis to stress (68).

Neonatal Stress
Maternal Separation

The most frequently studied and used model of ELS is the maternal separation (MS) model in which pups are separated from the dam and the rest of the nest every day during their first weeks of life. Multiple variations of the MS procedure have been described, with changes in the duration of the separation and the number of days of separation. Variations include MS until weaning whereas other protocols use a 24 h separation between post-natal day 3 (PND3) and PND4 (69). The most common protocol consists of about 12 days of separation, 3 h per day, starting from PND2 till PND14. This daily separation induces anxiety in the mother (70) leading to a discontinuity in maternal care and an abnormal mother-pups relationship (70, 71) and initiates the premature end of the hypo-responsiveness period through a rise of corticosterone in the pups (72). Short separation (< 60 min) is not harmful and separation of 15 min even diminishes anxiety-related behavior and the pup's response to stress later in life (73). Repetitive, short separations are reminiscent of the natural behavior of the mother who needs to gather food. However, longer separation mirrors caregivers neglect and physical and psychological abuse during childhood.

Pups who underwent the maternal separation, display an increased intestinal permeability as well as changes in the microbiota composition at the time of weaning which are associated with an increase in the basal level of corticosterone (74). Changes in the microbiota included a lower diversity of the microbiome with a decrease of the fiber-digesting bacteria, mucus-resident and butyrate-producing bacteria (74). Another study showed that, during the maternal separation (at PND9) in mice, the separated pups had an enhanced permeability with a decreased trans-epithelial electrical resistance and an increased transcellular permeability in the colon while the small intestine was not affected (75). In adulthood, at 2 to 3 months, animals who underwent the maternal separation protocol (12 days of separation 3 h/day) have an increased response to colorectal distension, which was more pronounced in mice than in rats (76). In fact, in rats, the MS protocol alone does not change visceral sensitivity but rather induces a susceptibility to develop visceral hypersensitivity when animals are re-exposed to an acute stressor later on during adulthood. Interestingly, this susceptibility is transmitted across generations through a mast cell-dependent mechanism (77). The latter is an important part of the immune cell infiltration characterized after MS in the intestinal mucosa. When activated, mast cells released mediators such as histamine and other inflammatory factors

including IL6 and nerve growth factor (NGF), which are able to sensitize the nerve endings located in the colonic mucosa which express ionic channels including the transient receptor potentiate vanilloid 1 (TRPV1). The modulation of this ion channel has been shown to be an important factor in the maternal separation stress-induced visceral hypersensitivity (78). O'Malley et al. also demonstrated an IL6-dependent hypersensitivity of the neurons from the submucous plexus, which are involved in the secretory and motility function of the colon (79). Local inflammatory mediators such as the myeloperoxidase activity (MPO), IL4, IL1β, or IFNγ are also associated with intestinal barrier dysfunction and to an alteration in morphology of the colon of adult rats (80). The increased permeability described in maternally deprived animals mainly involves the transcellular pathway and seems to involve an abnormal cholinergic regulation of the epithelial permeability (81). As previously mentioned, CRH receptors are expressed along the GI tract and CRH is one of the mediators of the GI effects of maternal separation. However, the two receptors for CRH have a differential effect on the intestinal physiology. Indeed, while maternally separated adult rats treated with CRH Receptor1 (CRHR1) antagonists displayed a decreased inflammation, the group treated with a CRH Receptor2 (CRHR2) antagonist showed an inhibited stem cell activity and injury repair. CRHR1 contributes to intestinal injury and modulation of the microbiota while CRHR2 promotes healing and repair of the intestine (82). Besides the well-documented colonic injury in animals submitted to maternal separation, studies also showed an alteration in gastric function characterized by enhanced susceptibility to gastric erosion (83) and a delayed gastric emptying associated with structural changes in the glial cells (84).

The separated pups develop an increased reactivity of the HPA axis in response to stress during adulthood (71), as shown by a decreased activity of the glucocorticoid negative feedback loop (85) and an increased adreno cortico trophic hormone (ACTH) response to a stressor (86). Several central neurotransmitter pathways are also affected by MS including the serotonergic (87), cholinergic (81) and glutamatergic (88) pathway. The serotoninergic pathway is altered with an increased 5-hydroxytryptamine (5HT, serotonin) concentration in the frontal cortex and increased expression of the 5HT 1A, 1B, and 2A receptors in the parietal cortex and the hippocampus (60, 89). Furthermore, MS rats showed increased activation of serotoninergic neurons in the raphe nucleus and the spinal cord. Rat studies have demonstrated the involvement of the 5-HT1A receptor in the pathophysiology of stress-induced visceral hypersensitivity as a treatment with the mast cell blocker Resveratrol was potentiated by a pre-treatment with a 5-HT1A agonist (90). In the same way, a therapeutic effect of anti-depressants targeting the monoaminergic system has been reported in this model (91). At the enteric nervous system (ENS) level, an increased number of enterochromaffin cells producing 5HT and increased expression of the serotonin transporter (SERT) were observed in MS rats (92). These findings may contribute to the observed altered sensorimotor function in FGID patients with a childhood abuse history, although the role of enterochromaffin cells and SERT has not

been studied in particular context. Increased noradrenaline levels, the main neurotransmitter of the sympathetic branch of the autonomic nervous system, in the cingulate cortex was associated with fear and anxiety in the MS model (93). In IBS patients, studies measuring heart rate variability confirmed an increased sympathetic nervous system activity and a decreased parasympathetic nervous system activity (94). Alterations have also been described in the glutamergic pathway, which is involved in emotion and cognitive behavior. Maternal separation induced a release of glutamate in the hippocampus which activated receptors leading to neuronal excitotoxicity (95). In the hippocampus of the MS rat, an increased expression of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and the N-methyl-D-aspartate (NMDA) receptors have been found to be associated with a remodeling of the synaptic plasticity (95, 96). Alterations in the hippocampus also concerns neurogenesis which explains the long-term consequences in adult behavior. Maternal separation affects the neurogenes which is very active during the SHRP and leads to impaired coping behavior in adulthood and the learning process (97).

