Interaction of psychological, physiological and neuronal processes in functional dyspepsia

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Contents

Abstract

Functional dyspepsia is characterized by postprandial fullness, early satiation, epigastric pain, bloating, and nausea symptoms in the absence of structural changes in the gastrointestinal tract. Numerous works have been performed to identify the peripheral characteristics of functional dyspepsia and its association with dyspeptic symptoms, including changes of gastric motility, visceral sensitivity, secretion of hormones, functions of immune system. However, the pathophysiological mechanisms involved and standard treatment strategies are still lacking. The role of the dysfunction of the brain-gut axis and the effect of the food ingestion in the gastrointestinal symptoms of functional dyspepsia patients have therefore been attracting more interest in recent years. How the food is processed differently in the peripheral and in the central nervous system in functional dyspepsia has, however, received little attention in comparison to other functional gastrointestinal disorders.

In this thesis, we used various approaches to examine the physiological and neuronal mechanisms in functional dyspepsia patients. We commenced by summarizing previous functional neuroimaging studies to establish their limitations. To bridge the resulting research gap, we investigated physiological and attentional responses to visual food cues, and measured the altered brain activity before and after the food ingestion in functional dyspepsia patients.

In the paper I, we reviewed the current status of brain research related to functional dyspepsia and were able to clearly show a knowledge gap regarding neural mechanisms of food-related factors in functional dyspepsia patients. In paper II, we introduced how to design the neuroimaging study and interpret the results of it to clinicians. In paper III, we report findings of an eyetracking and behavioral study on functional dyspepsia patients. The patients showed 1) greater dyspeptic symptoms even after ingestion of a lower calorie and food intake from standard

breakfast; 2) decreased pleasantness ratings to food images; and 3) reduced visual attention to food images in comparison to healthy controls. In paper IV, we report findings of a functional magnetic resonance imaging study during meal ingestion (yoghurt with different fat content and label info) in functional dyspepsia patients. The patients showed 1) greater abdominal pain, burning, and discomfort after high fat labeled yogurt ingestion than after low fat labeled yogurt ingestion irrespective of fat content, 2) increased activity in occipital areas before and after ingestion irrespective of fat content and label and increased activity in the middle frontal gyrus before ingestion, 3) increased functional connectivity between the insula and the precuneus after ingestion of yogurt with low fat label, and 4) greater nausea-related increased functional connectivity between the insula and the occipital gyrus after ingestion of high fat yogurt than of low fat yogurt. Furthermore, bidirectional influences between quality of life and depression, as mediated by dyspeptic symptoms and the impact of food craving on the amplitude of brain activity in the middle frontal gyrus, as mediated by depression in functional dyspepsia patients were recorded. In conclusion, the abnormal dietary behavior, reduced positive emotional response and visual attention to food images, and the role of cognitive perception of fat on the aggravation of dyspeptic symptoms should be considered in clinics and in research for functional dyspepsia.

1. Introduction

1.1. Definition of functional dyspepsia

Functional dyspepsia, the second most common functional gastrointestinal disorder after irritable bowel syndrome, is defined as the presence of symptoms localized in the gastrointestinal tracts without any structural or systemic diseases that might explain the symptoms [1]. Functional dyspepsia patients have a relapsing-remitting course of postprandial fullness, early satiation, epigastric pain, burning, nausea, and vomiting symptoms [2]. A large scale epidemiology study showed that the prevalence of functional dyspepsia ranges between 11 and 29.2% in general population [3] and a systematic review suggested that 20-70% of patients remain symptomatic by the end of the follow-up period of 1.5-27 years [4]. Although functional dyspepsia does not increase mortality, it should not be underestimated; its high prevalence and chronic nature cause a considerable social and economic burden and reduce work productivity in patients [5]. An outsized survey estimated that dyspepsia costs 0.5-1 billion pounds each year in the UK [6]. Furthermore, functional dyspepsia reduces disease-related quality of life of patients, and somatization, abuse history, and depression have been identified as the important risk factors for decreased quality of life in patients [7].

According to the ROME IV criteria [8], the most recent diagnostic criteria for functional gastrointestinal disorders, functional dyspepsia comprises postprandial distress syndrome and epigastric pain syndrome patients. Postprandial distress syndrome is characterized by meal-related dyspeptic complaints, and epigastric pain syndrome refers to epigastric pain and burning symptoms which do not exclusively occur after meal ingestion. There is also a considerable overlap between postprandial distress syndrome and epigastric pain syndrome patients in clinical practice. The definition of postprandial distress syndrome was therefore adapted from the ROME III to the

ROME IV criteria to include epigastric pain or burning, belching, and nausea as supportive remarks [8, 9]. Furthermore, a large overlap between gastroesophageal reflux disease [10-12], irritable bowel syndrome [13, 14], and functional dyspepsia causes challenges in research and in practice.

1.2. Diagnosis

Diagnosis of functional dyspepsia is challenging since it depends predominantly on subjective symptom reports by patients. Following a proposal for a classification for functional gastrointestinal disorders in 1990 [15], the first ROME criteria (the ROME I) was developed for irritable bowel syndrome in 1992 and for functional gastrointestinal disorders in 1994 [16]. Over the past decades, the definition of functional dyspepsia has evolved (the ROME II in 1999 [17]; the ROME III in 2006 [9]; the ROME IV in 2016 [8]), and the current standard diagnosis of functional dyspepsia is the ROME IV criteria. It comprises of a checklist of subjective symptoms with onset, duration, and frequency of symptoms (criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis, at least 1 or 3 days per week), and upper gastrointestinal endoscopy is also required to locate any structural abnormalities [8]. Subgroups of functional dyspepsia were defined as ulcer-like dyspepsia, dysmotility-like dyspepsia, and unspecific (non-specific) dyspepsia in the ROME II criteria, and have been divided into postprandial distress syndrome and epigastric pain syndrome from the ROME III criteria to this day. Furthermore, since a relationship between meal and dyspeptic symptoms has been revealed [18], it was described in the ROME III and IV criteria.

Although the standard criteria already existed, various issues also became the object of controversy. First of all, the term functional dyspepsia is not easily understood by patients, and clinicians also interpret it in different ways [1]. This may result in misdiagnosis of functional

dyspepsia patients as well as of other functional gastrointestinal disorders such as gastroesophageal reflux disease or gastroparesis. Furthermore, the definition of dyspeptic symptoms varies in most cultures and is also ambiguous. For instance, the term "discomfort" may or may necessarily be pain-related [19].

Tests and questionnaires

Standard diagnosis is based on subjective reports and upper gastrointestinal tract endoscopy. Nevertheless, clinicians and researchers have examined Helicobacter pylori infection, gastric emptying time with scintigraphy or magnetic resonance imaging, gastric accommodation using imaging techniques or drinking/nutrient challenge test, gastric sensitivity using barostat (mechanical stimulation) or nutrient infusion (chemical stimulation), and gastric motility using manometry or electrogastrography [20].

In addition to the ROME diagnostic questionnaire, several other questionnaires have been developed and validated for functional dyspepsia. The Nepean dyspepsia index questionnaire is a validated questionnaire for both functional dyspepsia symptoms and functional dyspepsia-specific quality of life [21, 22]. The original version of Nepean dyspepsia index consists of 15 items of a symptom checklist that measures the frequency, intensity and level of upper gastrointestinal symptoms, 25 items measuring quality of life during the prior 2 weeks, and a further 11 items measuring the importance of the above items using a 5-point Likert scale. Another two short forms of Nepean dyspepsia index were developed and contain 25 [21] and 10 items [23], respectively. Leeds Dyspepsia Questionnaire [24], Hong Kong index of dyspepsia [25], Functional dyspepsia-Related Quality of Life Questionnaire [26], Leuven Postprandial Distress Scale for patients with postprandial distress syndrome [27], Glasgow Dyspepsia Severity Score [28] have been developed and validated to measure dyspeptic symptoms and disease-related quality of life. In addition,

questionnaires on anxiety, depression, somatization, stress, sleep behavior, eating behavior, and other possible comorbidities have also been used depending on the research interests. Recently, Fujikawa et al. proposed a new questionnaire – the Naniwa scale – which has not yet been validated. It measures pain, burning, gastric acid reflux, fullness and bothersome nausea, belching, heaviness (food remains in the stomach for several hours after meals), and bloating symptoms using a 7-point Likert scale with an illustration of the eight upper abdominal regions and detailed descriptions of each symptom [29]. Since patients might not be familiar with the upper gastrointestinal tract anatomy and medical terms of symptoms, this approach would be an excellent opportunity to gather more reliable data from patients.

1.3. Pathogenic factors

Some of the pathophysiological mechanisms involved in functional dyspepsia remain unknown, suggesting that various physiological functions, pathogenic factors, and heterogeneous symptoms are at work. Symptoms of functional dyspepsia do not affirmatively indicate inherent pathophysiology, symptoms and gastric functions are even poorly correlated, and no physiological measurements or psychological tests have been validated for functional dyspepsia. So far, our knowledge of pathophysiological abnormalities in functional dyspepsia is practically limited to the functional abnormalities in the gastrointestinal tract, such as delayed gastric emptying, impaired gastric motility and intra-gastric meal distribution, visceral hypersensitivity to mechanical or chemical stimuli, changed hormone secretions, and immune cell functions. However, the prevalence of impaired gastric functions (particularly gastric accommodation and the gastric emptying) did not differ between postprandial distress syndrome and epigastric pain syndrome patient groups, nor did it explain symptom severity in patients with functional dyspepsia [30].

Since meal-related complaints, dietary behavior, and nutrition intake have become interesting topics in functional dyspepsia, more recent studies investigated the role of dietary habits in functional dyspepsia. Fat ingestion in particular is a potential factor in dyspeptic symptom triggering [31-33]. For instance, in a recent study, the most symptom-related food was fatty food (27.1%) followed by hot spices (26.4%) and carbonated drinks (21.8%) in patients with functional dyspepsia [32]. However, evidence on the amount, frequency, and composition of nutrients, meals, or snacks remains inconclusive.

Critics recently raised the issue that the stomach and the gastrointestinal system may not be responsible for dyspeptic symptoms. Only a small number of studies have investigated the psychological characteristics of functional dyspepsia patients and revealed the crucial role of anxiety, depression, and somatization [34]. Furthermore, the abnormality of the brain-gut axis (the mutual communication between the enteric nervous system and the central nervous system of neuronal and hormonal signaling) may be one of the key mechanisms behind functional dyspepsia [35]. Indeed, neuroimaging provided new findings on altered functional and anatomical changes in the brain of patients. One recent systematic review [36] showed that abnormal brain activity was frequently reported in somatosensory cortex, insula, thalamus, prefrontal cortex (sensory processing regions), hippocampus, and amygdala (limbic regions) in functional dyspepsia patients compared to healthy controls. Functional neuroimaging techniques now enable us to comprehend brain activity generated by signals from the gastrointestinal tracts as well as the effect of emotion and psychological factors in functional dyspepsia.

Furthermore, an earlier survey showed a significant effect of a family history in dyspepsia patients [37]. The role of genetic factor (G-protein β3 genotypes) in upper gastric symptoms [38] and in the impairment of the gastric emptying [39] in functional dyspepsia patients has also been demonstrated. It was also proposed that the g-protein β3 and cholecystokinin-A receptor genotypes

were involved in the pathogenesis of functional dyspepsia [40]. These findings suggested that genetic factors, dietary habits, and eating behavior of family contribute to the pathogenesis of functional dyspepsia.

Gastrointestinal motility, secretions, perception, and immune responses are regulated by the enteric nervous system. The latter receives considerable innervation from the autonomic nervous system, which is one of the control centers of digestive function. Heart rate variability has been measured extensively as a surrogate of sympathetic and parasympathetic activities to evaluate autonomic nervous system in patients with functional dyspepsia, and decreased parasympathetic activation [41] and vagal activity [42] were reported. However, we do not yet know whether the altered autonomic nervous system in patients cause dyspeptic symptoms or impaired gastrointestinal functions [43].

1.4. Changes in the gastrointestinal tracts

Impaired motor function, gastric accommodation, and emptying time

The gastrointestinal tract processes ingested food by motor functions of the proximal and the distal part of the stomach. A dysfunction of the proximal stomach as well as disturbances of gastric motor function, impaired gastric accommodation, and abnormal distribution of food in the stomach have been studied from an early stage of research in functional dyspepsia patients. The proximal stomach relaxes to allow an increase in intragastric volume without an increase of pressure. Patients showed a lower antral motor response and gastric relaxation to a test meal than healthy volunteers [44, 45]. The hypomotility of fundus may be involved in delaying the gastric emptying [46] and impaired accommodation [47]. This remains a controversial issue. Impaired gastric accommodation was associated with early satiation in the studies using barostat [48] and

scintigraphy [49]. However, other studies found neither impaired accommodation in patients [50] nor any association with the symptoms [51].

Impaired gastric accommodation may be caused by abnormal vago-vagal reflex since the accommodation reflex consists of a vago-vagal reflex pathway that affects smooth muscle tone in the proximity of the stomach [52]. Since motor neurons within the enteric plexuses control gastric motility, the inhibitory innervation may also be related to gastric accommodation. For instance, activation of Nitroxidergic pathways and inhibition of cholinergic pathways both contribute to gastric accommodation. Moreover, the central nervous system may affect gastric motility, for example, anxiety negatively affects the accommodation reflex [53].

The distal part of the stomach regulates the gastric emptying of food in cooperation with the proximal stomach and the small intestine. In a meta-analysis, the gastric emptying is slowed down in almost 40% of functional dyspepsia patients [54]. Moreover, fat in the stomach releases hormones such as cholecystokinin that increases pyloric sphincter tone and inhibits gastric emptying [55]. However, inconsistent results have been reported with regard to the relationship between dyspeptic symptoms and delayed gastric emptying in patients. Nevertheless, it is conceivable that fullness, nausea, and vomiting are mainly related to gastric emptying [56, 57]. Delayed gastric emptying is more frequent in female and low-weighted patients.

For the assessment of gastric accommodation, the barostat was developed to evaluate changes of pressure. Using single-photon emission-computed tomography, a three dimensional image of the stomach and its volume could be obtained. Gastric accommodation is determined by comparing fasting and postprandial volumes of the stomach. Magnetic resonance imaging and ultrasound are also available. The standard method of measuring gastric emptying is using

radioactive isotope method (scintigraphy). However, acid breath tests are now more widely used since these are non-invasive and without exposure to radiation [56].

Visceral hypersensitivity

Visceral hypersensitivity is an increased visceral sensation or a decreased threshold to mechanical or chemical stimuli. In functional dyspepsia patients, visceral hypersensitivity has been well established in gastric distension or nutrient infusion conditions. Expanding the balloon-type barostat in the gastrointestinal tracts, and infusion tests of lipid or acid are the most frequently used methods for mechanical and chemical stimuli, respectively. Both the volume of the meal (mechanical stimuli) and the absorption of nutrients in the meal (chemical stimuli) may be the main factors in meal-related dyspeptic symptoms, activating the mechanoreceptors and nutrient receptors responsible for the distension of gastric muscles, feeling of hunger/fullness/satiation, and secretion of hormones. A large-scale study using barostat distension showed that 34% of functional dyspepsia patients were suffereing from gastric hypersensitivity which was associated with pain, weight loss, belching [58] as well as with impaired accommodation [59].

Multiple studies have shown that functional dyspepsia patients showed higher visceral symptoms to the balloon or barostat distension [59, 60], altered brain activities during the balloon or barostat distension [61-63], higher nausea symptoms to the acid perfusion in duodenum [64, 65], and increased sensitivity to gastric distension after lipid infusion in duodenum [66, 67] than healthy controls.

Since barostat distension technique is invasive, it is unlikely to be used in clinics and is more suitable for pre-clinical research. Another point is that the somatic hypersensitivity to

cutaneous heat pain stimuli applied to the hand and foot was demonstrated, as well as the visceral hypersensitivity, in patients with irritable bowel syndrome [68]. Hyperalgesia to external pain stimuli has never been studied in functional dyspepsia. However, it is conceivable that the dysfunction of the central nervous system in pain processing leads to the somatic hypersensitivity in the functional gastrointestinal disorders. Further studies with regard to the origin of hypersensitivity in patients at the level of peripheral neurons in the gastrointestinal tracts, afferent neurons in the spinal cord, and subcortical or cortical neurons involved in processing pain signal may reveal the pathogenesis of visceral hypersensitivity in functional dyspepsia patients.

Lipid, carbohydrate, and acid have been infused in gastrointestinal tracts in functional dyspepsia patients to measure changes of visceral symptoms and plasma hormone levels after infusion. Functional dyspepsia patients showed more prevalent moderate to severe symptoms (particularly abdominal pain and distress) during intra-duodenal lipid and dextrose infusions than healthy controls, and they were associated with greater plasma level of Glucagon-like peptide-1 hormone [69]. Several studies have shown greater upper abdominal symptoms in response to lipid infusion [66, 70, 71]. However, infusions of nutrient might not induce the same kind of physiological responses as oral meal ingestion. Thus, a more recent study used standard meals of high fat and high carbohydrate and demonstrated the increased pain and nausea after high fat meal ingestion, as well as increased cholecystokinin and decreased peptide-YY in functional dyspepsia patients compared to healthy controls [72]. Furthermore, higher nausea symptom and lower motor response to duodenal infusion of hydrochloric acid were found in patients with functional dyspepsia than in healthy controls [64, 73].

Secretion of hormones

In response to food, the gastrointestinal tracts produce several hormones and peptides which are essential for the digestion of food. Ghrelin is a peptide secreted from the stomach mucosa. Secretion of ghrelin is maximized in the fasted state and suppressed by fat and carbohydrate ingestion, but not protein. Acylated ghrelin, a biologically active form of ghrelin, increases the sensation of hunger and initiates eating behavior by accelerating gastric contraction and emptying [74]. The relationship between the acylated ghrelin in plasma level and dyspeptic symptoms was significantly correlated [75, 76]. Furthermore, intra-venous injection of ghrelin twice a day for two weeks increased daily food intake in a small number of functional dyspepsia patients [77].