Limited Bedding
As mentioned above, the socio-economic status of caregivers affects the onset of FGID in children. To mimic poverty and precarious conditions in humans and also to limit the intervention of an external experimenter which is subject to variability, a model of limited cage bedding, mostly applied between the PND2 and PND9, was developed first in rats and later also in mice (98, 99). Some recent studies have proposed a variation by applying intermittent limited bedding from PND1 to PND7 or limited bedding from PND8 to PND12 (100). In this model, the female does not have access to any form of enrichment to build a nest. This altered environment is stressful for the mother and leads to a fragmentation of maternal care without changing the overall duration. The periods of maternal care are shorter and the behavior is more frequently shifted from one to another e.g., grooming, nursing, going in or out of the nest, self-licking, self-grooming… (101). The mother's stress can be modulated by varying the amount of nesting material introduced in the cage. With this protocol, the stress applied to the pups is chronic, unpredictable and uncontrollable which has good construct validity for stress-related disruption of parental care in a context of economic difficulties (69). The profound chronic stress induced by this protocol leads to a transient increase of the corticosterone concentration and hypertrophy of the adrenal glands, increased intestinal permeability and a fecal dysbiosis in 21-day old pups while these effects had disappeared at 12 months (69, 74). Although the concentration of corticosterone was strongly correlated with the hypertrophy of the adrenal glands, the elevated concentration of corticosterone was associated neither with the observed dysbiosis (lower diversity and increased abundance of genera of Gram positive cocci) nor intestinal permeability, for which a sex difference was observed with the females being more affected than the males (74). However, in adulthood, the rats submitted to this early life stress showed a reduction in social and exploratory behavior, impaired learning and memory processes, a decreased dendritic

branching in the hippocampus, an increased response to a stress challenge and visceral hypersensitivity (102, 103). The latter, depending on the method used for the colonic pain assessment, showed no sex difference or a higher colonic sensitivity in males (104). Similar results were observed for anxiety-like behavior. Moreover, differences in the brain connectivity of the thalamo-cortico-amygdala pathway during a painful stimulus have been reported to be altered in rats submitted to this protocol of ELS. The authors pointed out a sex difference with increased brain connectivity in the locus coeruleus/lateral parabrachial nucleus in females only (102). A recent finding showed that limited bedding affects the neuronal development of the dentate gyrus and depletes the stem cell pool in adult animals but does not influence neurogenesis (105).

Odor Shock Conditioning

Many mammals including rodents are born blind and deaf, and to stay warm and obtain the food and care needed to survive, they need to learn the odor of their caregiver, e.g., their mother. For this purpose, during the first 9 days of their life, pups display an enhanced capacity for odor preference learning through a stimulated release of norepinephrine, produced in the locus coeruleus, which binds to its receptors in the olfactory bulb (53). This allows the pups to learn the odor of their caregivers without associating it to fear or aversion. Furthermore, during this period, the pups display an inability to initiate a stress response. Once this period ends, the level of secreted norepinephrine decreases, associated with the development of the α2 inhibitory auto-receptors functionality and the downregulation of the α1 excitatory auto-receptors (53). In a second phase, called the conditional sensitive period, from PND10 till PND15, pups start to explore their environment and learn avoidance and fear for aversive stimuli in the absence of the mother. As we described earlier, the maturation of the HPA axis and maturation of fear behavior happens during the time-window in which neuronal circuits are located on the trajectory of the HPA axis maturation. Several studies showed that the rise of corticosterone levels starts during this period and is critical for the engagement of the amygdala and the learning of aversion and fear in response to a stimulus such as the odor of a predator (106). Interestingly, during this period, the presence of the mother can reengage the fear learning process (107).

Developed by and mostly used in the Greenwood-Van Meerveld lab, the model of odor shock-conditioning consists of predictable and unpredictable odor-shock pairings which mimic attachment to an abusive caregiver. This model uses the association between an odor and a modest electrical shock to the tail to reproduce the pup-dam interaction and creates an olfactory attachment to the conditioned odor in response to predictable or paired odor/shock. In practice, pups are exposed from PND8 to PND12 to an odor associated with an electrical shock 2 minutes after the odor exposition while controls are only exposed to the odor (104, 107–109). During adulthood, only female Long-Evans rats displayed an increased colonic permeability and a colonic hypersensitivity which persisted later in life and seemed to be directly linked to estrogen concentrations, as an ovariectomy in females subjected to

the odor shock conditioning model, rescued this phenotype (108). Moreover, increased expression of the CRF and the glucocorticoid receptor was found in the central nucleus of the amygdala which was involved in the maintenance of the colonic hypersensitivity (104). The use of linaclotide, a guanylate cyclase C (GC-C) agonist used in clinical practice to treat constipation-predominant IBS, restored both colonic hypersensitivity and permeability, proposing the GC-C/cGMP pathway as an important player in the peripheral regulation of the persistent visceral pain in adults exposed to this form of ELS (109).

Adult Stress
Physical Stress

Water avoidance stress (WAS) is one of the most frequently used models of stress in adult rodents either alone or combined with the maternal separation model. Several studies over the last decade have used it to characterize acute and chronic stress-induced GI symptoms and to study the effect of treatment, including nutritional and probiotic interventions (110, 111). During this protocol, the animal is placed on a platform (usually 10 × 10 for rats and 3 × 3 cm for mice) surrounded by water, either cold or at room temperature, until 1 cm below the platform. The water reservoir should be large enough to avoid the animal to jump out and thus give the animal the impression that no escape is possible. This protocol can serve as either an acute or a chronic stressor and mimics resilience to an uncomfortable situation. A large number of variations on the protocol have been described in the literature and often differ by their duration. WAS induces a robust activation of the HPA axis which transiently alters gut physiology (110). One day of stress induces an increased intestinal permeability (112, 113) while 3 days of stress are needed for colonic hypersensitivity to appear (114). Morphological changes, including the composition and the structure of the mucus layer, were present from the fourth day (115). Interestingly, the follicle-associated epithelium in the ileum seemed to be more affected than the colon (116). A fecal dysbiosis, including an altered composition and function of the microbiota, has been described after 10 days of stress in rats while in mice it already appears after 8 days in the small intestine (117, 118). Gastric contractions after a meal were increased in rats after two sessions of WAS, through the activation of the peripheral CRH1 receptors (119). Impaired gastric accommodation occurred after 2 days of stress and was mediated through the peripheral serotoninergic receptors 5HT2B (120). In mice, after four sessions, alterations in the brain occurred with an increase of the neuronal and glial activation in the hypothalamus, hippocampus, and amygdala. These structures are not only involved in the stress response but also in memory, pain and emotion pathways which are often found to be altered in IBS patients (121–123). A sex-difference has been described in the processing of emotional signals in healthy humans (124), in patients with FGID (125) and stress-related visceral hypersensitivity in rats (126).