Ever since the fat-specific responses in functional dyspepsia have been revealed, scientists have been showing increasing interest in the role of cholecystokinin. Cholecystokinin is released from entero-endocrine cells by the presence of fat and protein in the small intestine and is regarded as the satiety hormone which regulates food intake. Intra-venous injection of cholecystokinin produced significantly higher bloating, fullness, and nausea symptoms in functional dyspepsia patients than in healthy controls. Furthermore, oral administration of loxiglumide, a cholecystokinin-A receptor antagonist, relieved dyspeptic symptoms by intravenous administration of cholecystokinin in functional dyspepsia patients [78]. Plasma cholecystokinin level is significantly higher before meal ingestion and also increases more significantly after high-fat meal ingestion in functional dyspepsia patients than in healthy controls [72]. These findings suggest that the enhanced cholecystokinin secretion at the fasted condition and increased release of cholecystokinin in response to fat contributes to the pathophysiology of functional dyspepsia.

Infection and inflammation

Dysfunction of immune system has been investigated in functional dyspepsia due to the fact that a small number of patients develop their symptoms after a gastrointestinal infection. This

is known as post-infectious functional dyspepsia. The potential role of an infectious agent in functional dyspepsia initially focused on Helicobacter pylori. Although its role in the pathology of functional dyspepsia is unclear, Helicobacter pylori infection [56, 79] is still under consideration. It causes chronic inflammation in gastric mucosa and affects the production of ghrelin and mast cells in infected functional dyspepsia patients [80]. However, the relationship between the infection and gastric symptoms in functional dyspepsia patients does not seem to be significant [81]. Although the impact of Helicobacter pylori eradication in functional dyspepsia remains a contentious issue, it provides symptomatic relief in a small number of patients [82]. A recent systematic review reported small effect size of Helicobacter pylori eradication therapy which showed no short term benefit. Histologic changes of chronic gastritis did, however, appear to be relieved after therapy [83].

The prevalence of functional dyspepsia was significantly higher in patients with salmonella gastroenteritis than in the non-infected population [84], and a recent systematic review showed that diverse bacteria and viruses such as Salmonella spp., Escherichia coli O157, Campylobacter jejuni, Giardia lamblia, and Norovirus were associated with post-infectious dyspeptic symptoms [85]. Post-infectious functional dyspepsia patients showed focal aggregates of T cells and CD8+, reduced number of CD4+ T cells, and higher macrophage counts in the duodenum than functional dyspepsia patients with unspecific onset [86]. Furthermore, epigastric burning symptom was significantly correlated to the degree of histological duodenitis in post-infectious functional dyspepsia patients [87]. Changes of inflammatory cells were also reported in non-infected functional dyspepsia patients. Increased degranulation and clusters of eosinophils [87-90] and mast cells [89, 91, 92] in the duodenum of functional dyspepsia patients have been reported consistently in several studies. Investigation of immune cells in functional dyspepsia is a meaningful approach

as it shows the possibility of developing the objective measurement for the diagnosis and treatment of functional dyspepsia in the future.

1.5. Psychological and cognitive characteristics

The psychological aspects of functional gastrointestinal disorders have been reported from the mid-1980s and discussed vigorously since the 1990s. Of the many psychological factors involved in functional dyspepsia, anxiety and depression have been studied most often. In almost all studies, both were found to be more severe in functional dyspepsia patients than in healthy controls. Moreover, stress and coping style, psychological distress, sleep dysfunction and somatization, history of abuse, and traits such as perfectionism, hostility, and neuroticism have been studied in functional dyspepsia [37, 93-100]. Physical abuse history and somatization were associated with gastric discomfort threshold and gastric emptying time [101]. Moreover, both acute and chronic comorbid anxiety were associated with impaired accommodation in functional dyspepsia [102]. Epigastric pain was associated with neuroticism, somatization and abuse [103]. However, most of the studies used self-report questionnaires for assessment of psychosocial characteristics or the presence of psychiatric disorders rather than structured interviews or clinical decision process by well-trained psychologists.

The cognitive aspect is also involved in the development of dyspeptic symptoms. In an early study with a small number of patients, dyspepsia patients were served different muffins with or without high fat. Patients could not distinguish between the different muffins by taste and dyspepsia did not differ either. [104]. A more recent study also showed the effect of information about calorie (high or low calorie) on the level of plasma ghrelin and subjective satiety rating in healthy controls [105]. Another study with functional dyspepsia patients showed that a low fat meal

– under the pretense that it was high fat meal –caused more severe fullness and bloating symptoms than a low fat meal served with the correct fat information in FD patients [106]. This suggests that modified information about fat plays a prominent role in causing perceptual dyspeptic symptoms. These findings suggest that the effect of fat in gastric symptoms and functions in patients may be psychologically mediated and affected by the perception of fat rather than the ingested amount of fat. However, the size of impact of the cognitive perception of fat and the ingested amount of fat on symptom development needs to be studied further.

1.6. The brain-gut axis

The enteric nervous system

The enteric nervous system, also known as the second brain, is located in the walls of the gastrointestinal tracts and communicates with the central nervous system via autonomic nervous system and vagus nerve. It contains 200-600 millions of sensory, interneurons, muscle motor, and secreto-motor neurons [107, 108]. However, its function is highly independent of the central nervous system and the autonomic nervous system. It regulates gastric motility [109], exocrine and endocrine secretion, and immune system [108, 110]. More than 30 neurotransmitters comprised of small molecules (norepinephrine, 5-hydroxytryptamine, etc.), peptides, nitric oxide, carbon monoxide, and acetylcholine [108] are involved in this system. It is therefore one of the targets of pharmacological treatments in functional dyspepsia. For example, acotiamide, an acetylcholinesterase inhibitor that increases acetylcholine release in the enteric nervous system, is efficacious for postprandial distress syndrome by enhancing gastric contractility and accelerating delayed gastric emptying [111, 112]. Moreover, the gut microbiota, an ecological community of commensal, symbiotic and pathogenic microorganisms with a great impact on the gut functions,

regulates neuronal functions of the enteric nervous system [113]. Paroxetine enhanced the meal-induced relaxation of fundus, suggesting that selective serotonin reuptake inhibitor may be beneficial to patients with impaired postprandial fundus relaxation [114]. In a recent study of changes of neuronal function and structure of enteric nervous system, functional dyspepsia patients showed impaired neuronal activity (decreased calcium responses and lower peak amplitude) while healthy controls did not. FD also had a higher number of eosinophils and mast cells in submucosa plexus than healthy controls [115].

The central nervous system

Neuroimaging techniques and a growing interest in the psychosocial factors in functional disorders have accelerated the studies on the brain-gut axis in functional gastrointestinal disorders [116]. In irritable bowel syndrome, the most prevalent functional gastrointestinal disorder, changes of prefrontal cortex, somatosensory cortex, anterior cingulate cortex, insula, hippocampus, and amygdala activities are known to be associated with clinical phenotypes and symptom severity [117]. However, only very few studies have explored the structural and functional changes of the brain in functional dyspepsia patients, and conflicting results prevent us from achieving an integrative understanding [36]. Furthermore, the neuroimaging technique is an expansive, time-consuming, labor-intensive experimental tool that requires profound knowledge in physiology, pathology, neurology, physics, and program coding skills. As a matter of fact, the methods and results of functional neuroimaging studies are practically incomprehensible to people outside the field. Since it should provide novel methods of diagnosing and treating patients and improve our understanding on the features of the central nervous system in functional dyspepsia patients, it is

vital that clinicians and scientists from various fields cooperate with each other to conduct and interpret the results of neuroimaging studies [118].

The brain-gut interaction

A highly influential hypothesis to explain the functional gastrointestinal disorders is that the dysfunction of brain-gut signaling may contribute to these problems. The brain-gut axis is part of an interoceptive and homeostatic system and consists of the reward, affective, cognitive, sensorimotor systems in the central nervous system, enteric nervous system, autonomic nervous system, and vagus nerve. Ascending transmission of the information of visceral sensation and environment from the gut through the afferent pathway and descending modulation signals of psychological factors from the brain are responsible for gastrointestinal functions and symptoms. For instance, satiety and eating behavior [119], and gastric motility [120] are controlled by brain-gut axis.

In the neuronal pathways of brain-gut axis, the efferent pathway, consists of preganglionic parasympathetic fibers, travels along vagus and pelvic nerves and projects to the smooth muscles and enteroendocrine glands in the gut. The afferent pathway transmits the mechanical, chemical, and thermal information from the gastrointestinal tracts to the hypothalamus. After the information is integrated in hypothalamus, it is projected to several subcortical and cortical regions of brain such as thalamus, anterior and posterior cingulate cortices, amygdala, insula, somatosensory cortex, and frontal cortex [120].

The brain-gut pathway may explain how psychological states affect gastric symptoms and vice versa. A large scale longitudinal population-based study with a follow-up of more than 10 years revealed that anxiety was associated with the new onset of functional dyspepsia at follow-up

[121], and depression at baseline in a population without functional dyspepsia independently predicted dyspepsia symptoms at follow-up [122].

Recent studies have ascertained that the pathological changes of microbiota in the gut can even affect immune system, mind, emotion (especially anxiety and depression, the most common psychological problems in functional dyspepsia), cognitive development, and even human behavior through the brain-gut axis [123]. The alterations in the microbiota compositions in irritable bowel syndrome patients compared to healthy controls have been demonstrated. The microbiota may synergistically interact with infection and inflammation and enhance abdominal symptoms [124, 125] indicating the possible role of microbiota in functional dyspepsia. This theory requires further investigation.

1.7. Food, nutrition, and dietary behavior

Food is responsible for diverse changes in gastrointestinal tracts including visceral sensation, gastric motility, gastric volume, and hormonal release and also induces several gastrointestinal symptoms. Furthermore, a long-term negative experience with certain foods in functional dyspepsia patients may change the cognitive response to food by operant conditioning of food and symptoms.

The effect of fat in the impaired gastrointestinal sensitivity and symptoms is one of the well-known pathophysiological features in functional dyspepsia patients. Following ingestion of a high fat meal, nausea and pain symptoms were greater than after a high carbohydrate meal [72]. Food diaries revealed that functional dyspepsia patients consumed less fat and that their bloating symptoms were related to the amount of ingested fat [126].

Eating patterns of functional dyspepsia patients including size and frequency of meals, energy intake, and food intolerance have received little attention so far. Evidence showed that a smaller percentage of functional dyspepsia patients consumed three regular meals per day. They had a lower prevalence of eating large meals, ate snacks more frequently, and had a lower consumption of fiber and fat than healthy controls [126-129]. With regard to food intolerance, functional dyspepsia patients reported that high fat meals induced or exacerbated their symptoms. They exhibited more intolerance towards alcohol, fatty foods, fruits, spices, coffee, etc., than healthy controls [128-130].

However, conflicting results, lack of consented definition of 'meal', 'snack', 'frequency', and dyspeptic symptoms, and usage of diaries or questionnaires instead of in-depth interviews are the limitations of previous studies. To overcome these limitations, a few studies served fixed amounts of real meals to functional dyspepsia patients and investigated the gastric changes and meal-related dyspeptic symptoms [18, 131-133]. Furthermore, visual food images are a validated experimental tool that has been used to investigate food-related behavior in patients with obesity [134], anorexia nervosa [135], and binge eating disorder [136]. In general, food images are delivered as reward-related stimuli eliciting positive responses [137]. However, the evaluation of the reward value of food and food images, emotional and physiological responses to food and food images, and the effect of modification of eating behavior have yet to be demonstrated in functional dyspepsia patients.

1.8. Treatment and placebo response

Treatment of functional dyspepsia is still unsatisfactory due to the insufficient awareness of the disease on the part of both patients and physicians, difficulty in diagnosis, and lack of standard treatment guidelines. Therapies for functional dyspepsia have focused mainly on gastric

functions and relief of symptoms. Current treatment options include an eradication of Helicobacter pylori, prokinetic agents, histamine H2 receptor antagonists and proton pump inhibitors (acid suppression medications), tricyclic antidepressants, selective serotonin reuptake inhibitors, analgesics, complementary and alternative medicine (acupuncture and herbal medicine), and psychotherapies [138].

Pharmacological treatments which have been tested with regard to their efficacy and safety are currently not available for patients with impaired gastric accommodation. However, several options may be worth considering. Administration of sublingual glyceryl trinitrate improved proximal gastric accommodation and reduced pain, nausea, and total symptom score [139]. Sildenafil (used for smooth muscle relaxation) [140], paroxetine (a selective serotonin reuptake inhibitor) [141], and buspirone (5-hydroxytryptamine 1A receptor agonist) [142] have been tested and proved to increase gastric volume and enhance gastric accommodation, but only in healthy controls.

Current treatment options for functional dyspepsia do not take into account that dyspeptic symptoms are induced by food ingestion. To enhance the conventional therapies, a detailed interview of their eating patterns should first be conducted by physicians. If required, physicians might use the nutrient challenge test to measure meal-related symptoms in patients. On the basis of these data, physicians and patients could then discuss their eating behavior and decide how to modify it to alleviate their symptoms.

Placebo response in functional dyspepsia has been observed in clinical practice and clinical trials show that a substantial number of patients, ranging from 13-73%, respond to placebo treatment [143]. In an earlier study to determine predictors and contributing factors to the placebo response in functional dyspepsia patients, body mass index and the consistency of the most undesirable symptoms were found as predictors [1]. In a later study, lower baseline gastrointestinal

symptoms and increase of symptoms during the trial, and higher body mass index were found in placebo responders than in non-responders [144]. The relatively high response rate to placebo treatment in functional dyspepsia patients also shows the possibility of psychotherapies in symptom relief.

2. Functional neuroimaging studies in functional dyspepsia (Paper I, II)

Only a small number of studies have addressed the functional brain alterations of functional dyspepsia patients and conflicting results have been reported. We aimed to integrate the previous neuroimaging results in functional dyspepsia patients and present the important technical and practical issues of functional neuroimaging technique to clinicians. This might prompt functional neuroimaging studies in functional dyspepsia patients.

The systematic review (paper I) aimed to 1) find the brain regions assumed to be related to functional dyspepsia; and 2) establish a hypothesis of how altered brain activities are derived and interact with various factors in functional dyspepsia.

Sixteen articles were reviewed, and we found functional abnormalities of frontal cortex, somatosensory cortex, insula, anterior cingulate cortex, thalamus, hippocampus, and amygdala in functional dyspepsia patients. With behavior results, it is conceivable that the changes of brain activity of functional dyspepsia patients are induced from the repeated afferent signal from the gut and failure of central pain modulation.

In a second technical review study (paper II), we introduced the basic understanding of functional magnetic resonance imaging including the blood oxygen level dependent signal, hemodynamic response function, design, analysis procedure and software, and the technical terminology.

3. Physiological processing of and attentional bias to food images (paper III)

Chronic negative experience with food in functional dyspepsia patients may have a negative influence on the reward value of food and alter the autonomic and emotional response to it. Furthermore, food eating behavior and nutrient consumption have been studied in functional dyspepsia patients using diaries and questionnaires and need to be examined with the meal challenge test.

Visual food stimuli and the eye-tracking technique, which measures either the fixation of gaze or the path of gaze [145], have been used to investigate food-related attentional bias. Autonomic response and emotional state might change in functional dyspepsia patients: Attention might also be distorted while watching visual food cues. Activity of the autonomic nervous system and facial muscle contraction could be measured using skin conductance response, heart rate variability, and electromyography. Skin conductance response refers to changes in skin resistance in accordance with the activity of sweat glands. Since sweat glands are controlled by the sympathetic nervous system, it refers to the activity of sympathetic nervous system. Heart rate variability parameters are suitable for measuring different aspects of the autonomous nervous system. Face muscles are related to emotional response and several studies have shown that the pictures of positive and negative emotion are related to the greater activity of the zygomatic or corrugator muscle, respectively [146, 147]. In general, food images are positive reward cues [137].

We therefore aimed to determine the physiological and emotional responses and visual attention to food images after taking an ad-libitum meal. For this purpose, after a standard breakfast at which the participants could eat as much as they wished, five sets of high fat food, low fat food, positive, negative, and neutral images were presented with skin conductance response, heart rate, and facial electromyography measurements. Gaze data was also obtained during the presentation

of pairs of images of food and non-food images in functional dyspepsia patients and in healthy controls.

We observed that, in comparison to healthy controls, functional dyspepsia patients 1) had a higher food craving, depression, and anxiety score, 2) consumed smaller amounts of food (bread) and less calories and reported higher dyspeptic symptoms afterwards, 3) rated less pleasantness to both high and low fat food images, 4) showed lower sympathetic activation (ratio between low and high frequency components), and 5) fixated less time on food images than non-food images.

The results show that, despite the increased craving for food, functional dyspepsia patients can tolerate only small amounts of food. Decreased visual attention and pleasantness rating to food might reflect their disturbed perception of food.

4. Neuronal processing of fat and fat label (paper IV)

Due to the methodological difficulties of delivering a meal during scanning and matching the central response with the slow digestive process, the central responses following regular food ingestion have rarely been recorded [148]. In functional dyspepsia patients, fat content of food and modified information of fat content [106] as well as psychological factors such as anxiety, depression, and abuse history [34] influence dyspeptic symptoms. However, previous functional neuroimaging studies have discussed the resting state brain activity, brain response to visceral pain stimulation or acupuncture [36], and only very few of them examined the effects of anxiety, depression, and abuse history [62, 63, 149-151]. To date, no neuroimaging studies have been conducted on how the brain processes food and food-related information and how psychological/cognitive factors influence brain activity in functional dyspepsia patients.

In this study, we used functional magnetic resonance imaging to investigate how cognitive modulation of fat information and the amount of fat ingested influences the induction of dyspeptic symptoms and brain activities in functional dyspepsia patients. The resting state blood oxygen level dependent signal was recorded before and after the four types of yogurt ingestion. Functional dyspepsia patients and healthy controls were given a 200ml of high fat yogurt labeled 'high fat' or 'low fat', low fat yogurt labeled 'low fat' or 'high fat' during each visit (high fat=10%, low fat=0.1% fat). Dyspeptic symptoms were measured 4 times using a visual analog scale (to what extent do you feel fullness/satiety/epigastric pain/burning/nausea/vomiting).

We observed that 1) the low fat information relieved the abdominal pain, burning, and discomfort symptoms, in both high fat or low fat yogurt condition, 2) the resting state brain activity increased in the prefrontal, occipital and decreased in cingulate before yogurt ingestion, 3) resting state activity increased after yogurt ingestion in the cerebellum and occipital cortices, 3) functional connectivity of the insula-inferior occipital gyrus was higher in high fat condition than in low fat

condition and correlated with nausea symptom in functional dyspepsia patients, 4) functional connectivity of the insula-precuneus was higher in low fat label condition in patients than in healthy controls, 4) the bidirectional influences between the degree depression and disease-related quality of life which are mediated by dyspeptic symptoms, 5) there is a mediation effect of depression on the influence of food craving to the middle frontal gyrus activity in functional dyspepsia patients.

The results imply that the fat label has a significant effect on symptom aggravation, food craving on the higher cognitive brain region mediated by depression, and symptom (nausea) related functional connectivity from the insula to the occipital gyrus as well as on the reward context involved in the functional connectivity from the insula to the precuenus. The role of expectation of fat content in meals and psychological factors, particularly food craving and depression, may be crucial in the somatic symptoms induction and in the altered brain activity in functional dyspepsia patients.

5. Paper I. Functional neuroimaging studies in functional dyspepsia patients: a systematic review

Author contributions

The material of this chapter was published in *neurogastroenterology and motility* (Lee et al., 2016). All authors designed the study and interpreted the results. In-Seon Lee acquired and summarized all the data. In-Seon Lee wrote the manuscript with the help of Hubert Preissl and Paul Enck.

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REVIEW ARTICLE

Functional neuroimaging studies in functional dyspepsia patients: a systematic review

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Key Points

- By summarizing earlier functional neuroimaging studies, this systematic review proposes the FD-related brain regions and direction of future research.
- The functional abnormalities of frontal cortex, somatosensory cortex, insula, ACC, thalamus, hippocampus, and amygdala were reported in FD.
- Various neuroimaging tasks, interventions, precise diagnosis, and measurement of psychological factors could improve our understanding of FD.

Abstract

Background *There is increasing evidence in support of the presence of abnormal central changes (compared to healthy controls) in functional dyspepsia (FD) in addition to the peripheral changes in gastrointestinal tract.* ***Purpose*** *This systematic review aims to provide an integrative understanding of the abnormal functional brain activity, visceral sensation, dyspeptic symptoms, and psychological changes of FD. Electronic and hand searches were conducted to identify functional neuroimaging studies involving FD patients. Sixteen studies were selected and divided into three categories: 10 resting state studies, three visceral distention studies, and three acupuncture studies. Changes were reported in several brain areas in FD patients including the frontal cortex, somatosensory cortex, insula, anterior cingulate cortex, thalamus, hippocampus, and amygdala. These brain activity changes were associated with visceral hypersensitivity, dyspeptic symptoms, poorer quality of life, anxiety, and depression. The results show that FD is associated with functional abnormalities in sensory and pain modulation, emotion, saliency, and homeostatic processing regions. The diversity of conditions, heterogeneous results, poorly standardized diagnoses of FD, and various comorbidities may be responsible for the variability in the results.*

Keywords *brain imaging, fMRI, functional dyspepsia, PET, systematic review.*

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INTRODUCTION

Functional dyspepsia (FD) is defined as the presence of symptoms believed to originate in the gastroduodenal region without the evidence of any organic, systemic, or metabolic disease that might explain the symptoms.[1] Functional dyspepsia patients suffer from postprandial fullness, early satiation, epigastric pain, and burning.[2] This problem has now come into focus due to its high prevalence in the general population (11–29.2%),[3] unknown mechanism, heterogeneity of pathogenic factors and symptoms, poorer quality of life (QOL), and absence of management strategies. In addition to the studies on peripheral abnormalities (hypersensitivity, abnormal accommodation, gastric dysmotility), a hypothesis from the early 1990s proposed that abnormalities of the brain-gut axis (biochemical/neural communication system between the gut and brain) are one of the driving mechanisms behind FD.[4] The development of neuroimaging techniques and emerging evidence of the importance of psychosocial factors have also contributed to the study of the brain-gut axis impairment in functional gastrointestinal diseases.[5]

The thalamus, secondary somatosensory cortex (SII), prefrontal cortex (PFC), insula, and anterior cingulate cortex (ACC) all receive signals from the gastrointestinal tract via spinal or vagal afferents and process the sensory, affective, and cognitive information of visceral sensation.[6] The thalamus receives signals from the periphery and relays them to the insula, PFC, motor, and somatosensory area, the so-called visceral pain network.[7] Unlike the somatic sensation with its clear representation in the primary somatosensory cortex (SI), the visceral sensation is vaguely localized and diffused[8] and may be more strongly associated with the SII.[6] Furthermore, visceral sensation is closely related to the insula; a hub region responsible for the interoceptive function.[9,10] Insula, a monitoring center of our cognitive, affective, and homeostatic systems, is also considered to be a key region of salience network (the brain network of identifying the item among several stimuli to guide behavior[11]) with ACC.[12] Anterior cingulate cortex is involved in the motivation and motor aspect of visceral sensation, while insula is involved in the sensory part,[10] and pain modulation.[13–15] Prefrontal cortex is implicated in the attention and appraisal of stimuli and located in the highest hierarchy of visceral sensory network.[6,16] In short, thalamus and somatosensory cortex (SI and SII) are mainly associated with the first-order process of sensory information, whereas PFC, insula, and ACC tend to be rather associated with the higher order process of cognitive evaluation, attention, sensory-motor integration, and affective response.[6,16] In irritable bowel syndrome (IBS), one of the functional gastrointestinal disorders, changes of PFC, somatosensory cortex, insula, hippocampus, and amygdala activity are known to be associated with clinical phenotypes and symptom severity,[17] and various brain networks, including sensory and salience networks might be relevant.[18] However, only a small number of studies have addressed the functional brain alterations of FD patients, and conflicting results hinder the development of an integrative understanding.

This systematic review aims to (i) provide a comprehensive survey of the core brain regions assumed to be related to FD, (ii) establish a brain-gut axis model of how altered brain activities are derived and interact with various factors and clinical changes, and (iii) propose the direction of future research by summarizing current functional neuroimaging studies.

METHODS

Paper search

We used a systematic search strategy that followed the PRISMA guidelines for systematic reviews. Electronic searches were conducted in PubMed, EMBASE, MEDLINE, and Cochrane Library using the keywords 'FD', 'neuroimaging', 'functional magnetic resonance imaging (fMRI)', and 'positron emission tomography (PET)'. Search terms and methods were modified for individual databases (Table S1). Hand searching was performed by screening the reference lists of articles that met the inclusion criteria. The literature search was completed in October 2015.

Study selection and data extraction

Search results were screened on the basis of the title and abstract before the full text was assessed. Neuroimaging studies, including FD patients regardless of their characteristics (e.g. diagnosis, symptoms, age, gender, etc.) and imaging conditions (e.g. resting, distention, medical intervention, etc.), were incorporated.

We retrieved the first author's name, year of publication, characteristics and number of participants studied, subgroups of FD patients, imaging modality and conditions, analysis methods, behavioral outcomes (Table 1), and results of the brain imaging data (Tables 2 and S2). Results of behavioral and clinical outcomes are summarized in the text.

RESULTS

Study selection and description

Our research strategy retrieved a total of 314 articles, 104 of which were duplicates. These were discarded together with a further 194 after screening the title and abstract. Sixteen articles met the inclusion criteria and were incorporated in the systematic review (Fig. 1).

All articles[19–34] were published between 2007 and October 2015 (Table 1). We distinguished two research

Table 1 Overview of the functional neuroimaging studies in FD

No. Author (year)	Groups/*n* (male): Characteristics-subgroup/*n* (male)	Imaging modality/condition	Analysis	Behavioral measures	
				Somatic or FD symptom	Psychologic
1. Vandenberghe *et al.* (2007)	FD/13(3): hypersensitivity (+), helicobacter pylori (−) HC/11(5)*,†	$H_2{}^{15}O$ PET /baseline, distention, sham distention	1) Whole brain 2) Correlation with abdominal sensation	Distention threshold/gastric sensation, pain, discomfort, nausea, bloating	Anxiety, tension
2. Zeng *et al.* (2009)	FD/8(4): Rome III (PDS) HC/8(4): Age/gender matched	^{18}F-FDG PET-CT/baseline, after Acu	Whole brain	N/A	N/A
3. Van Oudenhove *et al.* (2010a)	FD/25(5) HC/11(5)*,† Similar to study 1	$H_2{}^{15}O$ PET /baseline, distention, sham distention	1) Whole brain 2) Correlation of ROI with anxiety	Distention pressure/ threshold/gastric sensation distention, DSS	STAI
4. Van Oudenhove *et al.* (2010b)	FD/25(5) Similar to study 1 -Normosensitive/12(5) -Hypersensitive/13(0) -Abused/8(1) -Non-abused/13(3)	$H_2{}^{15}O$ PET /baseline, distention, sham distention	Whole brain	Distention threshold/gastric sensation, DSS, PHQ-15	Abuse history, PHQ-9, STAI
5. Zeng *et al.* (2011)	FD/40(20) : Rome III (PDS) -FD milder/19(9) -FD severe/20(10) HC/20(10)	^{18}F-FDG PET-CT/resting	1) Whole brain 2) Correlation of ROI with SID, NDI-QOL	SID	NDI-QOL, SAS, SDS
6. Liu *et al.* (2012)	FD/16(6): Rome III -AD/8(3) -Non-AD/8(3)	^{18}F-FDG PET-CT/resting	Whole brain	DSS	NDI-QOL, SAS, SDS
7. Zeng *et al.* (2012)	FD+Acu/34(13) FD+sham Acu/30(12) :Rome III (PDS)	^{18}F-FDG PET-CT/baseline, after Acu	1) Whole brain 2) Correlation of ROI with SID, NDI-QOL	SID	NDI-QOL
8. Zhou *et al.* (2012)	FD/26(8): Rome III (PDS) HC/20(7): Age matched	fMRI/resting	FC	NDI-symptom	NDI-QOL, SAS, SDS
9. Zhou *et al.* (2013)	FD/29(19): Rome III (PDS) HC/16(7): Age/gender matched	fMRI/resting	1) ALFF 2) fALFF 3) ROI FC 4) Correlation of 1), 2), 3) with NDI-symptom, FD duration	NDI-symptom	NDI-QOL, SAS, SDS
10. Liu *et al.* (2013)	FD/49(18): Rome III (PDS) HC/39(14)	fMRI/resting	1) ICA of DMN 2) Correlation of ROI with NDI-symptom, SAS, SDS	NDI-symptom	NDI-QOL, SAS, SDS
11. Nan *et al.* (2013)	FD/50(25): Rome III (PDS) HC/50(23): Age/gender matched	fMRI/resting	1) MVPA pattern classification 2) Correlation of impaired connectivity with NDI-QOL 3) Correlation of connectivity severity with behavior	NDI-symptom	NDI-QOL, SAS, SDS
12. Liu *et al.* (2013)	FD/30(10): Rome III (PDS) HC/30(11): Age/gender matched	fMRI/resting	1) MVPA pattern classification 2) Correlation of ROI with NDI-symptom, FD duration	NDI-symptom	SAS, SDS
13. Nan *et al.* (2014)	FD/40(11): Rome III (PDS) -FD less severe/20(5) -FD more severe/20(6) HC/20(8)	fMRI/resting	1) ReHo analysis 2) Correlation of ReHo with NDI-symptom	NDI-symptom	NDI-QOL, SAS, SDS

(Continued.)

Table 1 *(continued)*

No. Author (year)	Groups/*n* (male): Characteristics-subgroup/*n* (male)	Imaging modality/condition	Analysis	Behavioral measures	
				Somatic or FD symptom	Psychologic
			3) Seed-based FC 4) Pattern classification		
14. Li *et al.* (2014)	FD/24(8): Rome III (PDS) HC/24(9)	fMRI/Acu	Whole brain	N/A	N/A
15. Nan *et al.* (2015a)	FD/40(8): Rome III -AD/18(3) -non-AD/22(5) HC/20(6)	^{18}F-FDG PET-CT/resting	1) Whole brain 2) Correlation with SAS, SDS	Dyspepsia symptom	NDI-QOL, SAS, SDS
16. Nan *et al.* (2015b)	FD/25(6): Rome III HC/25(11): demographic information matched	fMRI/resting	1) Small world properties 2) Network efficiency 3) Nodal metrics	DSS	SAS, SDS

*Data from another study, †Overlapping sample. Acu, acupuncture; AD, anxiety and depression; (f)ALFF, (functional) amplitude of low-frequency fluctuations; CT, computed tomography; DMN, default mode network; DSS, dyspepsia symptom score; FC, functional connectivity; FD, functional dyspepsia patients; FDG, fluorodeoxyglucose; fMRI, functional magnetic resonance imaging; HC, healthy controls; ICA, independent component analysis; MVPA, multivariate pattern analysis; n= number; N/A, no answer; NDI, nepean dyspepsia index; No., study number; PDS, postprandial distress syndrome; PET, positron emission tomography; PHQ, patient health questionnaire; QOL, quality of life; ReHo, regional homogeneity; ROI, region of interest; SAS, Zung self-rating anxiety scale; SDS, Zung self-rating depression scale; SID, symptom index of dyspepsia; STAI, state-trait anxiety inventory; VAS, visual analogue scale.

groups (Group 1: Studies 1, 3, 4; Group 2: Studies 2, 5–16), on the basis of authors and affiliations. Group 1 focused on the central processing of visceral stimuli (by distention of a gastric balloon) in FD patients using PET in comparison to healthy controls (HC). The influence of moderating variables (anxiety, gastric sensitivity, and abuse history) on brain activity in FD subgroups (normosensitive/hypersensitive and abused/non-abused) was also investigated. Group 2 reported resting state activity (n = 10) and brain activity following acupuncture (n = 3) with fMRI. Group 2 applied several analysis methods for resting state activity, including whole brain, region of interest, correlation analysis with behavioral outcomes, functional connectivity, (functional) amplitude of low-frequency fluctuations ((f)ALFF), independent component analysis (ICA), multivariate pattern analysis (MVPA), regional homogeneity (ReHo), and topological brain network analysis. They also measured the resting state brain response before and after the acupuncture treatment, and during the acupuncture stimulation.

Participants

A total of 504 FD patients (460 of whom participated in the neuroimaging scan, 181 males) and 294 HC (120 males) were investigated. Twelve studies included FD patients between 20 and 30 years of age only, and the mean age of patients in the other four studies (in which the inclusion criteria for the age was not stated) ranged from 22.5 to 35.1 years. The mean duration of FD symptoms or diagnosis ranged from 15.25 to 82.78 months. Thirteen studies (all by Group 2) included FD patients who met the Rome III diagnostic criteria for functional gastrointestinal disorders,[2] and 10 of these studies contained postprandial distress syndrome patients only (one of the subgroups of FD patients in accordance with the Rome III criteria).

Five studies divided FD patients into subgroups (Studies 4–6, 13, 15). Among the three gastric distention studies, Study 1 included FD patients with visceral hypersensitivity, and Study 4 divided FD patients into normo- and hypersensitive or abused and non-abused groups. To identify the symptom-related functional brain activity, patients were divided into milder (or less severe) and severe (or more severe) groups in Study 5 and 13. In Studies 6 and 15, patients were divided by the score of anxiety and depression (AD). In Study 7, FD patients were randomly assigned into two groups for acupuncture and sham acupuncture treatment.

With the exception of Studies 1, 3, 4, 6, and 7, healthy volunteers were used in the other studies as

Table 2 Brain imaging data of frequently reported brain areas

	FD *vs* HC			FD subgroups	
	Resting	Sham distention	Other conditions	Resting	Distention>baseline
SI/SII	↑(5, 15) Interhemi FC↑(8)	↓(3)	↓Distention>baseline(3)	–	Normosensitive> hypersensitive(4)
PFC	↑(5, 10, 15) Interhemi FC↑(8) ReHo↑(13)	↓(3)	↓acupuncture(14)	Abused>non-abused(4) Severe>milder(5) AD>non-AD(6, inf) Non-AD>AD(6, sup/med)	Non-abused>abused(4)
OFC	↑(5, 15) ↓(2, 10) ReHo↑(13)	↓(3)	↓acupuncture(14)	–	
Insula	↑(5, 10, 15) Interhemi FC↑(8) fALFF↑(9)	↓(3)	↑acupuncture(14)	Severe>milder(5) AD>non-AD(6)	
ACC	↑(5, 10, 15) ↓(2) Interhemi FC↑(8) ReHo↑(13) FC with OFC↑(13) FC with insula, PFC↓(13)	–	↓acupuncture(14)	Severe>milder(5)	
Thalamus	↑(5, 10, 15) Interhemi FC↑(8) ReHo↓(13) FC with cerebellum(9), PFC(med, 13)↑ FC with insula, PFC (inf/mid/sup)↓(13)	–		Severe>milder(5) AD>non-AD(6)	
Hippo/amygdala	↑(15)	–	↑Sham>baseline(3)	Non-abused>abused(4)	Abused>non-abused(4)

ACC, anterior cingulate cortex; AD, anxiety and depression; fALFF, functional amplitude of low-frequency fluctuations; FC, functional connectivity; FD, functional dyspepsia patients; HC, healthy controls; Hippo, hippocampus; inf, inferior; intermehi, interhemispheric; med, medial; mid, middle; OFC, orbitofrontal cortex; PFC, prefrontal cortex; ReHo, regional homogeneity; sham, sham distention; SI(II), primary (secondary) somatosensory cortex; sup, superior; (), study number; ↑, greater than healthy controls; ↓, lower than healthy controls.

the control group for FD patients. In Study 1 and 3, the demographic, behavioral, and brain data of FD patients were compared with the HC of a previous study.[35] In Studies 4, 6, and 7, the data of FD subgroups without HC group were compared.

Imaging modality, analysis, and conditions

Functional magnetic resonance imaging is the most frequently applied brain recording technology (n = 8). This is followed by PET-CT (n = 5) and PET imaging (n = 3). PET and PET-CT studies conducted whole brain analysis and correlation analysis with behavioral data. Functional magnetic resonance imaging studies performed analyses of the whole brain, functional connectivity, (f)ALFF, ICA, MVPA, ReHo, and topological brain network analysis.