As for the maternal separation, several models of physical constraint have been developed over the years, among them three versions of physical constraint: the partial restraint stress,

full restraint stress and cold restraint stress. The extent and the duration of the stressor differ amongst the protocols. With a full body restraint stress applied in rats for 30 min, studies have shown neuronal activation over several brain structures and nuclei including the supraoptic nucleus, locus coeruleus, the ventrolateral medulla, the medial division of the central amygdaloid nucleus, nucleus of the solitary tract and even the dorsal nucleus of the vagus nerve, structures involved in food intake and the stress response (127, 128). Interestingly, in those nuclei and structures, the activated neurons also expressed nesfatin-1 and/or phoenixin, two peptides involved in the regulation of food intake and anxiety behavior (129, 130). Nesfatin-1 is mainly expressed in the hypothalamus and brainstem where it colocalized with CRF (131). When administered directly into mice brain, nesfatin-1 led to an increase of plasmatic ACTH and corticosterone levels, as well as an activation of neurons expressing CRH, noradrenalin and serotonin, indicating both a central and a peripheral response to stress. Furthermore, the nesfatin-1 system is activated when rats are submitted to restraint stress for 1 h (132). In mice, a protocol of 60 min of full restraint stress showed a CRF-dependent increase of pellet output which was abolished by central injection of a specific CRFR1 antagonist—while a CRFR2 specific antagonist had no effect—(37) and also by a systemic injection of peptide YY (133). Partial restraint stress is another common form of the model which consists of restriction of the upper body movements. In this model, the shoulders, upper forelimbs, and thoracic trunk of the animal are wrapped in a confining harness of paper tape or cloth to restrict, but not to prevent, body movements (134). This protocol is mostly used as an acute stressor with a 1 to 2 h period of restraint. However, this short exposure already promoted (1) colonic hypersensitivity (134–136), (2) an increased influx of immune cells in the mucosa, mostly consisting of mast cells and eosinophils (135), (3) an intestinal hyperpermeability through the reorganization of the cytoskeleton in epithelial cells (134), (4) a delayed gastric emptying associated with the stress-induced sympathetic activation, increased CRF (36, 137) and associated peptides (138) as well as active ghrelin concentration (137, 139, 140), and (5) changes in colonic morphology and a decrease of enteric glial cells especially in the submucosa plexus (135). By using this model for 14 days, Yi et al. could demonstrate the implication of the insular cortex in stress-induced visceral hypersensitivity, a region found to be abnormally activated in FGID patients (19) and more in general in patients with chronic pain (141).

Social Stress

One of the important findings of the last century in the field of psychobiology is the stress-buffering effect of social relationships, with an important role for oxytocin (142). Social buffering conceptualizes the idea that social support can attenuate the stress response and reduce the release of stress hormones (143). As we discussed in the previous section, the more powerful demonstration of this concept is the mother's social buffering of the offspring in which the mother and the pups can influence each other's corticosterone concentration (144).

However, the positive effect of this social buffering depends on the nature of the relationship between individuals as well as on the social organization of the species and/or gender. Many species, including humans and rodents, live for almost their entire life in a group with a strong hierarchy. As a result, any disturbance in this social order or abuse is a potential source of stress. In rodents, stress models used either the isolation of one animal from the rest of the group, i.e., social isolation stress, or, at the other end of the spectrum, an overpopulation within a small area, i.e., crowding stress. Often used as models for anxiety and depression-related disorders alterations of the GI physiology have also been studied in rodents submitted either to the social isolation stress or the crowding stress models. Crowding induces a strong competition for space, food, and water and leads to a strong increase of the corticosterone concentration in the first days associated with early transient alterations in the nitrergic system in the hippocampus, prefrontal cortex and hypothalamus, an increase of iNOS expression in all structures and increased of nNOS in the hippocampus and hypothalamus. These changes are normalized over time through a habituation process but remains higher than in normal housing conditions. This protocol is used for 2 to 9 weeks depending on the strain and type of rodent and is often combined with other types of a stressor to simulate the combination of chronic and (sub)acute stressors that naturally occurs in humans. Work from our group demonstrated that 14 days of crowding stress in Wistar rats induced increased permeability in the jejunum which correlated with plasma corticosterone levels. However, mast cell density was only increased in the colon (145). In Wistar Kyoto rats, a strain sensitive to anxiety, a crowding stress protocol applied for 15 days induced a transient increase of the small intestine and colon permeability associated with a transient rise of MPO activity and altered mitochondrial activity (146) as well as mast cell infiltration (colon) and activation in the GI tract (small intestine and colon) (146).

Conversely, in the social isolation stress (SIS) model, the animal is isolated from the rest of the cage. Often applied just after the weaning, SIS modifies the development of the brain and influences the nitrergic system in several brain areas such as the hippocampus, the frontal cortex by increasing the nNOS expression in the hippocampus and hypothalamus and iNOS in the prefrontal cortex (147). A decrease production of BDNF, and an activation of the HPA axis which produce more corticosterone.. Mice exposed to SIS, have an impaired reactivity to stress with an overreaction to another stressor together with increased anxiety- and depression-like symptoms (148). In the GI tract, regional differences have been pointed out between the colon and the rectum concerning MUC2/TFF3 expression and in the IL-18 pathway in mice exposed to 16 days of SIS (149).

Combined Stress and Chronic Mild Unpredictable Stress

Although the various animal stress models developed over the last years have provided critical information about the influence of stress on physiology, humans are usually not exposed to only one stressor during their life and a combination of stressors is often present in patients with FGID. Also, physiology can

adapt to one homotypic stressor in humans and rodents, leading to habituation and absence of effect after repeated exposure. As a variety of animal models of stress are available, a wide range of stress combinations can be used to better understand the pathophysiological mechanisms behind stress-induced FGID symptoms. Combinations of unpredictable mild stress are also often used with a rotation between different stressors such as light/dark cycle, isolation, crowding, predator odor exposure, shock, cold environment, restraint stress. . . .

An often-used combination in rats is the maternal separation combined with one session of water avoidance stress in adult rats. In contrast to mice, maternal separation is not always sufficient to induce GI symptoms in rats (76) but increases the susceptibility for GI symptoms upon subsequent exposure to stress (150) which is transmitted to the next generation (77). In another type of combination of early life stress, i.e., odor shock conditioning, and water avoidance, a sex-difference was observed with a more pronounced female susceptibility to develop visceral hypersensitivity, which is in line with the female predominance in FGID (151).