Behavioral and clinical outcomes

Fourteen studies reported behavioral and clinical outcomes, while two acupuncture studies (Studies 2, 14) reported brain imaging data only. The behavioral outcomes were classified into three categories: somatic symptom, FD symptom, and psychological outcomes.

Somatic symptom outcomes Somatic symptom outcomes were measured in three distention studies (Studies 1, 3, 4) as balloon distention threshold (pain or unpleasantness), gastric sensation, or on a visual analog scale for pain, discomfort, nausea, and bloating during distention. Gastric sensation during baseline, distention, and sham distention were higher in FD patients than in HC in one study, with lower distention pressure (Study 3). Gastric sensation was higher in the hypersensitive and the abused group than in the normosensitive and the non-abused group, respectively (Study 4). Distention pressure threshold was also lower in the hypersensitive than in the normosensitive group, but did not differ between the abused and non-abused groups.

FD symptom outcomes Functional dyspepsia symptoms were measured in twelve studies. The Nepean dyspepsia index (NDI) was reported in six studies (Study

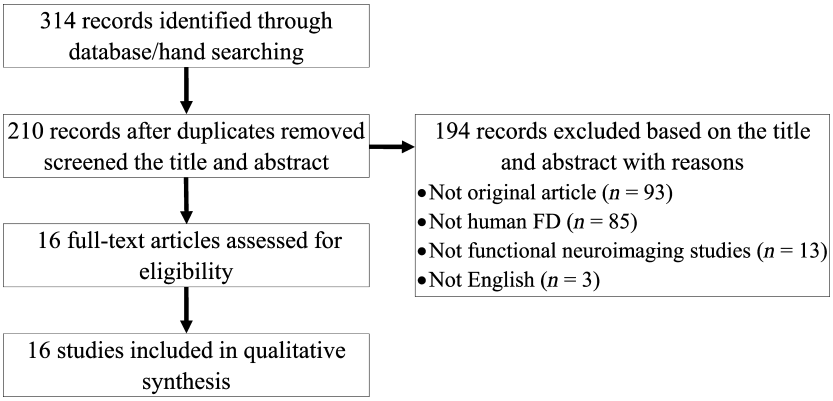

Figure 1 Flow diagram of literature search.

8–13), dyspepsia symptom score (DSS) in four studies (Studies 3, 4, 6, 16), the symptom index of dyspepsia (SID) in two studies (Studies 5, 7), and one study reported symptoms on a 4-item custom score (Study 15). Somatization severity was also measured with the Patient Health Questionnaire (PHQ-15) in Study 4. The NDI questionnaire, which was the most frequently used questionnaire for FD symptom assessment in this review, could record both FD symptoms and FD-specific QOL and was validated.[36,37]

Functional dyspepsia symptom scores were higher in FD patients than in HC. Functional dyspepsia symptom scores were also higher in the severe (or more severe) groups than in the milder (or less severe) groups and in the AD group than in the non-AD group, respectively (Studies 5, 6, 13). Both the AD and the non-AD groups showed higher symptom scores than HC (Study 15). The DSS and PHQ-15 scores correlated with gastric sensation during baseline, distention, and sham distention. PHQ-15 scores—but not DSS—were higher in the hypersensitive than in the normosensitive group (Study 4).

Functional dyspepsia symptom scores before and after acupuncture treatment are shown in Study 7. Symptom index of dyspepsia scores for postprandial symptoms and NDI-QOL scores improved after both acupuncture and sham acupuncture treatments, whereas SID scores for early satiety improved in the acupuncture group only.

Psychological outcomes The influence of psychological factors in FD symptoms was reported in all but the three acupuncture studies. Zung self-rating anxiety scale (SAS) and Zung self-rating depression scale (SDS, Studies 5, 6, 8–13, 15, 16), state-trait anxiety inventory (STAI, Studies 3, 4), PHQ-9 (Study 4), and the level of anxiety and tension during distention (Study 1) were reported. Quality of life (NDI-QOL, Studies 5–11, 13, 15), abuse history, and somatization (PHQ-15, Study 4) were also measured.

Although anxiety and depression scores were higher in FD patients than in HC (Studies 5, 8–12, 16), no differences were found between normosensitive and hypersensitive, or between abused and non-abused groups (Study 4). No differences were detected between the milder FD and severe FD patients in Study 5, but higher scores were observed in the more severe than in the less severe group (Study 13). Quality of life scores were higher in HC than in FD patients (Studies 10, 11), in the milder (or less severe) than in the severe (or more severe) group (Studies 5, 13), in HC than in the non-AD, and, finally, in the non-AD than in the AD group (Studies 6, 15).

Brain imaging data

Brain data can be divided into three main categories: resting state activity, activity following gastric distention, and activity with acupuncture. Resting state activity includes the results of resting state or baseline conditions in studies except Study 3, due to the balloon in the stomach during baseline. Activity following gastric distention includes brain response during the distention of a balloon in the stomach and sham distention (information about distention without actual distention). Activity with acupuncture refers to the brain activity before and after, or during the acupuncture and sham acupuncture. The activation of the most frequently reported brain areas, frontal cortex, somatosensory cortex, insula, ACC, thalamus, hippocampus, and amygdala, are summarized in Table 2. We made no distinction between the data of PET/PET-CT and fMRI, and the statistically significant results of each study are described with the corresponding *p*-values.

Resting state brain activities Ten studies (Studies 5, 6, 8–13, 15, 16) performed resting state brain imaging and one study (Study 2) reported baseline data. According to the resting state brain analyses, the activation of the PFC, somatosensory cortex, insula, and thalamus was

consistently greater in FD patients than in HC, while brain activities of the orbitofrontal cortex (OFC) and the ACC are inconsistent. The severe FD group showed higher activity in ACC, midcingulate cortex (MCC), insula, thalamus, and cerebellum than the milder FD group (Study 5). The AD group showed higher activity in SI, insula, thalamus, and parahippocampal gyrus, and lower activity in frontal cortices and MCC than the non-AD group (Study 6).

Functional dyspepsia patients showed higher interhemispheric connectivity (synchronized activity between the same brain areas in opposite hemispheres) of ACC, insula, thalamus, and cerebellum than HC (Study 8). Pattern classification analyses were also applied to distinguish FD patients from HC. Classification accuracy was sufficiently high, and discriminative regions were the medial PFC, OFC, ACC, MCC, insula, thalamus, hippocampus, and cerebellum based on MVPA pattern classification. Anterior cingulate cortex and thalamus distinguished FD from HC, or less severe from more severe FD, respectively (Studies 11–13). In a recent study (Study 16), a new approach to topological changes of the brain network revealed a higher clustering coefficient and local efficiency in FD patients than in HC. Furthermore, nodal efficiency in the ACC was found to be positively correlated with dyspeptic symptom and duration.

Seven studies performed correlation analyses between resting state brain activity and behavioral measures (Studies 5, 9–13, 15). A positive correlation between ACC activity and symptom score was observed in four studies (Studies 5, 10, 12, 13). Anxiety scores positive correlated with ACC, MCC, and insula in Study 15 only. Depression score and FD duration correlated poorly with brain activity in five studies (Studies 9–12 and 15).

Distention-related brain activities The three distention studies were conducted by Group 1 and therefore had similar balloon distention procedures (Studies 1, 3, 4). The ventral PFC, OFC, SI, and temporal lobe were commonly activated during the balloon distention. Significant correlations of upper abdominal sensations with these areas were reported (Study 1). Study 3 reported a deactivation during distention in dorsal PFC, medial OFC, ACC, hippocampus, amygdala, and several regions in the parietal, temporal, and occipital lobes in FD patients.

Group comparison of [distention>baseline] condition revealed that activity in the mid brain, cerebellum, and dorsal pons was greater, and activity in SI and SII was lower in FD patients than in HC (Study 3). The normosensitive group showed greater activation in SII, MCC, and precuneus than the hypersensitive group. Functional dyspepsia patients with an abuse history showed greater activation of the hippocampus, parahippocampal gyrus, and amygdala, and lower activation of dorsal PFC, insula, caudate, and cerebellum than the non-abused group (Study 4).

Sham distention did not elicit any brain (de)activation in FD patients (sham distention *vs* baseline), but in comparison with HC, FD patients showed higher hippocampus and amygdala activity under [sham distention>baseline] condition (Study 3).

Acupuncture-related brain activities The initial acupuncture study (Study 2) compared the resting state of FD patients before and after the acupuncture treatment. The second study (Study 7) compared the influence of acupuncture and sham acupuncture, while the third study (Study 14) compared the brain response during acupuncture stimulation in FD patients and HC.

After five sessions of manual acupuncture, brain activity in FD patients increased in PFC and precuneus, but decreased in SI, pons, and cerebellar tonsil (Study 2). After 20 sessions of electro-acupuncture, brain activity in FD patients increased in SI and precuneus, but decreased in anterior/mid/posterior cingulate cortex, insula, thalamus, putamen, hippocampus, and cerebellum (Study 7). The inconsistent results from these two studies might be due to the different characteristics of FD patients, stimulation type (manual or electro-), number of sessions, or comparatively small sample size. Correlation analysis showed that changes of ACC, insula, thalamus, hypothalamus are positively correlated with changes of symptom score, and negatively correlated with changes of QOL score in the acupuncture group. In the sham acupuncture group, changes of QOL score were negatively correlated with fewer areas than the acupuncture group that included thalamus and brainstem (Study 7).

During manual acupuncture stimulation at the acupoint ST36, FD patients showed greater brain activity in SI and insula, and lower activity in PFC, OFC, and ACC than HC (Study 14).

DISCUSSION

Sixteen articles were taken into consideration in this review and functional brain activity (resting state, visceral distention, acupuncture conditions) and behavioral/clinical outcomes were measured. The abnormal brain activity was frequently found in SI, PFC, insula, ACC, thalamus, hippocampus, and amyg-

dala. When compared to HC, FD patients showed greater activation in PFC, insula, cingulate cortex, and thalamus during the resting state, and altered activation in the somatosensory cortex, OFC, hippocampus, and amygdala during distention and acupuncture-related conditions. According to the pattern classification analysis, FD patients and HC could be distinguished using the activity pattern of ACC and thalamus. The behavioral data showed that FD patients experienced visceral hypersensitivity (low balloon distention threshold with higher gastric sensation/pain) during the balloon distention. Furthermore, the anxiety and depression scores were higher in FD patients than in HC, and QOL scores varied on the FD symptom severity, anxiety, and depression scores.

Visceral sensations are involved in the ascending visceral pain pathway and processed in the somatosensory cortex.[6,38,39] The somatosensory cortex activity during both the resting state and visceral distention was higher in FD patients than in HC. Moreover, brain activity during acupuncture (external somatic stimulation) also showed a greater increase in SI activity in FD patients than in HC, implying that the hypersensitive to somatic stimulation like IBS patients.[40] The increased activity of somatosensory cortex—even in the absence of visceral stimulation—could support the hypothesis of cortical sensitization in FD patients.[41,42] Central sensitization, increased brain response to various stimuli, may be one of the underlying pathophysiologic features in fibromyalgia, migraine, IBS, and FD patients.[42] Since FD is a chronic disease, somatosensory cortex receives afferent ascending visceral pain signals from internal organs repeatedly and consequent sensitization of the brain could result in an abnormal central modulation of sensory information and peripheral abnormalities such as visceral hypersensitivity. However, FD symptoms during resting state measurement, which could affect the brain activity, were not reported in any studies. The sensitization hypothesis therefore still requires further confirmation. Unlike the resting state activities, HC showed a higher somatosensory cortex activation than FD patients in [distention>baseline] and [sham distention>baseline] conditions. This could be due to the increased activity in resting state (ceiling effect), attenuated increase from chronic visceral sensation in FD patients, or different visceral distention pressure between groups (lower in FD patients). Although visceral distention pressure was lower in FD patients than in HC, this is not enough to explain the increase in somatosensory cortex activation in HC in [sham distention>baseline] condition. This is worth bearing in mind as evidence of abnormal sensory processing in FD patients. Further research on the sensitization or attenuation of brain activity during resting or internal/external stimuli is required to gain an understanding of sensory processing in FD patients.

The frontal cortex is associated with executive and integrative control functions. The integration of information from peripheral, cognitive modulation of pain (medial, dorsolateral), and appraise or response to affective aspect of pain sensation (medial, ventrolateral) are processed in the PFC.[5,43,44] Orbitofrontal cortex is also involved in cognitive pain modulation, inhibition of pain-related emotional response, sensory discrimination, and monitoring,[45–47] and is closely related to psychological disorders such as anxiety[48] and depression.[49] It is also associated with the endogenous opioid analgesia systems in conjunction with ACC.[15] The activation pattern of PFC is similar with somatosensory cortex during resting (FD>HC) and sham distention conditions (HC>FD), whereas PFC activity did not differ from HC during distention despite low distention pressure in FD patients. One may speculate that this is due to an overlapping influence of chronic ascending sensory processing, cognitive and descending pain modulation, attention, and anticipation for visceral sensation on the frontal cortex activity in FD patients. However, in contrast to the previous studies which showed the close connection between OFC and anxiety or depression,[48,49] the relation between psychological factors and OFC activity was not observed in the current review.

Insula, ACC, and thalamus were already in the focus of early functional gastrointestinal disease studies. The insula is involved in interoceptive processing, homeostatic function, emotion, affective state, and awareness.[10,50] In our review, the insula is activated during visceral distention in FD patients ([distention>baseline]), where it showed greater activation than in HC during baseline and sham distention in all but one study. This implies that the abnormal excitement of the insula could be derived by the residual influence of chronic visceral sensation, psychological state, homeostatic imbalance (supported by greater brain activation in FD than in HC in the baseline condition), and anticipation of distention (supported by comparison in the sham distention condition) in FD patients. ACC, one of the core regions of medial pain system, is particularly important for cognitive pain modulation, attention to pain, endogenous opioid system-related placebo analgesia, and regulating the affective component of pain experience.[13–15] Various analyses, including whole brain, interhemispheric functional connectivity, topological brain network, ReHo, classification, and correlation analysis reported the abnor-

mal functional activity of ACC in FD patients. It is feasible that the altered activity of ACC causes diminished central pain modulation and subsequent hypersensitivity in FD patients. Although various functions and distinct neuroanatomical regions within the insula and the ACC are not covered in this review, it is still important to note that the insula is not a homogeneous region,[51] and neither is the ACC.[52]

The role of the limbic area (hippocampus, amygdala, hypothalamus, parahippocampal gyrus, etc.) has been discussed in terms of pain sensitivity, stress, and anxiety in IBS patients.[53,54] In particular, the studies evaluated in this review reported a coactivation of hippocampus and amygdala in FD patients. Amygdala is associated with the emotional memories with hippocampus, emotional evaluation of sensory stimuli, recognition of emotion, and nociceptive pathway.[55–58] In FD patients, the activity of amygdala and hippocampus could be interpreted as the anticipation or response to the visceral stimuli, and the recall of previous negative memories (pain, unpleasant, anxiety, etc.). Furthermore, the volume and synaptic changes of hippocampus and amygdala by chronic pain[59,60] could also affect the functional activity in FD patients. Greater activity is recorded in these regions in FD patients than in HC during both sham distention and [distention>baseline] condition and in abused than in non-abused group, supporting the hypothesis that negative emotional memory influences brain activity in FD patients.

On the basis of our results, it can be assumed that the changes of brain activity, response to visceral stimulation, and cognitive state of FD patients are due to the repeated afferent signal from the periphery and failure of central pain modulation leading to the dysfunction of the pain (SI, SII, ACC, insula, thalamus, amygdala) and salience network (ACC and insula). The evaluation, integration, and response to salient stimuli were altered in chronic pain and IBS patients.[11,61–63] Moreover, a saliency of stimuli varies according to the emotional state and pathological condition. We can therefore also assume that the visceral sensation, dyspeptic symptoms, emotion, and cognitive processing of dyspeptic symptoms have different saliency in FD patients than in HC. In summary, we propose that FD, like IBS, can also be considered as the functional chronic pain syndrome in which pain and salience processing are impaired[64,65] and that the ACC and insula play critical roles in FD.[66] Constant sensory signal from the gut (bottom-up) and abnormal central modulation (top-down) of pain and gut functions might be key features of FD, showing that peripheral changes could originate from abnormal brain activities through the brain-gut axis (Fig. 2).

By summarizing previous neuroimaging studies, we also suggest further research of FD. Since only two research groups have performed functional neuroimaging studies in FD patients, this review could potentially be biased. In addition to the limited number of research groups, there is still a lack of appropriate tasks. In early studies, visceral distention was applied to patients and healthy participants to investigate the visceral sensitivity-related activation of the brain. However, balloon distention is invasive and further peripheral changes are also related to the FD. Different kinds of tests such as water load test or real food intake are therefore required. Although FD patients have increased dyspeptic symptoms after eating food,[67] neuroimaging studies during or after food ingestion are relatively rare compared to those on obesity or IBS patients. New paradigms to overcome the practical problems involved (e.g. discomfort and dyspeptic symptoms of patients during food ingestion, amount and type of food) could augment our understanding of FD. Furthermore, the only intervention in which neuronal mechanism in FD has been investigated is acupuncture, and careful interpretation is necessary due to the poor reliability and validity of sham acupuncture.[68] Although acid-suppressive drugs, prokinetic agents, antidepressants, and psychotherapy are prescribed for FD patients, various phenotypes of patients and unknown underlying mechanisms often disrupt the standardized treatment strategy. Neuronal mechanism studies could therefore be helpful. Moreover, the improved and unified methods of measuring the psychological factors in FD, such as more specific definition (e.g. trait or state anxiety, anxiety for symptom or experimental environment, anxiety of present or previous week) and well-structured interviews rather than self-rating questionnaires are also important.[69] It is also worth investigating further psychological, behavioral, and lifestyle factors, including somatization,[42,70] stress,[71,72] fatigue,[73] food behavior,[74] sleep behavior, and comorbidities (IBS, other functional pain syndrome diseases, anxiety, depression). Finally, a more representative sample of FD patients should be included in further studies. In this review, although diagnoses of FD usually depend on Rome III criteria, a number of studies did not describe the diagnostic procedure. Although the peak prevalence of FD is distributed around the middle age,[3] many studies included only patients in their twenties. Representative and homogenous sample recruitment, where age, symptom severity, comorbidities, and gender are taken into consideration, could improve the reliability of the research.[75]

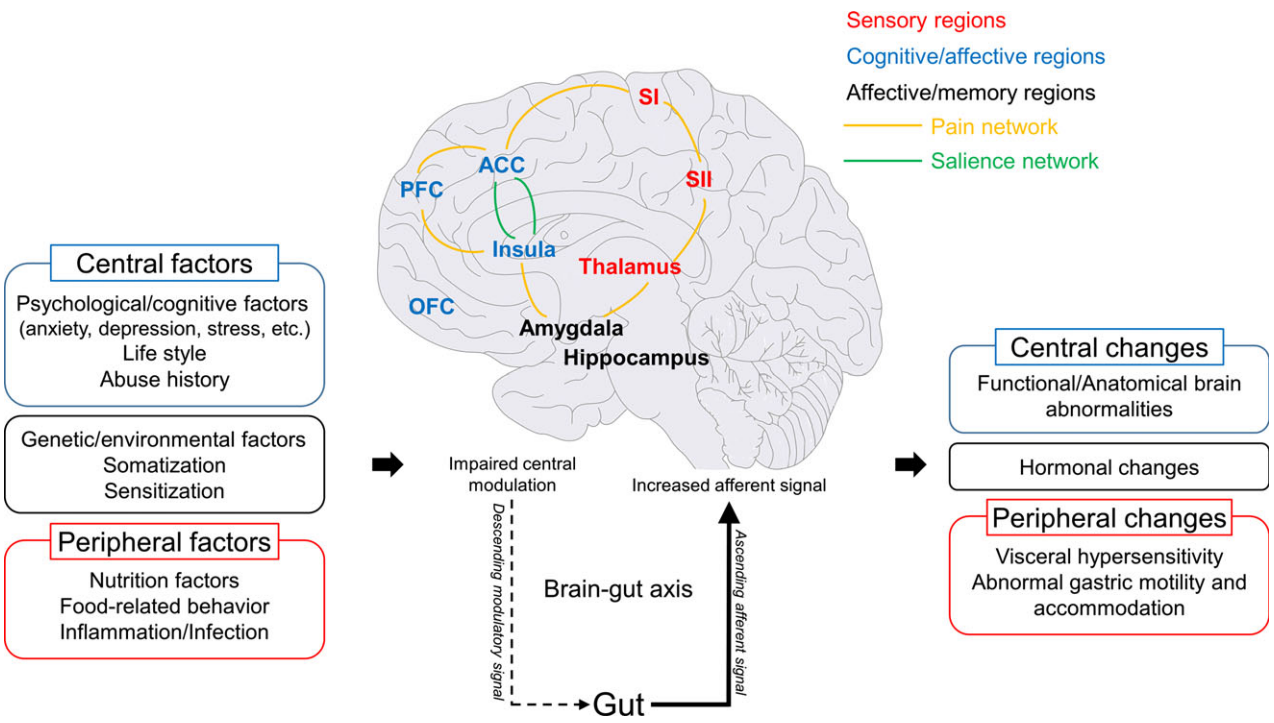

Figure 2 Pathological mechanisms of functional dyspepsia. Various factors involved in the brain, gut, and brain-gut axis in functional dyspepsia patients. Sensory, cognitive, and affective related brain regions showed altered functional activities in functional dyspepsia patients compared to healthy controls. Repeated visceral sensory signal from the gut (bottom-up) and abnormal central modulation (top-down) of pain and gut functions might be involved in functional dyspepsia. It also suggests that peripheral changes could be derived from abnormal brain functions through the brain-gut axis. ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; PFC, prefrontal cortex; SI (II), primary (secondary) somatosensory cortex.

In summary, in comparison to the body of research in patients with IBS, where much more data on both central and peripheral functions and genetic and microbiotic contributions allow to draw a complex network theory of the disease,[18] our current knowledge about brain activity in FD patients is still fragmentary. Whether brain activation is similar or different (in extent and brain areas activated/deactivated) between IBS and FD has never been studied (nor is it studied here). However, given the different symptoms between both functional gastrointestinal disorders, differences are liable to exist and even similar activations may be based on different peripheral or central processes.

CONCLUSIONS

The results of this review show the functional abnormalities of frontal cortex, somatosensory cortex, insula, ACC, thalamus, hippocampus, and amygdala, demonstrating the altered pain and salience network in FD patients. The chronic suffering from gastrointestinal symptoms, psychological problems, and subsequent abnormal brain functions could be the key clinical features of FD. As many pathogenic factors and physical changes of FD remain to be discovered, more diverse neuroimaging tasks, state-of-the-art interventions, precise diagnosis and measurement of psychological factors could improve our understanding of FD.

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CONFLICTS OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

PE designed the study; ISL performed the paper search, paper selection, data extraction; ISL, HP, YC, HW, and PE discussed the results and wrote the paper.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site:
Table S1 Search terms used in each database.
Table S2 Brain imaging data of functional neuroimaging in FD

6. Paper II. How to perform and interpret functional magnetic resonance imaging studies in functional gastrointestinal disorders

Author contributions

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Technique Review

How to Perform and Interpret Functional Magnetic Resonance Imaging Studies in Functional Gastrointestinal Disorders

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Functional neuroimaging studies have revealed the importance of the role of cognitive and psychological factors and the dysregulation of the brain-gut axis in functional gastrointestinal disorder patients. Although only a small number of neuroimaging studies have been conducted in functional gastrointestinal disorder patients, and despite the fact that the neuroimaging technique requires a high level of knowledge, the technique still has a great deal of potential. The application of functional magnetic resonance imaging (fMRI) technique in functional gastrointestinal disorders should provide novel methods of diagnosing and treating patients. In this review, basic knowledge and technical/practical issues of fMRI will be introduced to clinicians.
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Key Words
Brain; Functional magnetic resonance imaging; Functional neuroimaging; Gastrointestinal diseases

Introduction

Functional gastrointestinal disorders (FGIDs) are associated with functional and histological changes of gastrointestinal compartments such as gastric motility, visceral sensitivity, and inflammation. Our understanding of the underlying pathophysiological mechanisms is, however, limited. The advent and development of functional neuroimaging techniques in humans has facilitated the investigation of bottom-up processes—brain activations generated by signals from the periphery—and top-down processes—the effect of cognitive and psychological factors—in healthy volunteers. Functional neuroimaging is now recognized as an objective and accurate tool in the exploration of the central mechanism of functional disorders. Over the past few years, evidence from functional neuroimaging studies has endorsed the hypothesis that the dysregulation of the brain-gut axis (neuronal and hormonal interactions between the brain and the gut) is a key factor in FGIDs. According to previous reviews,[1,2] the functional alterations in sensory, emotional, pain-related, and homeostatic brain areas (changes of the brain function in frontal cortex, somatosensory cortex, insula, anterior cingulate cortex, thalamus, hippocampus, and amygdala) are the important

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pathogenic factors in FGIDs. Most present-day studies involve patients with irritable bowel syndrome (IBS) and functional dyspepsia (FD) and although several other functional neuroimaging methods are available, functional magnetic resonance imaging (fMRI) has proved to be the most frequently applied technique. Functional MRI is completely non-invasive, sensitive to task-related or non-task-related (resting state) brain activation, with high spatial (a few millimeters) and acceptable temporal (a few seconds) resolution, and facilitates deep brain structure and brain stem-imaging. Moreover, due to the availability of standard analysis tool boxes and tremendous advances in analysis methods, from univariate to multivariate analysis, fMRI has become increasingly popular in cognitive and clinical neuroscience studies.

In this review, we present the technical and practical issues of fMRI and show its application in FGIDs-related studies—with emphasis on IBS and FD patients—to improve clinicians' understanding of the merits of fMRI studies as well as of their possible limitations. Subsequently, we also propose future approaches in this field to further knowledge of FGIDs.

Brief Overview of the Functional Magnetic Resonance Imaging Technique

MRI has already been used to investigate tissue properties. In the 1990s, MRI was also deployed to measure the blood oxygen level dependent (BOLD) contrast in the investigation of functional activations in the brain.[3] Activation of neurons in the brain leads to the consumption of oxygen as well as to an increased flow of blood in the surrounding area (hemodynamic response). These changes result in magnetic field distortions in the brain tissue. To record these changes, the different relaxation times of the protons are measured by a constant magnetic field (nowadays, most fMRI systems use 1.5-7.0 Tesla, the strength of the constant field is a major determinant of the signal strength) and a superimposed gradient magnetic field. A BOLD fMRI signal (increased signal intensity of T2*-weighted images) is determined by a combination of blood flow, volume, and relative oxygenated hemoglobin level. The temporal signal recorded by BOLD fMRI (Fig. 1B) lies in the range of seconds and does not correspond directly to neuronal activity, but provides a hemodynamic proxy. For the analysis and interpretation of BOLD fMRI, the hemodynamic response function (HRF; Fig.

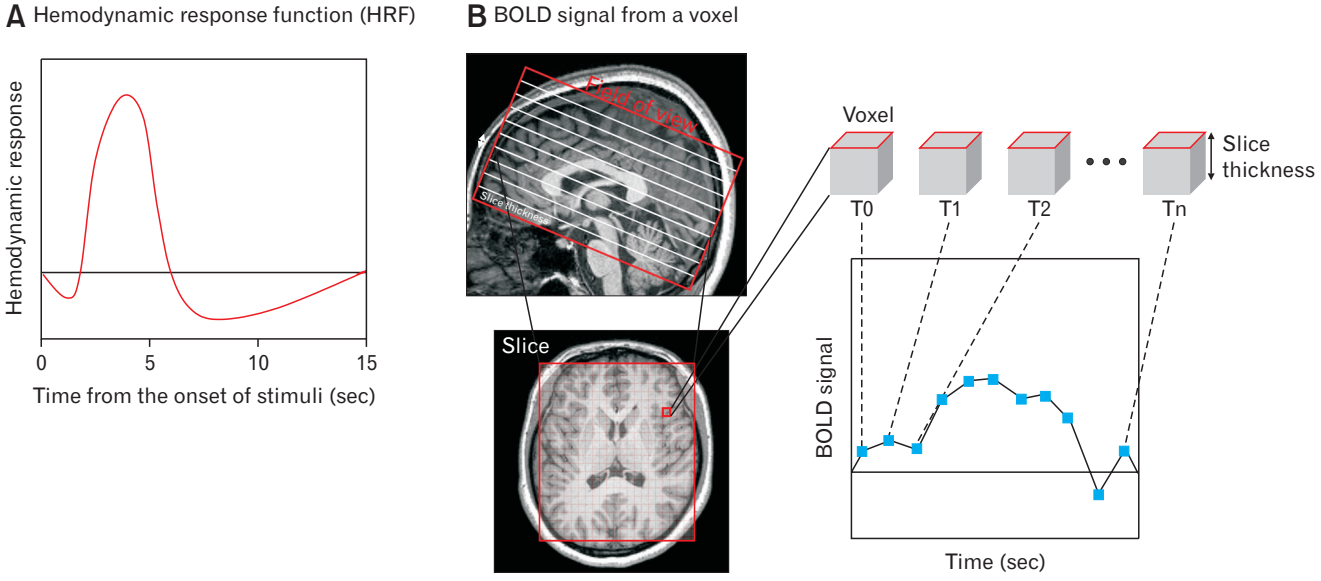

Figure 1. Example of hemodynamic response (A) and time series blood oxygen level dependent (BOLD) signal from a voxel (B). (A) Neurons respond rapidly to internal or external changes and allow the alterations of blood flow and oxygenation in the close area (hemodynamic response) that drives the peak of BOLD signal few seconds after the onset of internal or external changes. BOLD signal slowly returns to baseline level following an undershoot. (B) Within the field of view, each slice consists of a certain number of voxels determined by the size of the measurement matrix. The BOLD signal of each voxel is recorded at consecutive time points and this time trace is further analysed to interfere with functional brain activation.

1A) that describes the temporal derivative of the BOLD signal related to the neuronal activity must be determined. Most studies now use a homogenous HRF for the whole brain; a fixed model of temporal changes of BOLD signal due to the neuronal activity responding to external stimuli or changes of internal states, which peaks roughly 4-5 seconds after the neuronal event. HRF generates the anticipated BOLD signal which identifies the activation map of brain function (see below, Analysis of Functional Resonance Imaging Image section), and various methods have been proposed with which more spatially or temporally accurate HRF could be retrieved so as to improve fMRI analysis.[4,5]

To derive changes in neuronal activity, relative changes of signal intensity (contrast) are measured rather than absolute fMRI signal intensity. Furthermore, fMRI can be used to obtain not only the relative BOLD signal but also quantitative perfusion measurements. Arterial spin labeling is used to measure the cerebral blood flow by detecting the signal of magnetically labeled arterial blood.[6,7] The use of a quantitative measure enables us to more easily draw comparisons between studies. In this review, we will focus on BOLD contrast. Glossary of terms for fMRI is summarized in Supplementary Table.

How Is an Functional Magnetic Resonance Imaging Study Performed?

Design of an Functional Magnetic Resonance Imaging Study

Not all fMRI study designs are identical, and the designs are adapted depends on the type of research (basic/translational/clinical research, uncontrolled or controlled clinical trials, case reports, etc) and the purpose of the study. At present, most task-related study designs are either block (Fig. 2A) or event-related designs (Fig. 2B). Traditionally, various cognitive tasks, such as perception, attention, learning, memory, language skill, emotion, and motor related tasks, were applied in fMRI studies to identify the location or network of cognitive functions in the brain. However, interest in non-task-related brain activations, known as resting-state fMRI (rs-fMRI) in which participants' brain are imaged during resting without any specific tasks, has increased.

Task functional magnetic resonance imaging and resting-state functional magnetic resonance imaging

In early fMRI studies, fMRI signal responses to the repeated task (or stimulation) during a relatively short time interval were averaged and compared. For example, several blocks of Task A (or Stimulus A) and resting (no task; Fig. 2A, Example 1) or Task A,

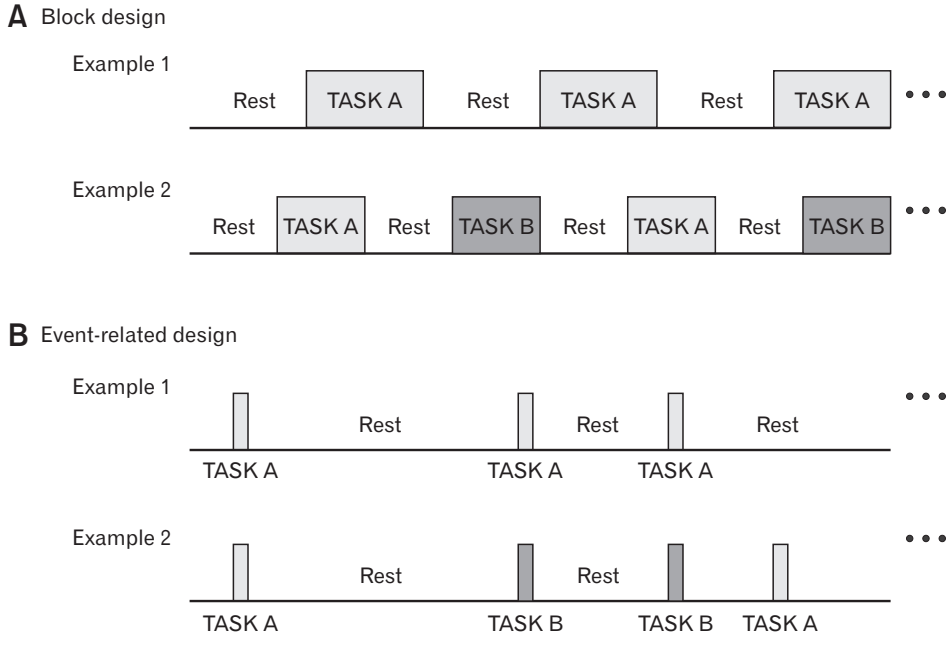

Figure 2. Examples of block design (A) and event-related design (B). (A) Example 1 shows the block design with a single task (Task A) and Example 2 with multiple tasks (Task A, B). (B) Event-related design with a single task (Example 1, Task A) and multiple tasks (Example 2, Task A, B). In both designs, the number of tasks and time durations are laid down in accordance with the type of task, hypothesis, and planned analysis scheme.

Task B (control condition), and resting (Fig. 2A, Example 2) are presented alternately. In the former case, averaged fMRI signals of blocks of Task A were compared to signals of blocks of resting to show Task A-related increase (Task A > resting) or decrease (resting > Task A) of BOLD signal in the brain regions. In the latter case, a comparison between the baseline-corrected signals during Tasks A and B revealed that different brain activities were associated with each task. In some cases, two different types of task are delivered simultaneously, eg, pain stimulation during the attention demanding task,[8] or the basic condition of participants, eg, hunger or satiety, could be modified.[9] Due to its comparatively high statistical power and large signal changes, block design is an efficient and sensitive method for detecting task-specific brain activations.[10,11] In a block-design fMRI study, a series of identical tasks (stimuli) are delivered in single block, whereas an event-related design measures the fMRI signal of each single task (stimulation). This approach improves the flexibility of the design by order randomization (which suppresses participants' prediction of the following task) or by post-hoc subgroup analysis (eg, correct vs incorrect tasks).

Design of functional magnetic resonance imaging studies in Functional gastrointestinal disorders

In fMRI studies, visceral distention is the most frequent stimulation performed on patients with FGIDs. The balloon distention method now consists of a bag-type balloon which is placed in an upper or lower gut compartment and distended (supra- or subliminally) by a barostat.[12] This measures the brain response to visceral stimulation in, for example, patients with IBS.[13-44] Auditory[22,45] and somatic pain stimuli[19,36] were also delivered to patients with IBS in fMRI studies. The results indicate that dysfunction of brain responses in patients is caused not only by visceral sensation but also by non-visceral stimuli, auditory and somatic pain. Cognitive tasks, such as affect matching paradigm,[46] Wisconsin card sorting test,[47] emotion recognition paradigm,[48] and attention network test,[49] have also been investigated in patients with IBS. Psychological factors such as anxiety and depression were also examined and correlated with brain activation or network parameters in IBS or FD patients. Moreover, fMRI results were reported as the primary outcome in case report[50] and clinical trials,[37,51,52] and brain responses to the treatment itself[37,53] were examined to ascertain the effect or neuronal mechanisms of pharmacological or non-pharmacological treatments (acupuncture, moxibustion, hypnosis, etc). In such cases, fMRI data were usually obtained before, during, and after the treatment (repeated measurements).