With the use of unpredictable stress models, which consist of applying a stressor (SIS, restrain, WAS. . .) at unpredictable moments of the day for a few days, the involvement of nerve growth factor, endorphin, beta-adrenergic pathway, BDNF and mast cells mediators and the toll-like receptor 4 (TLR4) pathways have been demonstrated in stress-induced visceral hypersensitivity (107). Recent studies suggested a role of an altered microbiota in the anxiety and depression-like behavior in animals exposed to unpredictable mild stress with a strong correlation between the alterations in the microbiota and colonic serotonin concentrations (152, 153).

LOW GRADE INFLAMMATORY, POST-INFECTIOUS AND POST-INFLAMMATORY MODELS OF FGID

Infections and inflammation are among the best-characterized triggers for FGID symptoms. Although the pathophysiological mechanisms are not yet fully understood, a low-grade inflammation is considered as the main explanation for the symptoms in so-called post-infectious (PI) IBS and FD (154). Evidence from IBD patients in remission has also brought some more arguments for this mechanism of persistent low-grade inflammation triggering IBS-like symptoms (155). Psychological factors have been shown to be associated with the prevalence of PI-IBS as well as somatization which, when happening during the infectious period, is positively correlated with the incidence of IBS symptoms (156).

Low Grade Inflammatory Models
Low dose injections of inflammatory factors like the bacteria-derived lipopolysaccharide (LPS), injected systemically at the dose of 1 mg/kg can also trigger FGID features such as rectal allodynia and colonic hyperpermeability. Visceral allodynia appears 3 h after injection last up to 12 h and is mediated by mast cell degranulation, IL1β and TNFa (157). When

Authors performed a subdiaphragmatic vagotomy, they observed an increased allodynia compared to sham animal which suggest that the rectal allodynia seen after LPS injection is controlled by the vagus nerve (158). In another study, both the hyperpermeability and allodynia observed 3 h after injection, were normalized with antagonist of TLR4 and IL1β as well as with a CRFR2 specific agonist and Astressin a non-selective CRFRs antagonist suggesting an important role of the CRF in the effect of LPS injection on visceral sensitivity and permeability (159). Moreover, allodynia and hyperpermeability were abolished with peripheral injection of a selective CRFR2 agonist and with an non-selective inhibitor of CRF receptors (157). Losartan, an angiotensin II blocker, and lovastatin reversed the permeability and allodynia, dependent on the macrophage peroxisome proliferator-activated receptor gamma (PPARγ) and the endogenous opioid, dopaminergic and nitrergic system, potentially opening the door for novel therapeutic strategies of FGID (160, 161). Similarly, the tricyclic antidepressant imipramine reversed LPS-induced allodynia and colonic hyperpermeability (162).

Several models have also been developed using a low concentration of dextran sodium sulfate (DSS) to induce low-grade inflammatory changes (163). This model contrasts with the post-inflammatory model of high-dose DSS followed by a recovery period, discussed in the next paragraph. An overexpression of the T-type calcium channel cav3.2 in this model, which was also observed in colonic biopsies of IBS patients, was associated with colonic hypersensitivity in this model (163, 164).

Post-inflammatory Models
Rodent models of post-inflammatory GI disorders attempt to simulate a post-inflammatory situation mostly represented by the resolution of an acute infection. Most of the models presented below originally are models for IBD but can be instrumental to study certain aspects of the development of GI symptoms after resolution of the acute inflammation. The development of post-inflammatory GI disorders occurs in 25 to 100% of the treated animals depending on the trigger used. Furthermore, the severity of symptoms and functional alterations developed in the post-inflammatory period—except for the visceral hypersensitivity—seems to be independent of the severity of the initial inflammation (165).

An acute treatment with a high percentage of DSS followed by a DSS-free period (166) creates a remaining low-grade inflammation which is associated with visceral hypersensitivity and SERT downregulation leading to gut dysmotility. The expression of TRPV1, another ionic channel, has been shown to be increased in the recovery phase only in the colonic mucosa and linked to the persistent visceral hypersensitivity (167). However, other studies have shown a quick restoration of the original phenotype without colonic hypersensitivity to mechanical stimuli (152).

Trinitrobenzene sulfonic acid (TNBS), leads to a Th1 immune response with ulcers in rodents within the first 3 days after instillation. Two weeks after instillation, the inflammation is resolved, but a visceral hypersensitivity, motility dysfunction

due to a persistent long-term smooth muscle hyperactivity to acetylcholine, an increased mast cell infiltration in the mucosa, an upregulation of the NMDA-NR1, as well as galanin and tachykinin expression in the mucosa and myenteric plexus, a barrier hyperpermeability and a decreased secretory function through a cyclooxygenase-2 (COX-2) dependent mechanism can be demonstrated (168). The long-lasting symptoms, such as visceral hypersensitivity, are present up to 17 weeks after the induction and involve overexpression of the NMDA receptor NR1 in the spinal cord as well as changes in the distribution and the sensitivity of the colonic afferents. A TRPV1 antagonist, a guanylate cyclase agonist, melatonin and a probiotic (*Bifidobacterium infantis* 35624) were able to rescue this phenotype (169). Mast cells, through their main mediator histamine and its receptors H4R and H1A, play a substantial role in post-inflammatory visceral hypersensitivity (170). Recently, Winston et al. demonstrated a gastric hypersensitivity in 8 weeks-old rats previously exposed to TNBS (171) which positioned this model as a general model for FGID and a good model for patients with FD and IBS overlap symptoms, which is common in clinical practice (172).

Acid acetic, Zymosan and mustard oil are irritants administered directly into the colon of adult or neonatal animals by enema. When administered in pups, long-lasting visceral hypersensitivity has been reported for 8 to 10 weeks for Zymosan and mustard oil and up to 12 weeks for acid acetic (168) without histological damage in adult rats (173). Seven days after acid acetic induction in adult rats, when the inflammatory phase has subsided, a defect of the intestinal barrier function was reported with altered occludin and ZO-1 protein expression in a miR-144-dependent manner (174). The same barrier defect was also observed in rats submitted to the neonatal protocol. When administered directly into the submucosal layer of the stomach using 15 to 20 injections in adult rats, acid acetic promoted gastric hypersensitivity (175). Zymosan and mustard oil sensitized the mechanoreceptor and other neurons present in the colonic wall which persisted after the inflammatory phase (176, 177). Both Zymosan and mustard oil, induced neuronal changes in the spinal cord and the brain by increasing the neuronal excitability as shown by an increased presence of c-FOS positive neurons (178). The latter was associated with an altered expression pattern in the NMDA receptors in females which might be responsible for the female predominance in visceral sensitization following the mustard oil model (179). The stimulating effect of mustard oil on GI motility has been documented in both the upper and lower GI tract (180). Although the increased neuronal activation in the central nervous system in those models would suggest behavioral changes, anxiety-like symptoms have only been described in the zymosan model in which these behavioral symptoms were present during the inflammatory phase and remained present for 2 weeks after the induction, associated with increased c-FOS expression in different brain regions such as the amygdala, prefrontal cortex, periaqueductal gray... and increased TNF-α levels in the colonic mucosa (181).

Iodoacetamide is an alkylating agent administered by gavage in neonate rodents to induce a mild inflammation of the gastric mucosa that is associated with acute changes in sensory and motor function. Inhibition of the glucocorticoid receptors, adrenergic receptors, BDNF or the nerve growth factor (NGF) during the neonatal period suppressed the induced gastric hypersensitivity (171). The inflammation phase in pups is characterized by a thickening of the neuromuscular layer without increased MPO levels. During adulthood, histology and inflammation levels are comparable to control animals while the gastric sensory and motor dysfunction remained present up to 8 weeks after the treatment (182).

Post-infectious Models

Within the GI tract, the host and billions of micro-organisms are co-existing, creating a unique symbiosis. Products secreted by the microflora influence the gut function by their effect on neurotransmitters, epithelial function, secretion, or muscle contraction (183). The composition of the gut microflora depends on different factors such as diet, geographic position, genetics, and gender. The balance is strongly influenced by changes in the diet, travel or bacterial and parasitic gastrointestinal infections. The occurrence of FGID symptoms after an infectious episode has been found in a range of 3 to 36% of an infected population (156, 184, 185). The latter has been found to alter gut physiology through different mechanisms including the triggering of an inflammatory reaction, alterations at the neurochemical level and immune function, and alterations of the nerve distribution (186). Some of the parasites and bacteria that can infect the human GI tract, can also infect rodents and trigger symptoms or GI abnormalities reminiscent of human FGID.

Trichinella spiralis is an intestinal parasite found in humans, rodents, and pigs and is used as a model of post-infectious IBS in mice. For this purpose, mice are infected with 200 to 300 larvae in one gavage. During the acute phase, parasites are evacuated from the organism triggering a Th2 inflammatory phase in both the mucosa and muscular layers (187, 188). The post-infectious phase is defined at 4 weeks post-infection when the inflammatory phase has subsided (188). However, Akiho et al. found that transforming growth factor (TGF)-β remained overexpressed during this post-inflammatory phase and the smooth muscle cells were still hyperreactive to an immune challenge (primary culture of smooth muscle cells incubated with Th2 cytokines TGF-β1 and COX2) in a COX-2 dependent manner (187). Moreover, the long-lasting effects of the infection, such as visceral hyperalgesia which has been reported up to 70 days after infection, were inhibited by selective and non-selective COX-2 inhibitors (187, 189). Data on the effect of COX-2 inhibitors in humans are still lacking. In the post-infectious phase, the small intestine smooth muscle contractility, as well as the mucosal transport, remained altered. The latter shifted from a predominantly cholinergic in normal conditions to a non-cholinergic regulation (188).

Other animal models used infections with *Nippostrongylus brasiliensis* and *Cryptosporidium parvum*, which are both characterized by mast cell hyperplasia, visceral hypersensitivity, motility dysfunction with an increased motor response to excitatory agonists due to a remodeling of the nerve pattern as found in PI-IBS patients long after the infection. Other bacteria have been used including *Campylobacter rodentium*, *Campylobacter jejuni* or *Salmonella enterica*. However, those

infections are less well-characterized regarding their FGID features or have a low success rate in rodents (168).

Overgrowth of *Escherichia coli* in the ileum of IBS and IBD patients has been associated with the expression of the human bacterial colonizing receptor CEACAM6 (190). Expression of the human CEACAM6 in the murine GI tract induced colonization and growth of pathogenic Escherichia coli upon gavage and led to an infection. In this model, mice are treated with the bacteria for 3 days, leading to a transient inflammation, intestinal hyperpermeability, and colonic hypersensitivity. The latter was present until 3 weeks after infection and was associated with a remaining low-grade inflammation and overexpression of the purinergic receptors P2X receptors in the colon (191).

FOOD-RELATED MODELS OF FGID

Food indigestion, intolerance or allergy are major triggers for abdominal symptoms. Due to genetic predisposition, infection or stress, oral tolerance, which is critical in avoiding immune reactions against food antigens, may be disrupted, leading to FGID symptoms. In IBS patients, the ingestion of certain food compounds triggers FGID symptoms (192) and elimination diet strategies, such as low FODMAP or gluten-free diets, are effective in some patients (193).

Food allergy represents a break in the oral tolerance and the consumption of allergens trigger a Th2 response and the activation of mast cells through the IgE pathway. FGID manifestations such as low-grade inflammation, visceral hypersensitivity, increased permeability, have been reported in rodent models of food allergy (194, 195). In those models as well as in humans, gender differences have been described. However, studies are diverging on the effect of the gender with female rodents being more affected than males while in children the prevalence is higher in males. Those differences might be related to the difference in the immune system response to allergens which is strongly influenced by sex hormones (102, 195). Those models differ by the allergen used, which can be egg proteins, peanut components, milk or seafood extracts, but all follow the same pattern with a phase of allergy-induction and a re-challenge with the same allergen several days or weeks after the induction (195). Many of the validation criteria for a rodent allergy model are comparable to the evaluation of the FGID related changes, e.g., presence of histological changes with local and systemic inflammation and activation of eosinophils and mast cells (195). In an elegant study, Aguilera-Lizarraga and Florens demonstrated that the establishment of oral tolerance can be impaired due to stress or intestinal infection, two major triggers of FGID symptoms, without triggering a systemic allergic reaction. Their results showed that this impaired tolerance triggered a mast cell activation through local (but not systemic) IgE, leading to colonic hypersensitivity and hyperpermeability (196, 197).