Resting-state fMRI has already been carried out in a number of studies with IBS[54-61] and FD patients[62-68] and its use continues to increase. Functional connectivity, (fractional) amplitude of low-frequency fluctuations ((f)ALFF), regional homogeneity (ReHo), independent component analysis (ICA), clustering, and graph theory analysis (see below, Advanced analysis) have been used as well as correlation analyses between the effect of adverse history, anxiety and depression, symptom severity, and the brain activity.

Analysis of Functional Magnetic Resonance Image

The initial goal of fMRI analysis was to identify voxels in the brain that show significant differences between tasks or against rest. In the history of fMRI analysis, great emphasis has always been placed on reducing noise and artifacts and on developing methods to deal with the multiple comparison problem caused by the large number of voxels. The localization of those specific brain regions activated during experimental conditions and its interaction with behavior and cognitive function data (task outcomes, physiological measurements, subjective ratings, questionnaire values, symptom severity, etc) were the primary goals of early fMRI studies (task-fMRI). A newly developed approach to fMRI analysis reveals patterns of fMRI signals such as temporal correlation-based functional connectivity, (f)ALFF, ReHo, ICA, clustering, and graph theory analysis in both task-based and rs-fMRI. For example, if a fluctuation of a time series signal of voxels corresponds to the timing of a certain task in task-based fMRI, then we can detect these voxels with general linear model (GLM). On the basis of the availability of the HRF and the known onset and duration of tasks, an anticipated BOLD signal could be generated (input function × HRF = expected BOLD response; Fig. 3A). The expected BOLD signal is utilized to estimate the task-specific activation of voxels. For example, in GLM, the linear relationship between observed (from voxels, dependent variable, blue signal in Fig. 3B) and expected (from HRF, independent variable, red signal in Fig. 3B) BOLD signal is estimated. The voxels whose observed BOLD signal corresponds significantly to the expected BOLD signal, as in Figure 3B, could be defined as the activated voxels following the task.

The sequence of any fMRI analysis is (1) preprocessing, (2) single subject analysis, (3) group analysis, and (4) additional analysis and visualization. A number of software programs and scripts have been developed for each step of an fMRI analysis. In general, statistical parametric mapping (http://www.fil.ion.ucl.ac.uk/spm/), FMRIB software library (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), analysis of functional neuroImages (https://afni.nimh.nih.gov/afni/),

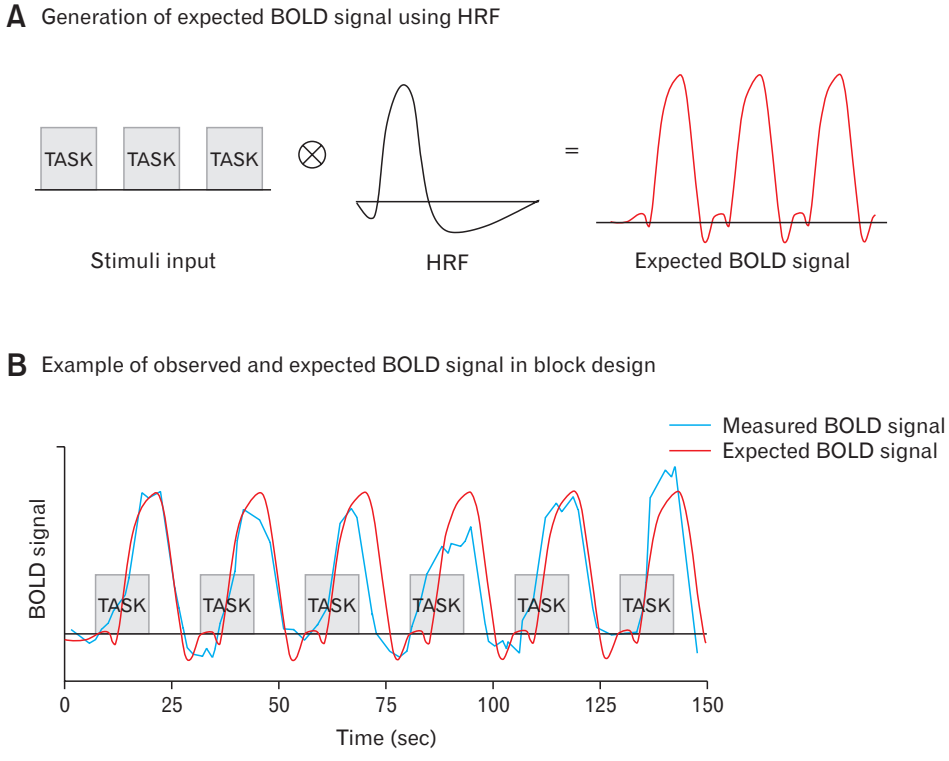

Figure 3. Illustration of expected and measured blood oxygen level dependent (BOLD) signal from single voxel in task functional magnetic resonance imaging. (A) Example of expected BOLD signal using hemodynamic response function (red). (B) Illustration of measured BOLD signal in task-specific activated voxel (blue) and simulated BOLD signal (red) from (A). In the general linear model, the linear relationship between observed (blue) and expected BOLD signal (red) is estimated.

BrainVoyager (http://www.brainvoyager.com/), and additional toolboxes for certain analysis are used. Since the terminology and the steps of analysis differ considerably between the various kinds of software, we will confine ourselves to describing the process of analysis on the basis of the BOLD signal analysis with statistical parametric mapping.

Statistical Power

As with other types of studies, it is prudent to perform a statistical power analysis before conducting the main fMRI study. To obtain an optimal statistical power (the probability of rejecting the null hypothesis when it is false), it is vital that the effect size and the sample size be taken into consideration. The size of effect is influenced by the sequence parameters, type of task, study design, inter/intra-variability of the sample data, and the sample size. The latter can easily be controlled by the experimenter. If the anticipated effect size is taken from pilot data or open source data from fMRI databases, a power analysis can be conducted before embarking on the main study to determine the optimal sample size.[69,70] Desmond and Glover[71] tested simulated fMRI data to estimate the statistical power. They ascertained that a minimum of 12 subjects is required to ensure 80% power at $\alpha = 0.05$ at the single voxel level and almost twice as many are necessary to achieve the same power level after multiple comparison correction. However, Yarkoni[72] claimed that the results in fMRI studies with a small sample size were overestimated and proposed that 50 is a reasonable sample size. At present, sample sizes below 20 are generally considered to be rather small.

Task Functional Magnetic Resonance Imaging

Preprocessing

Preprocessing is necessary to modify the recorded fMRI signal into statistical analyzable data by correcting artifacts and noise generated either by the MRI scanner (acquisition timing) or by participants (head motion, inter-participant variability in anatomical features).

(1) Slice timing correction (temporal preprocessing): the brain in the field of view is repeatedly scanned every few seconds and one scan image is composed of several slices (planar image) of the brain. In other words, the slices in one scan image are not collected concurrently (Fig. 1B). To increase the time-sensitive effects, all times series of each slice are adjusted to the acquisition time of one slice (reference slice).

(2) Realignment (spatial preprocessing): participants' head motions, which produce signal noise and voxel mismatch between scans, are corrected. Since larger movements (> 2 mm, > 2 degree

rotation) can produce significant non-amendable noise, slices with large head motion are usually discarded. Smaller movements can be corrected or the movement can be taken into consideration during the statistical analysis.

(3) Co-registration (spatial preprocessing): registration of an anatomical image to match the functional image is required for further analysis.

(4) Segmentation (spatial preprocessing): segmentation of an anatomical image to separate brain tissues, cerebral spinal fluid, white matter, and gray matter.

(5) Normalization: individual images are normalized into standard space to correct between subject variability. This step increases sensitivity, and facilitates the generalization of results and comparisons between studies.

(6) Smoothing: a smoothing filter, such as Gaussian kernel, is applied to blur the images and reduce the number of independent observations based on random field theory. This process suppresses noise, increases sensitivity, and makes images more appropriate for single-subject and group analysis.

Single subject and group analysis

In a single subject analysis, also known as subject level or first level analysis, design and contrast of all experimental conditions are defined. In order to specify the experimental design, information about the onset and duration of each task is required. F-contrasts (effects of interest) or T-contrasts (the contrasts between tasks or task and resting condition) are defined according to the design and purpose of the analysis. Movement parameters and other regressors are also determined in case they are required.

In group analysis, also known as second level analysis, t tests, ANOVAs and other general linear model analyses with covariates or regressors can be performed. In the event of a specific hypothesis about the correlation between the clinical symptoms, task performance, personality, or duration of the disease and brain activation, multiple regression analysis using covariates could identify those brain regions that positively or negatively correlate with the covariates. Contrasts for group analysis must also be defined to report group level results. In general, the analysis is performed as a whole brain analysis. For region-of-interest (ROI) analysis, the equipped ROIs in the toolbox library (Automated Anatomical Labeling atlas[73]) or newly generated ROIs using center coordinates and radius or number of voxels are used. A ROI-based approach should be used only if clear hypotheses are available and the multi-comparison correction should be taken into account if more than one ROI is used. Having set a statistical threshold and multiple comparison correction thresholds to correct false positives (family-wise error rate or false discovery rate is generally used), one can export the results into figures, tables, or time series signal data.

Resting-state Functional Magnetic Resonance Imaging

Once rs-fMRI data is preprocessed in a similar way to task-fMRI, procedures of single subject and group analysis differ from task-fMRI. In resting state analysis, the spontaneous low frequency fluctuation (0.01-0.10 Hz) is of major interest. Several approaches, including ALFF and (f)ALFF, were developed specifically for rs-fMRI analysis in an effort to extract an amplitude or ratio of spontaneous low frequency fluctuation from the BOLD signal, indicative of a regional intensity of activation.[74,75] Functional connectivity, ReHo, and ICA are also applicable in rs-fMRI as well as in task-fMRI. Further toolboxes and scripts for rs-fMRI were also developed.[76,77]

Advanced Analysis

Various advanced analyses have been introduced in fMRI analysis. Here, we briefly introduce the analysis technique which has been used of late in FGIDs studies.

Functional connectivity, one of the most widespread analysis techniques, is defined as 'temporal correlation between the different parts (voxels, clusters, or ROIs) of the brain.[38,44,56,57,78] It enables us to estimate the connection of brain regions and to compare its patterns between groups. Effective connectivity provides us with additional information as to which brain areas induce a direct causal influence over others.[48,51,79] Dynamic causal modeling is an example of the effective connectivity analysis method and shows how the effective connectivity (causal influence) between brain regions is modulated by experimental conditions.[47,80] Graph theory analysis, ie, the analysis of the properties of connections (edges) between functionally connected brain regions (nodes) to account for the complex characteristics of a network, is a further form of connectivity analysis.[61,68,81] ReHo is basically a voxel-based connectivity analysis that measures the regional similarity of the signals between the specific voxel and its neighboring voxels.[59,67,82]

Of all the multivariate analyses applied in FGIDs studies, ICA pattern classification is the most familiar.[29,38,58] ICA works on the assumption that an fMRI signal is linearly composed of several (spatially or temporally) independent signals, and that the original fMRI signal is separated into independent groups.[83] Since ICA is one of the data-driven analysis methods, it can reveal an intrinsic structure of the original signal and can therefore also be utilized to generate hypotheses.

Interpreting Functional Magnetic Resonance Imaging Results in Functional Gastrointestinal Disorders

In most studies, the list of brain regions (coordinates and statistical information) displaying increased or decreased activity in certain conditions or groups is reported in a voxel-wise or a ROI-wise manner. In some instances, a group of the brain areas involved in the same function (eg, pain processing) is identified as a 'network.' For example, albeit opinions are deeply divided on this issue, somatosensory cortex, insula, anterior cingulate cortex, and thalamus are termed a 'pain network.'[84] The most frequently reported brain regions in FGIDs studies are the prefrontal cortex, somatosensory cortex, insula, cingulate cortex, and thalamus. The contributory networks to FGIDs are known as the sensory-motor network, salience network, autonomic network, and cognitive/affective network.[1,85]

Functional MRI data may allow us to elucidate the basic neurophysiological and pathophysiological mechanisms in brains which is associated with clinical information. For example, the activation map following rectal balloon distention can indicate the altered neural processing of visceral pain in the somatosensory cortex, frontal cortex, cingulate cortex, insula, thalamus, and (pre)motor cortex with higher pain sensation (visceral hypersensitivity) in patients than in controls.[15,17] Anxiety and depression were associated with the brain activation in the cingulate cortex and prefrontal cortex,[28] and history of abuse affected the brain activation in the cingulate cortex.[27] Several studies have attempted to identify the specific mechanisms of treatment[86] and neuroimaging biomarkers for further disorders.[87] The inhibition effect of pain-related brain activation in IBS patients by amitriptyline (tricyclic antidepressants)[20] identified the central mechanism of antidepressants in the reduction of rectal distention pain. The brain activity during acupuncture suggested the modulation of serotonin pathway at insula and the higher cortical regulation of affection as potential neural mechanisms of acupuncture treatment.[34] Furthermore, correlation analysis between fMRI data and psychological indices such as anxiety and depression may demonstrate the influence of the psychological state on patients.[28,35] When interpreting the fMRI results on interventions, the blinding issue, changes of symptoms, co-morbidities, quality of life, non-specific effect, and placebo response should also be taken into consideration carefully.

Limitations and Future Approaches of Functional Magnetic Resonance Imaging Studies in Functional Gastrointestinal Disorders

Functional MRI measurement is not only expensive and time consuming, but also requires extensive skills and resources. Researchers should be aware of the variety of factors which affect the brain imaging results before performing experiments, and it is only when valid tasks or stimuli, well-structured procedures, controlled populations of participants, and proper analyses come together that reliable data can be gained. The unusual environment of MRI must also be taken into consideration. Patients with a metal implant or with claustrophobia should not participate. No movement, particularly no head movement, is permitted inside the scanner. Recent studies have demonstrated in both IBS and in healthy controls that visceral pain perception is higher within the MRI environment than outside.[88] Investigators and participants must therefore adapt themselves to the MRI environment.

Until now, all neuroimaging studies in FGID have used a correlation approach. This does not permit us to make any causal inference about the direction of influence (central to peripheral, peripheral to central, or both). At present, inconsistent study designs, analysis methods and statistical principles make it difficult to compare or integrate fMRI data in FGIDs across studies using meta-analysis. However, because FGIDs lack biomarkers such as neurohormones, cytokines, and genes, functional neuroimaging may provide further information to elucidate the symptoms in patients. Furthermore, fMRI studies may help us to better fathom the role of emotional feelings and cognitive functions by combined with other neuroimaging techniques or with autonomic response, genetic and epigenetic approaches, and neurotransmitter research to identify key components of the disease, or to differentiate between subtypes.

In summary, fMRI is a unique research tool that provides information on neuronal mechanisms of symptoms and treatment effects in the patient population, and physiological processing in healthy volunteers. It should, however, be utilized prudently in research, and its pros and cons should be weighed up carefully.

Since neuroimaging has been applied in FGIDs for less than twenty years and analysis methods are developing and improving rapidly, future approaches hold tremendous potential. As yet, only experimental pain stimulation and a few cognitive tasks have been implemented in FGIDs patients. Besides the pain and anxiety/depression scores, FGIDs patients may have many other pathological, behavioral and somatic characteristics; such as impaired affective

memory, heightened vigilance, abnormal eating behavior, increased stress sensitivity, disordered autonomic regulation, dysbiosis of the gut microbiota, additional bowel symptoms such as nausea, bloating, urgency, and autonomic and somatic co-morbidities. It may be advisable to examine the effects of pharmacological or non-pharmacological therapy, and the influence of such therapies on brain activity may help to establish novel treatment strategies. Albeit still a far cry from clinical application, neuroimaging data will nevertheless one day be used to perform subgroup analyses in patients (eg, hypersensitive vs normosensitive or even hyposensitive patients) or to distinguish patients from healthy controls.[89] The neuroimaging data with more numerous tasks, behavioral measurement, and therapies could improve our understanding of the pathophysiology of FGIDs and lead to more appropriate treatment options for patients in the future.

Conclusions

The advent of the fMRI technique has not only provided information on regional brain activities and the interaction of different brain areas, but has also improved our understanding of the neuronal changes and its relationship with symptoms and cognitive/affective changes in many patient groups. Although its usage in basic or clinical neuroscience research in FGIDs patients has been reported in only a limited number of studies, and despite its requiring an intensive level of knowledge in neurology, physiology, pathology, physics, and program coding, it does have considerable potential. An accurate understanding and application of fMRI technique in FGIDs will hopefully lead to new methods of diagnosing and treating patients.

Supplementary Material

Note: To access the supplementary table mentioned in this article, visit the online version of *Journal of Neurogastroenterology and Motility* at http://www.jnmjournal.org/, and at https://doi.org/10.5056/jnm16196.

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7. Paper III. Attentional and physiological processing of food images in functional dyspepsia patients

Author contributions

The material of this chapter was submitted to *Scientific reports (2017 July)*. All authors designed the study and interpreted the results together. In-Seon Lee acquired and analyzed all the data with the help of Katrin Giel and Kathrin Schag. Results were discussed with the help of Hubert Preissl, Katrin Giel, Kathrin Schag, and Paul Enck. In-Seon Lee wrote the manuscript. Hubert Preissl, Katrin Giel, Kathrin Schag, and Paul Enck revised the manuscript.

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Attentional and physiological processing of food images in functional dyspepsia patients

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Abstract

The food-related behavior of functional dyspepsia have been attracting more interest of late. This study aims to provide evidence of the physiological, emotional, and attentional aspects of food processing in functional dyspepsia patients. The study was performed in 15 functional dyspepsia patients and 17 healthy controls after a standard breakfast. We measured autonomic nervous system activity using skin conductance response and heart rate variability, emotional response using facial electromyography, and visual attention using eyetracking during the visual stimuli of food/non-food images after standard breakfast ingestion. In comparison to healthy controls, functional dyspepsia patients showed a greater craving for food, a decreased intake of food, more dyspeptic symptoms, lower pleasantness rating of food images (particularly of high fat), decreased low frequency/high frequency ratio of heart rate variability, and suppressed total processing time of food images. There were no significant differences of skin conductance response and facial electromyography data between groups. The results suggest that high level cognitive functions rather than autonomic and emotional mechanisms are more likely to function differently in functional dyspepsia patients. Abnormal dietary behavior, reduced subjective rating of pleasantness and visual attention to food should be considered as important pathophysiological characteristics in functional dyspepsia.

Keywords functional dyspepsia; eye-tracking; food images; fat

1. Introduction

Functional dyspepsia (FD) is defined as a disorder that includes unexplained symptoms originating from the gastroduodenal region such as postprandial fullness, early satiation, epigastric pain and burning[1, 2]. So far, our knowledge of pathophysiological abnormalities in FD had been limited to functional abnormalities in gastrointestinal tract (visceral hypersensitivity, abnormal accommodation, delayed gastric emptying and gastric dysmotility), and only a small number of studies had investigated the psychological characteristics of FD patients and revealed the crucial role of anxiety, depression, and somatization[3].

More recent studies investigating the role of dietary habit and nutritional intake in FD patients suggest that fat ingestion is a potential factor in symptom triggering[4-6]. Although it is already known that FD patients tolerate only small amounts of food, evidence on the extent of nutritional intake of daily meals remains inconclusive[7]. One of the limitations of previous studies in FD patients was that a food diary or questionnaire was used to measure their dietary habit, which may have caused a recall bias[8-10]. In studies using real food in FD patients, specific amounts of solid or liquid type meals were served to determine the meal-related dyspeptic symptom, gastric accommodation, or hormonal changes[11-14]. Furthermore, the psychophysiological response and cognitive processing of food stimuli in FD patients are not well established despite the fact that these are important determinants in the pathophysiology of eating disorders such as anorexia nervosa, binge eating disorder, and obesity[15, 16]. Since a close relationship between the exacerbated FD symptoms and meal ingestion has been reported[11-14], chronic negative experience of eating may cause abnormal behavioral and cognitive response, i.e., avoidance or an aversive response rather than a positive approach to food stimuli.

Visual food stimuli have been used to investigate food-related behavior in patients with obesity[17], anorexia nervosa[18], and binge eating disorder[19]. Food images are generally used as pleasure or incentive stimuli eliciting a positive response or causing an attentional bias[20]. The eye tracking technique, which measures the gaze parameters such as initial fixation and total duration of fixation on images[21], is well suited to the investigation of initial saliency and the later cognitive processing of images. In addition, autonomic nervous system function and facial movements can provide further support for altered homeostatic and emotional changes during food image processing in FD patients. Skin conductance response (SCR) and heart rate variability (HRV) have been used as the parameter of the arousal level of the sympathetic branch of autonomic nervous system and of the balance of sympathetic and parasympathetic activity, respectively. Electromyography of facial muscles measuring the intensity of the contraction of the corrugator supercilii and zygomaticus major muscle has been used to quantify negative and positive facial emotional response[22].

In the current study, we aimed to determine the physiological/emotional response and visual attention to food images in FD patients. We evaluated the physiological, emotional, and attentional response of FD patients to high fat food, low fat food, and non-food images after taking an ad-libitum breakfast. We hypothesized that, in comparison to healthy controls, 1) FD patients consume a smaller amount of food, but have higher dyspeptic symptoms afterwards; 2) FD patients show negative emotional response and increased arousal level to food images, particularly to high fat food images; 3) FD patients show decreased visual attention to food images, especially to high fat food images.

2. Methods

2.1. Participants

15 FD patients (3 male, aged 41±4.72 years) and 17 age- and BMI-matched healthy controls (HC, 5 male, aged 39.65±4.02 years) were included in the study. The age range was 18-75 years and body mass index (BMI, weight/height^2) range 19-29 kg/m^2. FD patients were diagnosed on the basis of ROME III criteria[23] and an unsuspicious endoscopy documented in their medical records. Participants with visual impairment, severe psychiatric illness, intake of antidepressants or antipsychotics, and any food allergy or intolerance were excluded. The study was approved by the ethics committee of the Medical Faculty, Tübingen University, Germany (041/2016BO2). All participants provided informed consent and all experiments were conducted ethically according to the principles of the Declaration of Helsinki.

2.2. Procedure

Study was conducted at the Universitätsklinikum Tübingen, Tübingen, Germany. Participants were asked to fast from 10 pm of the evening prior to the study. The study commenced at 8 a.m. the following morning. The participants began by rating their physical condition such as hunger, appetite, abdominal fullness, satiation, nausea, vomiting, abdominal pain, abdominal discomfort, burning, and bloating symptoms (baseline) on a visual analogue scale (VAS; 0=not at all, 10=very much). They were then served a standard breakfast consisting of bread (2 slices, 110g), butter (36g), jam (46g), milk (1.5% fat, 500ml), orange juice (500ml), and water (total calorie 402.09kcal, fat 14.52g, carbohydrate 53.61g, protein 12.98g). The participants could eat as much as they wished within 10 minutes. VAS ratings were assessed again immediately after breakfast (Post1), between **Experiment 1** and **Experiment 2** (Post2, 20-25 minutes after the meal), and at the end of the experiment (Post3, 45-50 minutes after the meal, Figure 1A.). The remaining food from each participant was weighed and calorie intake was calculated.

Experiment 1. Emotional and physiological response to food and non-food images

Skin conductance response was measured with two electrodes attached to the index and middle finger of the left hand. Three electrodes were placed on the chest region to measure the electrocardiography (ECG) signal. For facial electromyography (EMG) measurement, three electrodes were attached on both the corrugator supercilii and zygomaticus major muscles on the left side of the face[24]. The data were recorded with a Biopac MP36 system and Acknowledge software ver. 4.1 (Biopac Systems Inc., Goleta, USA).

Five fixed-order sets of image stimuli (neutral, positive, negative, high fat, and low fat food images, n=30, respectively), were each presented in a randomized order for 180 seconds (6 seconds for each image) followed by 5-second rest with visual cross fixation between each set. Subjective

pleasantness to each image was measured by pressing a button from 1 to 10 on a keyboard (1=very unpleasant, 10=very pleasant). Participants were also requested not to move or talk while the measurements were being carried out (Figure 1.B.).

Experiment 2. Visual attention to food and non-food images

After **Experiment 1,** gaze data were recorded with the eye tracking system iView X Hi-Speed (SensoMotoric Instruments GmbH, Berlin, Germany). Each participant received a standardized 13-point calibration procedure to ensure optimal gaze data quality. Following calibration, 24 pairs of images (different from those used in **Experiment 1**) composed of food (high fat n=12, low fat n=12) and non-food images (household items, n=24), were randomly presented. Each pair of stimuli images was presented for 3 seconds and a fixation cross at the center of the screen was shown for 2 seconds between each pair. Participants were requested to freely explore the presented pictures and to fixate the cross when shown (Figure 1.C.).

After the eye tracking experiment, the anticipated FD symptoms (postprandial fullness, early satiation, abdominal pain, and burning sensation) at each food image (high fat n=12, low fat n=12) was assessed using VAS (0=not at all, 10=very much). At the conclusion of the study, each participant's dyspepsia symptom intensity and disease-related quality of life were assessed using Nepean Dyspepsia Index (NDI)[25]. Depression and anxiety levels were evaluated using Beck Depression Inventory (BDI-II)[26] and State-Trait Anxiety Inventory (STAI)[27], respectively. Furthermore, the Eating Disorders Examination questionnaire (EDE-Q)[28], Food Craving Questionnaire (FCQ)[29], and Fat Preference Questionnaire (FPQ)[30] were used to identify eating behavior.

2.3. Materials and apparatus

Experiment 1. Positive and negative images were selected from the International affective picture system (IAPS)[31]. Taking the diversity of food and color-matching between food and non-food images into consideration, we selected neutral household items and food images from food image databases[32]. Images were presented and subjective pleasantness rating was recorded with Presentation® (version 16.5, www.neurobs.com). Physiological signals were recorded with a Biopac MP36 system and Acknowledge software 4.1 (Biopac Systems Inc., Goleta, USA). SCR, ECG and EMG signals were sampled at 1 kHz. For SCR, a low pass filter of 10Hz, for ECG a bandpass filter between 0.5 and 35 Hz, and for EMG a bandpass filter between 30 and 250 Hz were applied.

Experiment 2. A validated image set for the eye tracking experiment was used in this study[33]. The food and non-food stimuli were matched in color, brightness, and contrast. The complexity, valence, and arousal levels of the images were rated in a previous study[34]. Eye movements were recorded with the IViewX Hi-Speed and IViewX 2.8 software (SensoMotoric Instruments, Berlin, Germany) and sampling rate was set at 500 Hz.

2.4. Data processing

Experiment 1. We analyzed the raw SCR signal using Ledalab software (www.ledalab.de). Raw SCR was smoothed and event-related activation was extracted when the signal exceeded 0.01muS. For the computation of the standardized ratio, the total amplitude of SCR of each block (neutral, positive, negative, high, and low fat cues) was divided by the total amplitude to normalize individual differences.

Rectified EMG was derived from raw EMG data, while integrated EMG was defined as the area under the curve of the rectified EMG signal. Muscle activation was located every 30ms automatically and visually ascertained. To calculate the standardized ratio, we divided the whole EMG signal from all muscle activations located in each block by the total amplitude (Acknowledge software 4.1, Biopac Systems Inc., Goleta, USA).

ECG was analyzed with Kubios HRV software (version 2.2, http://kubios.uku.fi/)[35]. Following QRS detection, a medium level of artifacts correction was applied and trend components were removed using the smooth priors method (Lambda 500, f=0.035 Hz). Frequency bands were set at 0.04-0.15 Hz for LF (low frequency) and at 0.15-0.4 Hz for HF (high frequency), and Fast Fourier Transform-based power spectrum estimation was applied.

Experiment 2. The raw gaze data was analyzed using BeGaze 3.0 software (SensoMotoric Instruments GmbH, Berlin, Germany). The areas of interest (AOIs) were defined for food and non-food images and fixation cross. The initial fixation position was defined by the geographical position of gaze on AOIs and fixation duration was calculated as the sum of the duration time of fixation inside each AOI[36]. Any trials in which participants did not fixate on the cross at the onset of the trial were ruled out. Two variables were defined to test the hypothesis: 1) the coefficient percentage (%) of initial fixation on food versus non-food images: (number of initial fixations on

food images–number of initial fixations on non-food images)/(number of initial fixations on food images+number of initial fixations on non-food images)*100 (%), 2) the coefficient percentage (%) of total fixation duration on food versus non-food images: (fixation duration on food images–fixation duration on non-food images)/(fixation duration on food images+fixation duration on non-food images)*100 (%). All authors had access to the study data and reviewed and approved the final manuscript.

2.5. Statistical analysis

All statistical analyses were performed with IBM SPSS statistics 24.0 (IBM Corp. New York, USA). Independent two sample t-tests were used to assess any differences between study groups (FD and HC) that were related to anthropometric data, food consumption data, and questionnaire scores. A two-way repeated measures analysis of variance (ANOVA) with the groups (FD and HC) and the time factors (baseline, Post1, Post2, Post3) was used to identify the changes in meal-induced FD symptom ratings. A two-way ANOVA with the group (FD and HC) and type of image (2x5: neutral, positive, negative, high fat food, low fat food; 2x2: high fat, low fat food) factors were applied to the pleasantness rating, SCR, EMG, HRV variables, eye tracking data, and anticipated FD symptom rating. Two-tailed partial Pearson correlation analysis was also computed between BMI, fat and total energy intake, NDI_Symptom- and NDI_QOL (quality of life) scores, BDI-II, STAI, FCQ, and FPQ scores, and the eye-tracking variables while controlling for age and BMI. The statistical significance level was set at $\alpha=0.05$ and Bonferroni correction was applied to account for multiple testing if necessary.

3. Results

3.1. Sample characteristics

Sample characteristics and scores of questionnaires are presented in Table 1. No significant differences in age and BMI between groups ($p>0.5$) was shown. Eleven FD patients showed both postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) (9 females), 3 patients (all females) had PDS only, and 1 patient (male) had EPS only. FD patients showed a significantly higher NDI_Symptom score ($P<.001$) and a lower NDI_QOL score ($P<.001$) than the HC group. FD patients also showed significantly higher depression and anxiety levels ($P<.05$ and $P<.01$, respectively) and higher FCQ-state score ($P<.05$) than HC. The total and subscale scores in EDE-Q and FPQ did not differ significantly between groups.

3.2. Food/energy intake and FD symptoms

Following overnight fasting, FD patients ate significantly less bread than HC group (FD: 61.6±5.07g; HC: 76.71±6.95g, $P<.05$). Albeit FD patients consumed less fat (FD: 9.25±1.12g; HC: 10.38±1.63g), carbohydrate (FD: 51.85±6.61g; HC: 62.53±6.60g), and protein (FD: 8.56±1.64g; HC 9.27±1.36g) than the HC group, these differences were statistically not significant. Overall, FD patients consumed significantly less total energy than the HC group (FD: 332.19±37.77kcal; HC 389.76±38.03kcal, $P<.05$).

FD symptom ratings of baseline, Post1, Post2, Post3 are described in Supplementary Table 1. Hunger rating decreased immediately after breakfast, and increased subsequently in both groups. Appetite also decreased initially and increased gradually afterwards, but FD patients had significantly lower appetite at Post3 than HC ($P<.05$). Abdominal fullness in the FD group was only slightly higher than HC before breakfast, but significantly higher immediately after breakfast

(P<.05). Significant main effects of group (FD>HC) were found in abdominal pain (P<.05), discomfort, burning, and bloating symptoms (P<.01).

Table 1. Baseline characteristics of the study sample

	Healthy controls	FD patients	P value
Gender (m/f)	5/12	3/12	-
Subgroup	-	PDS: 2/12, EPS: 3/9	-
Age (year)	37.65±4.02	41±4.72	NS
BMI (kg/m^2)	24.95±0.73	23.27±1.19	NS
NDI_Symptom	10.56±1.90	70.62±9.51	P<.001
NDI_QOL	46.8±1.35	23.62±2.33	P<.001
EDE-Q Total	1.25±0.26	1.05±0.29	NS
Restraint scale	1.07±0.28	0.84±0.23	NS
Eating concern	0.33±0.14	0.31±0.15	NS
Weight concern	1.31±0.32	1.13±0.34	NS
Shape concern	1.58±0.35	1.45±0.33	NS
BDI-II	3.94±1.61	9.77±2.44	P<.05
STAI_state	31.06±1.64	43.46±3.21	P<.01
STAI_trait	31.81±2.06	44.54±3.34	P<.01
FCQ_state	31.94±3.09	42.93±3.31	P<.05
FCQ_trait	83.94±7.28	92.08±9.90	NS
FPQ_TASTE	55.89±5.26	65.98±4.81	NS
FPQ_FREQ	52.10±5.05	57.26±4.83	NS
FPQ_DIFF	3.79±1.73	8.72±2.23	NS

Mean±standard error

BDI: Beck depression inventory; BMI: body mass index; EDE-Q: Eating disorder examination questionnaire; EPS: epigastric pain syndrome; f: female; FCQ: Food cravings questionnaire; FD: functional dyspepsia; FPQ: Fat preference questionnaire; FPQ_TASTE: how much better high fat food taste, FPQ_FREQ: how much high fat food eaten more often, FPQ_DIFF: high fat restriction (FPQ_TASTE-FPQ_FREQ); m: male; NDI: Nepean dyspepsia index; NS: statistically not significant; PDS: postprandial distress syndrome; QOL: quality of life; STAI: State trait anxiety inventory

P value: two sample t-test FD vs HC

3.3. Experiment 1. Measurement of physiological response

Physiological response and pleasantness ratings of food and non-food images in FD patients and HC are summarized in Supplementary Table 2.

Pleasantness rating: ANOVA analysis for the 5 image sets showed that there was a significant main effect of image ($P<.001$). In accordance with the post-hoc analysis, pleasantness of negative images was significantly lower than of any other images ($P<.001$). Pleasantness of high fat food images was significantly lower than of positive images ($P<.001$). Low fat food images and positive images were rated significantly higher than neutral images ($P<.001$) in both groups. Subsequent analysis on high fat and low fat food images showed significant main effects of group and image ($P<.05$). Pleasantness ratings of food images in FD were significantly lower than in HC, and pleasantness of high fat food images was rated significantly lower than that of low fat food images in FD ($P<.05$).

SCR: ANOVA analysis for 5 image sets resulted in a significant main effect of image ($P<.001$). Post-hoc analysis showed that, in both groups, SCR standardized ratio for negative images was significantly higher than for other image (vs neutral, positive, high fat images, each $P<.001$; vs low fat images $P<.01$). There were no significant differences between groups for either ANOVA.

EMG corrugator supercilii: ANOVA analysis for 5 image sets showed a significant main effect of image ($P<.001$). Post-hoc analysis showed that the EMG response to negative images was significantly higher than to any other image (positive, high fat food, low- fat food images, all $P<.001$; neutral image $P<.01$). There were no significant differences between groups from either ANOVA.

EMG zygomaticus major ANOVA analysis for 5 image sets showed that there was a significant main effect image ($P<.001$) and interaction of group*image ($P<.05$). Post-hoc analysis showed

that the zygomaticus major muscle EMG response to high fat food images was significantly higher than to negative ($P<.01$) and low fat food images ($P<.05$). EMG signal was significantly higher to positive images than to negative, neutral, and low fat food images (all $P<.001$). No differences were found between groups from the 2X5 ANOVA. A 2X2 ANOVA analysis for high fat and low fat images showed a significant main effect of image ($P<.01$). EMG activation was lower in FD patients than in HC and significantly higher to high fat food images than to low fat food images in HC ($P<.01$).

HRV SDNN 2X5 and 2X2 ANOVA analysis showed that there was a marginal main effect of group (p=.058, p=.059, respectively) and FD patients showed higher SDNN values than HC group.