Obesity is one of the main health problems of our society and affects an increasing number of people over the world. Obesity is often associated with metabolic disorders such as diabetes or hypertension and also with FGID (198). IBS is three times more frequent in obese patients compared to the general population (199) and patients report both upper and lower GI complaints (200). Moreover, studies have reported an increased incidence of GI symptoms among fast-food and western-diet consumers (201, 202). Although, no animal model of FGID includes eating habits or genetic background leading to obesity, several observations in obesity models have pointed out a chronic GI low-grade inflammation and hyperpermeability induced by high fat, high sugar diet (HFHS) (203) which is associated with changes in the microbiota composition (204). Therapeutic strategies targeting the microbiota (205) as well as dietary strategies (204, 206) have shown some promising results in this model on the immune dysfunction and colonic hyperpermeability. Although the effect of obesity on low-grade inflammation, neuropathy, and hyperpermeability is not specific to the gut—some studies reported epigenetic changes in several organs (207)—it will be interesting to further investigate the effect of obesity on the gastro-intestinal features of FGID and on how it affects the outcome and/or the development of FGID.

The imbalance between some bacterial phyla such as Firmicutes/Bacteroidetes has been reported in IBS patients (208). The firmicutes are the predominant butyrate and other short-chain fatty acids (SCFA)-synthesizing bacteria within the colon. Although the benefits of SCFA has been demonstrated, rectal butyrate instillation in animals has been linked to visceral hypersensitivity (209, 210). This hypersensitivity without inflammation involved the enteric glial cells-derived NGF pathway which sensitized the nerve fibers within the colonic wall (211).

SPONTANEOUS MODELS OF FGID

Spontaneous animal models sharing key characteristics with human FGID are of great value to unravel the complex chain of events ultimately leading to symptoms and to aid in preclinical drug development. Only a few spontaneous models for FGID have been described (212–214) often sharing common features for FD and IBS, mostly in rats.

The BioBreeding rat (BB-rat) is an inbred colony originating from Wistar rats and have been selected for their ability to spontaneously develop type 1 diabetes (215). The BB-rat consists of a diabetes-resistant (control) and a diabetes-prone (BB-DP) strain of which about 50 to 90% develops hyperglycemia depending on their environment (216). Originally mostly used as a model for type 1 diabetes, several groups have demonstrated GI alterations closely mimicking FGID. Indeed, Neu et al. have described changes in intestinal morphology and intestinal permeability before the onset of diabetes (217). In the last 5 years, our lab has described a gastrointestinal phenotype in those BB-DP animals which did not develop diabetes, at all levels of the GI tract. The intestinal changes closely resemble the alterations found in patients with FD and IBS (213, 218–220). Based on these observations, we proposed the normoglycemic BB-DP rat as a spontaneous animal model for FGID. A natural history study of the small intestine demonstrated that the earliest abnormality was

an increased intestinal permeability at 50 days of age, followed by an immune cell infiltration, progressing from the mucosa to the myenteric plexus in animals from 110 days onwards (213). This myenteric plexitis is associated with a loss of nitrergic neurons and disturbed motility (213, 220). Concomitantly, an impaired gastric accommodation, like in functional dyspepsia, has been observed in young normoglycemic rats (220). The immune infiltration is affecting the stomach, the small intestine, and the colon and is characterized by activated mast cells and eosinophils (221). Although the barrier defect precedes the infiltration of the immune cells in the small intestine, we observed that in the colon, the infiltration of the immune cells, which is present at the same age as in the jejunum, precedes the hyperpermeability suggesting a different mechanism in both locations. At both levels, we found a positive correlation between mast-cells density and mucosal permeability. Following this immune activation, we also demonstrated colonic hypersensitivity to colorectal distention and anxiety-like behavior in older BB-DP rats, which, however, was not associated with the increased permeability or immune infiltration in the colon (219). Altogether, the BB-rat model is a valid spontaneous animal model for FGID, recapitulating the permeability defect, eosinophil and mast cell predominant immune activation, motility disturbances at different levels of the GI tract, visceral hypersensitivity and behavioral alterations, similar to human FGID.

The Wistar Kyoto (WKY) rat, originally used as a control normotensive strain for the spontaneously hypertensive rats (SHR), has been studied in the last decades as a model of brain-gut dysfunction. Those rats display an exaggerated response to chronic stress compared to Sprague Dawley rats (222–224), associated with a higher susceptibility to develop anxiety-like and depression-like symptoms (225). Regional differences in monoamines concentration within the brain may explain their susceptibility to anxiety and depression (226). As described above, stress is a potent trigger for alterations in gut physiology, especially through the central expression of CRH in key structures involved in stress but also in pain and emotion regulation. Wistar Kyoto rats have an increased response to colorectal distension associated with increased neuronal activation in the cortex (227). Specific inhibition of the CRH pathway in the central amygdala and inhibition of central and peripheral 5HT2B inhibits the increased response to colorectal distension (87, 228). Besides the well-described colonic hypersensitivity (87, 228, 229) and impaired intestinal permeability (223, 224), the WKY rats also display gastric alterations such as an impaired gastric accommodation and a higher sensitivity to gastric distension (230). Interestingly, O'Malley et al. have compared the WKY to Sprague Dawley in a maternal separation paradigm and showed that the susceptibility to gastrointestinal dysfunction in stressed Sprague Dawley is comparable to what is found in non-stressed WKY (231).

The Flinders Sensitive Line (FSL) rat originates from selective breeding from Sprague Dawley and has been selected for their resistance to the choline esterase inhibitor, diisopropyl fluorophosphate (232). Used first as a model of cholinergic-adrenergic hypothesis depression (233), they are now more generally used as a model of depression without comorbidity

of anxiety and with a female predominance (234). The effect of the microbiota composition has been studied in those rats showing that their microbiota composition is different (235) and might represent a target to improve the depression phenotype (236). However, only a few studies have investigated the gastrointestinal features in those rats. Some characteristics of functional dyspepsia including a delayed gastric emptying but not an impairment of the gastric accommodation have been described (214). Taking into consideration the link between depression and FGID, a more detailed study of the GI features in the FSL rats may bring some new insights for the link between GI symptoms and depression in FGID.

INTERVENTIONAL MODELS OF FGID

During abdominal surgery, the opening of the skin and the abdominal cavity triggers adrenergic reflexes involving a spinal loop which temporarily blocks GI motility. Considered as an iatrogenic disorder, postoperative ileus (POI) occurs in most patients undergoing abdominal surgery and is characterized by a transiently impaired GI motility. However, when recovery of bowel function is delayed for more than 3–7 days, this disorder is defined as an illness (237). Several rodent models exist to induce POI: briefly, either the abdominal cavity is opened and exposed to room temperature air for 3 h (238) or the intestinal tract is gently manipulated during 5–10 min (239). In both cases, an impaired GI motility affecting the stomach, small intestine, and colon, an intestinal inflammatory response, and hyperpermeability have been reported. The inflammatory response is associated with increased production of TNFα, IL1α, and IL6 in the early stage, followed by an increase of myeloid cell-derived cytokines, e.g., IL1β and CCL2. During this early stage, the inhibitory reflex pathway is activated, inhibiting gut motility. The transit time is delayed from 12 h up to 1 week after the surgery (240). At 24 h after the surgery, small intestine and colonic transit were delayed and associated with increased cytokine expression within the smooth muscle layer. The role of mast cells in the POI is still controversial as some studies found a mast cell-dependent mechanism in the POI-induced intestinal hyperpermeability and bacterial translocation (241) while other studies found no involvement of these cells by using another type of mast cell knockout (KO) mice (242). While intestinal permeability to bacteria is increased in this model, the TLR2/4 pathway does not seem to be involved (243). Although the role of mast cells is still unsure, the infiltration of resident macrophages through their expression of the alpha7 nicotinic acetylcholine receptors plays a critical role (244). The early cytokines released from the drop of temperature and dry stimulation due to the opening of the abdominal cavity, are potent activators of the macrophages. Furthermore, the afferent nerves activated by the manipulation of the intestine may also activate the resident macrophages and trigger an inflammatory response. In this context, pharmacological or electrical stimulation of the cholinergic anti-inflammatory pathway has been presented as an attractive option to reduce POI (245). Moreover, stimulation of the vagal nerve has been shown to reduce the severity of the POI

in animal models (246). As mentioned before, the decrease of the ambient temperature plays an important role in the inflammatory response and also for the activation the thyrotropin-releasing hormone (TRH) in the brain which stimulates gastric motility and secretion for the activation of gastric myenteric cholinergic neurons (247). Several hormones expressed both in the brain and the GI tract are involved in the pathophysiology of POI, including ghrelin, nesfastin-1, somatostatin, TRH, CRF and calcitonin gene-related peptide (CGRP). In the POI model, lower ghrelin concentrations were observed which—like CRF and CGRP—leads to a delayed gastric emptying (248–250). Pharmacological inhibition of somatostatin, a hormonal modulator, in the POI model induced elevated ghrelin levels (251). Centrally, POI activates brain nuclei (supraoptic nucleus, locus coeruleus, paraventricular nucleus of the hypothalamus & rostral raphe pallidus) expressing the nucleobindin2/nesfatin-1 complex which contribute to the decrease in food intake and intestinal transit (252).

Manipulations of the central nervous system have also been described as potential models of GI disorders associated with anxiety-like symptoms. As described before, the limbic system and especially the amygdala are strongly involved in stress-induced colonic hypersensitivity associated with anxiety-like symptoms and the manipulation of this brain region is sufficient to induce colonic pain (253). Direct delivery of corticosterone through a surgically implanted cannula in the central nucleus of the amygdala (CeA) induces a persistent colonic hypersensitivity, which is dependent on CRH, mineralocorticoid and glucocorticoid receptors (169). The activation with a specific agonist of one of those receptors in the CeA, has the same effect on colonic sensitivity as stress (254, 255). Furthermore, the infusion of corticosterone directly into the amygdala leads to epigenetic modifications that enhance the expression of those receptors in a long-term and transmissible manner (256).

MISCELLANEOUS MODELS OF FGID

Genetic models of FGID include specific KO animals for several receptors, ion channels, and cellular pathways. These models have provided important knowledge of FGID pathophysiology. Mostly used to better understand the pathophysiology of colonic pain, they have demonstrated the importance of BDNF, guanylate cyclase, serotonin transporters, and ion channels. The latter have been extensively described in the literature to be involved in the intestinal mechanoreception and inflammation (257, 258). Therefore, all compounds capable of activation/inhibition of those channels can trigger IBS-like symptoms and especially colonic pain (169), the full description of which is beyond the scope of the current review. Studies in mice deficient for the protease-activated receptor 2 (PAR2) highlighted the importance of this receptor in colonic sensitivity (259), and immune response, notably against *Trichinella spiralis* (260). Other key components of innate immunity, e.g., the TLRs, such as TLR4, are expressed in the intestinal tract and the CNS (261). The activation of TLR4 leads to the activation of inflammatory

cascade but also pain behavior through its expression in the spinal cord. Furthermore, TLR4 has been found to be upregulated in the GI tract of patients with IBS (262, 263). Studies in TLR4 KO mice demonstrated a role of the central expression of TLR4 in visceral hypersensitivity following maternal separation stress (264). More generally, in the CRH-induced colonic hypersensitivity and hyperpermeability, TLR4 is a pivotal factor for CRH-mediated modulation of the immune system (159).

Several studies have demonstrated a cross-sensitization between different abdominal organs. In the spinal cord and the brain, the convergence of the sensory neuronal pathways of the different organs is one of the mechanisms underlying this sensory visceral crosstalk (265). Furthermore, within the abdominal cavity, all organs are linked to each other through physical contact and blood circulation. The best-documented model of cross-sensitization is the interaction between the bladder and the colon, in which inflammation in one of the two will affect the other partner as well. Similar to FGID, bladder pain syndrome and bladder hyperactivity syndrome has a female predominance and is often associated with IBS (266). Animal models of bladder irritation, e.g., triggered by protamine sulfate, display an increased colonic hypersensitivity and permeability (267, 268).

NON-RODENT MODELS

Although most of the research focuses on rodent models, some other species have been used to investigate the FGID pathophysiology. The guinea pig is a good model to study intestinal motility and the enteric nervous system. The models used are similar to what we previously described in rodents, with the use of stress models such as water avoidance and CRH injection (269, 270). As a model of altered GI transit, several chemical approaches have been used in guinea pigs such as the gavage with mustard oil and serotonin or the injection of TRH. Mustard oil, given orally, induces elongation of the transit time in the upper GI (esophagus) and a decreased transit time in the lower GI part (colon) (271). Ricinoleic acid-induced defecation in the guinea pig is suppressed by a specific tachykinin receptor NK2 antagonist (272).

In rabbits, intracolonic infusion of Zymosan leads to colonic hypersensitivity (272) which is reduced by a tachykinin NK2 receptor antagonist.

Pigs have a comparable GI system to humans, with an equivalent size, anatomy, development and diet preference, which are evidently very different in rodents (273). Also, the enteric nervous system phenotype is comparable to the human counterpart with more complex inter-neuronal connections and plexi compared to rodents (274). It has been shown that pigs have a more highly developed CNS with a complex behavior response to psychosocial stimuli (275) and therefore are more suitable as a model for the response of the GI tract to early life stress in humans. In pigs, the weaning itself is considered a very stressful event (both psychological and physical) which promotes an intestinal barrier defect (276). In a model of early weaning, the piglets are separated from their sow 1 week earlier

TABLE 2 | Strengths and limitations for common models of Functional GI disorders.

Model	Strengths	Limitations	Part of the GI tract studied
Pre-natal stress	Allows to study epigenetic changes	Individual variation among animals, around 80 to 95% of animals are sensitized by the stress applied	Small bowel and colon
Maternal separation	Reproduces maternal neglect and mistreatment of FGID patients		Colon
Limited bedding	Non-interventional model, avoids experimenter influence		Colon
Odor shock conditioning	Specifically mimics alterations in learning and fear conditioning		colon
Water avoidance	Strong acute models reproducing a strong stressor and mimicking resilience in an uncomfortable situation	Limited construct validity: physical constraint is not a factor commonly encountered in the etiology of FGID in patients	Stomach Ileum (more affected) colon
Partial restraint stress			Stomach and colon
Crowding stress	Models capture the social component in stress-induced FGID	Social organization, and individual reactions to stress are obviously less standardized and more complicated in humans	Small intestine and colon
Social isolation			Colon
Combined stress	Good model to reproduce anetiology commonly found in human	Because of the more complex interaction of stressors and depending on the protocol used, results tend to be more difficult to reproduce	Colon
Post-inflammatory	Reproduces low-grade inflammation often found in FGID e.g., after infection or in IBD in remission	Limited construct validity: interventional models using irritants/chemicals	Local effect depending on the targeted organ: mainly Stomach and colon
Post-infectious	Model for post-infectious FGID which allow a detailed study of dysbiosis involved in FGID	Different infectious agents compared to humans; most models have used parasitic infections which is uncommon in human FGID	Depending on the infection, small bowel or colon
Food allergy	Murine immune response in case of loss of oral tolerance closely resembles the human counterpart	The nutritional pattern differs between rodents and humans; evidence for immune reaction to food is still limited in human FGID	Colon
Spontaneous models	Non-interventional models, good face and construct validity	Sensitivity to environmental factors (food, stressors…) which makes these model more difficult to reproduce	Stomach, small bowel, colon
Postoperative Ileus	Good construct validity. Allows to study of the mechanisms of interventional surgery as a trigger of intestinal alterations	Intervention is highly operator and experimental condition dependent	Small bowel
Manipulation central nervous system	Suitable for mechanistic studies of the involvement of the central nervous system	Limited construct validity: far from human etiology	Mainly colon
Genetic model	Ideal models to study a specific genetic target and its role in FGID	Compensation phenomena; human FGID is not monogenetic	If KO: all levels of the GI tract If conditional KO: organ targeted
Cross sensitization	Understanding of the overlap in neuronal pathways which is common in human FGID	Interventional models using irritants	Depending on the organs targeted (mainly bladder and colon)

KO, knockout; FGID, functional gastrointestinal disorders; IBD, inflammatory bowel disease.

than usual. In this model, adult pigs display a defect in the small intestinal and colon mucosal barrier function with an elevated electrogenic transport activity, chronic diarrhea associated with an enhanced mast cell activation and an upregulation of the enteric cholinergic population (277, 278) Pretreatment of the stressed animals with a CRH antagonist abolished the stress-induced elevated secretory activity and increased intestinal permeability in jejunum and colon (279). *Ex-vivo* experiments demonstrated that CRH increased permeability via a TNF-α dependent mechanism (280). Similar to rodent models and

humans, female pigs are more affected than males by this stress paradigm (276). Despite the differences listed above, pig and rodent models reach the same conclusions on the effect of stress on the GI tract, confirming the critical and harmful effect of early life stress across species.

SUMMARY AND CONCLUSION

Functional gastrointestinal disorders are complex and multifactorial disorders involving a complex interaction between

biological, psychological and social variables that none of the current animal models can reproduce perfectly. The strengths and limitations of the varied models are listed in **Table 2**. The main limitation of those models remains the societal component of the FGID pathophysiology that is extremely difficult to reproduce in animals. Nevertheless, animal models have brought pivotal insights into the pathophysiology of FGID, including the complex interaction between the gut and the central nervous system, and represent essentials tools for identifying novel therapeutic targets and testing of new generations of pharmaceutical and non-pharmaceutical therapies. Over the years, the improved understanding of the FGID pathophysiology has stimulated the conception of new animal models, which are now more complex and include a combination of causes triggering FGID features, which more closely resembles the human condition. However, with the development of those new multidimensional models using a multitude of slightly different protocols—sometimes not explained in detail in the literature—comes a lack of reproducibility hampering further progression. Several guidelines have been created to address this problem and to enhance scientific rigor (281, 282).

Most of the published studies still suffer from two limitations which weaken their translational relevance. First, most of the available studies focus on the lower GI tract (**Table 2**), while increasing evidence points out the overlap between the different FGIDs. Many of the models described above are characterized as IBS models but might also be suitable as FD models if alterations in the upper GI tract would be investigated. The second limitation is that the large majority of the pre-clinical studies are performed in male animals to avoid the "hormonal fluctuation" problem although FGID are mainly affecting women. Moreover, recent findings about the impact of sex hormones on the immune response suggest that estrogen is an important player in the onset and development of FGID. For each model presented in this review, at least one study performed in females was available, but often studies comparing both genders were lacking. The early life stress models more frequently addressed the impact of gender because of their methodology, since the stress is applied to pups in a stage when the sex is more difficult to determine. Although the field is slowly changing, studies including females are still underrepresented and those taking the hormonal parameters into account are even fewer. In order to improve the construct validity of the animal models capturing the female predominance of human FGID these studies are awaited in the near future.

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All authors contributed to the first draft of the manuscript, critically revised subsequent drafts, and approved the final version.

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