HRV HF No significant main effect was registered for either the group or the images of HF value.

HRV LF/HF ratio 2X5 and 2X2 ANOVA analysis showed that there was a significant main effect of group ($P<.01$, $P<.05$, respectively) and FD patients showed significantly lower LF/HF ratio than HC group.

3.4. Experiment 2. Eye tracking experiment

Initial fixation (coefficient %): There were no significant differences according to the ANOVA (high fat: FD -24.78±7.53, HC -24.87±6.19; low fat: FD -32.80±5.62, HC -31.33±3.86, Figure 2.A.)

Fixation duration (coefficient %): There was a significant main effect of group and both high and low fat food images were fixated significantly less by FD patients than by HC (high fat: FD 2.77±5.18, HC 15.07±5.16; low fat: FD 0.60±5.34, HC 12.01±5.53; $P<.05$, Figure 2.B.).

Anticipated symptom rating: There was a significant main effect of group on anticipated symptom rating, with FD patients showing higher ratings to high fat food images used in **Experiment 2** than HC ($P<.001$). Post-hoc analysis showed that FD patients anticipated significantly higher pain and burning sensation than the HC group ($P<.05$, $P<.01$, respectively)

and there were no differences in fullness and satiation between groups. As for the low fat food images, none of the symptoms differed between groups (Supplementary Table 3.).

3.5. Correlation analysis

Pearson correlation analysis revealed significant negative correlations between the fat intake and BDI-II (r=-.88), fat intake and FCQ_DIFF (r=-.93), energy intake and FCQ_DIFF (r=-.95), and STAI_state and FCQ_state score (r=-.91, $P<.05$) in FD patients.

4. Discussion

We investigated physiological responses and the visual attention to food and non-food images in FD patients and healthy controls. Food craving, depression, and anxiety scores were significantly higher in FD patients than in HC. After food intake, FD patients experienced more symptoms of bloating, nausea, vomiting, abdominal pain, abdominal discomfort and burning sensation, despite lower total food/energy (kcal) consumption than the HC group. FD patients rated significantly lower pleasantness of both high and low fat food images than HC group. Although there was no difference in the initial orientation bias between groups, FD patients also had a significantly lower total attentional processing time of food images versus non-food images than HC group. The depression score with the consumption of fat, fat restriction score with fat/total energy intake, and anxiety level with the food craving state score were negatively correlated in FD patients only.

In this study, FD patients showed higher meal-induced FD symptoms after consuming less food and energy than healthy controls. It is noteworthy that pain and burning sensation in FD patients subsided immediately after meal ingestion and then gradually increased again. These results suggest that food ingestion can not only aggravate but also alleviate FD symptoms. According to a previous study[12], the intensity of each FD symptom increased significantly following meal ingestion. These inconsistent results may be due to the different composition of meals and instructions (“eat everything” vs “eat as much as you want”), and sample characteristics. We also found that FD patients also suffered from FD symptoms (pain, discomfort, burning, bloating) even when they were in a fasted state. FD patients are known to eat more frequently, but take smaller portions and are unable to finish a normal meal portion. This may be due to dynamic changes of symptoms in a state of hunger or fullness.

As often reported in earlier studies, FD patients had significantly higher anxiety and depression levels than HC group. In the current study we detected a negative correlation between the food craving state score and the state anxiety score, and between the depression score and the amount of fat intake in FD patients. Food craving is known to be less related to hunger than to the restraint or deprivation of food[37]. Lower energy consumption in FD patients also suggests that food craving may be induced by deprivation. A further explanation is that the food craving is more related to a negative mood, such as anxiety[38]. Although a clear conclusion cannot be drawn from correlation analyses, the results may show the mutual influences of a state of anxiety, food craving, depression, and eating behavior in FD patients.

High HRV and decreased sympathetic activation in FD patients were observed regardless of the type of pictures, which is akin to the results of previous studies [39, 40]. The reduced HRV and increased sympathetic activation may therefore be an intrinsic characteristic of FD patients rather than a response to external stimuli. Furthermore, the emotional response during the visual stimulation of food and non-food cues did not differ significantly between groups. This can be interpreted along with the eye tracking results, which showed a similar tendency of initial attention with HC group and a lower total attention processing time (fixation duration) to food images in FD patients than in the HC group. While visual food images may not immediately induce negative emotional and avoidance responses, a late cognitive processing of the images by higher cognitive function may cause the avoidance response to food images in FD patients while processing food images. These results suggest that high level cognitive functions rather than autonomic and emotional mechanisms can operate differently in FD patients. Furthermore, a decreased fixation duration on food images in FD patients is at variance with earlier findings in patients with obesity and binge eating disorder[33, 41] (where increased duration on food images was reported) and is

similar to the results in anorexia nervosa patients[42] suggesting a positive and negative perception of food cues in eating-related diseases.

The reduced pleasantness of and attentional bias to visual food stimuli in FD patients could be a key to future psychotherapeutic intervention and research. Various treatment options have been proposed for FD, such as H. pylori eradication, prokinetic agents, acid suppressive medications, antidepressants. Nevertheless, a standardized treatment strategy for FD patients has yet to be established and cognitive behavioral therapy remains an unexplored area[43]. A new therapy that includes self-restraint response to food, emotional management, and eating behavior modification could be considered for patients who do not respond to conventional therapies. Furthermore, how FD patients perceive, encode, store, and recall the value of food and how food memories influence their food-related decision making are interesting topics for future studies.

In the interviews conducted before the study, almost all FD patients complained about the changes in their eating behavior and their poor quality of life. Most patients avoided symptom-related foods, such as fatty foods, bread, pasta, or alcohol, which varied from person to person and almost all patients requested advice as to what food they should be eating. Fatty foods aggravated the symptoms in some patients, whereas others remained unaffected. Nevertheless, the high fat restriction score was significantly related to the lower intake of fat and total energy in FD patients only and they anticipated more severe symptoms to high fat food images than HC. Previous negative memory of the aftereffect of eating could be extended to the restriction of food intake and the attentional avoidance[20]. This fact needs to be better recognized in clinics and clinical studies, and food consultation might be instrumental in improving the quality of life and establish healthier eating guidelines for patients.

A limitation of this study was the difficulty in finding one particular item of food that might be either symptom-related or symptom-unrelated to each patient. We therefore used standard images for all participants. This may be the basis for the similar autonomic and emotional responses to high fat and low fat images in our study. However, since this first-of-its-kind study investigates the basic physiological response to food in FD patients, we tried to include various measurements with diverse images from established databases. Moreover, our sample size was not large enough to conduct further subgroup analysis and we did not examine any differences between PDS and EPS, patients with severe and mild FD/depression/anxiety symptoms. Due to the lack of knowledge on the food-related behavior, cognitive, emotional, and physiological responses of FD patients, further studies with large sample size are necessary.

5. Conclusion

We observed an increased food craving, decreased amount of food intake, food ingestion-induced aggravation of FD symptoms, and abnormal visual processing time and perception of food-related pleasantness in FD patients. The effectiveness of conventional therapies in FD patients might be enhanced by taking dietary consultation and modification of psychological response to food as well as somatic symptoms, and future studies on the evaluation of food may identify the underlying pathophysiology of FD.

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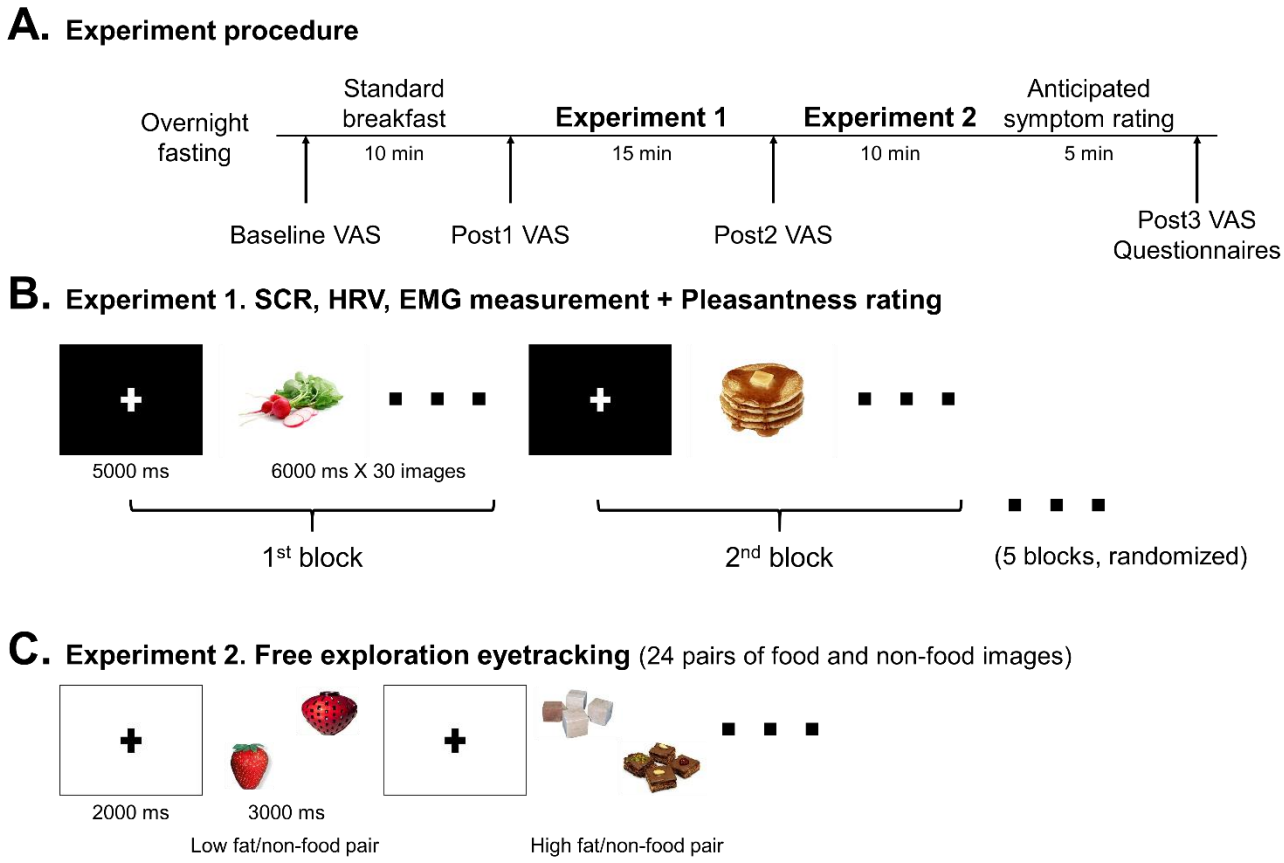
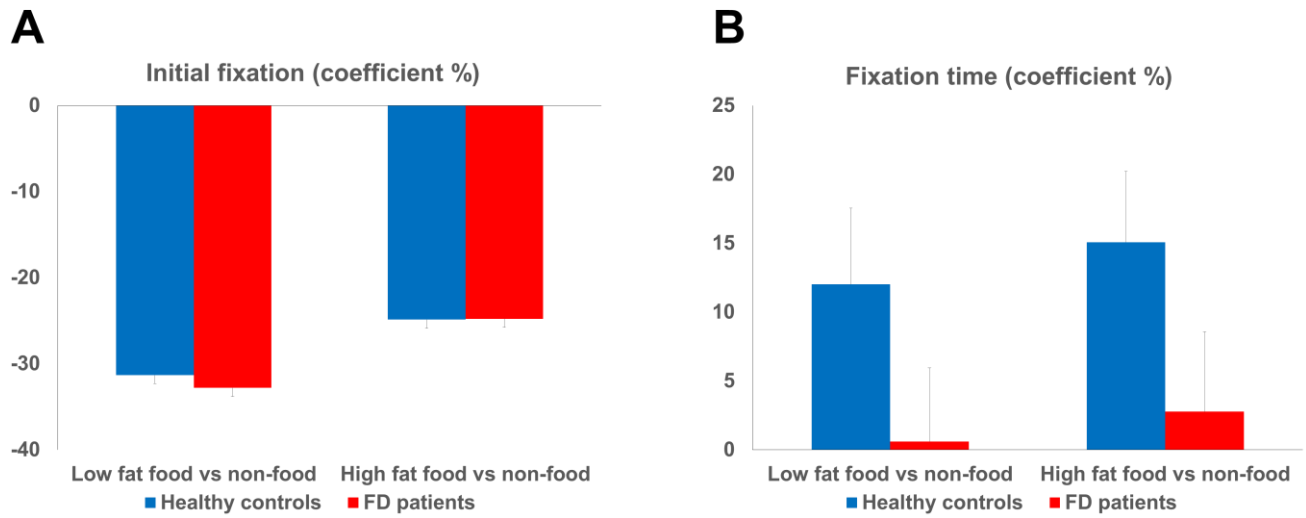
Figures and figure legends

Figure 1. Experimental protocol



A. Experimental procedure of the study. B. Illustration of the Experiment 1 including skin conductance response, heart rate, electromyography measurements and pleasantness rating to food and non-food images. Randomized order of 5 blocks of images (neutral, positive, negative, high fat, and low fat food images, n=30, 6000ms for each image) with fixation cross (5000ms) between each block were presented. C. Schematic presentation of the eye tracking experiment using free exploration paradigm. Low fat food and non-food pairs and high fat food and non-food pairs (n=12, respectively) were presented for 3000 ms with 2000 ms of fixation cross between pairs. Location of the images (1^{st}-4^{th} quadrant) was balanced.

Figure 2. The coefficient percentage of initial fixation and total fixation time in FD and healthy controls



Mean and standard error of coefficient % of initial fixation (A) and total fixation duration (B) on low fat food and high fat food images compared to paired non-food images in FD patients and healthy controls. There were no significant differences of initial fixation between groups and images. Total fixation time was significantly lower in FD patients than in HC for both high and low fat food images ($P<.05$).

Supplementary Table 1. FD symptom ratings before and after breakfast

		Baseline	Post1	Post2	Post3	P value (ANOVA)
Hunger	HC	5.09±0.65	1.14±0.40	1.16±0.40	2.62±0.67	main effect of time p<0.01
	FD	4.5±0.85	0.71±0.28	1.7±0.58	1.75±0.42	
Appetite	HC	4.68±0.52	1.82±0.55	2.31±0.60	3.65±0.74	main effect of time p<0.05
	FD	4.33±0.89	1.5±0.48	2.2±0.69	1.89±0.53*	
Fullness	HC	1.16±0.42	2.29±0.60	2.38±0.53	1.97±0.43	main effect of time p<0.05
	FD	2.67±0.86	4.82±0.92*	3.03±0.69	3.36±0.76	
Satiation	HC	1.97±0.58	6.54±0.59	5.50±0.63	4.71±0.74	main effect of time p<0.01
	FD	2.07±0.45	5.57±0.77	3.73±0.72	5.14±0.90	
Abdominal pain	HC	0.24±0.08	0.14±0.06	0.13±0.05	0.18±0.07	main effect of group p<0.05 (FD>HC)
	FD	1.93±0.75*	0.82±0.39	0.90±0.31*	1.54±0.60*	
Abdominal discomfort	HC	0.21±0.06	0.14±0.06	0.22±0.10	0.21±0.10	main effect of group p<0.01 (FD>HC)
	FD	3.83±0.83***	3.04±0.80**	2.53±0.75**	2.64±0.67***	
Burning	HC	0.50±0.20	0.21±0.08	0.22±0.10	0.18±0.07	main effect of group p<0.01 (FD>HC)
	FD	2.47±0.97***	1.39±0.62**	1.17±0.64**	2.93±0.82***	
Bloating	HC	0.29±0.13	0.39±0.16	0.38±0.17	0.41±0.19	main effect of time p<0.05 main effect of group p<0.01 (FD>HC)
	FD	2.50±0.83*	4.43±0.87***	3.07±0.77**	4.32±0.83***	
Nausea	HC	0.53±0.20	0.78±0.06	0.31±0.15	0.29±0.14	main effect of time p<0.05
	FD	1.47±0.66	1.07±0.64	1.23±0.41*	1.64±0.62*	
Vomiting	HC	0.18±0.06	0.14±0.06	0.38±0.21	0.24±0.10	Not significant
	FD	0.77±0.33	1.07±0.64	0.77±0.26	0.71±0.22*	

Mean±standard error

ANOVA: analysis of variance; Baseline: baseline VAS rating before breakfast; HC: healthy controls; Post1: VAS rating after breakfast; Post2: VAS rating 20-25 minutes after breakfast; Post3: VAS rating 45-50 minutes after breakfast; FD: functional dyspepsia patients

*, **, ***: two sample t-test FD vs HC. p>0.05, >0.01, >0.001, respectively

Supplementary Table 2. Physiological response to and pleasantness rating of emotional and food images in FD patients and healthy controls

		non-food emotional images			food images		P value (ANOVA)	
		Neutral	Positive	Negative	High -fat	Low -fat	General effect (2X5)	Fat effect (2X2)
Pleasantness	HC	5.08± 0.39	7.46± 0.38	2.17± 0.18	6.61± 0.40	7.38± 0.27	Main effect of image p<0.001	Main effect of group p<0.05
	FD	5.27± 0.52	7.94± 0.30	1.97± 0.20	5.62± 0.47	6.79± 0.39		Main effect of image p<0.05
SCR (ratio)	HC	0.92± 0.17	0.61± 0.11	1.69± 0.27	0.88± 0.12	0.83± 0.16	Main effect of image p<0.001	NS
	FD	0.85± 0.17	0.83± 0.24	1.55± 0.24	0.72± 0.08	1.05± 0.17		
EMG_corrugator supercilii (ratio)	HC	1.03± 0.19	0.26± 0.10	1.96± 0.39	0.90± 0.17	0.69± 0.20	Main effect of image p<0.001	NS
	FD	0.94± 0.21	0.77± 0.20	1.69± 0.31	0.66± 0.18	0.91± 0.15		
EMG_zygomaticus major (ratio)	HC	0.90± 0.17*	1.35± 0.25	0.41± 0.10	1.67± 0.35	0.59± 0.14	Main effect of image p<0.001	Main effect of image p<0.01
	FD	0.37± 0.08	2.00± 0.38	0.40± 0.11	0.89± 0.26	0.52± 0.12	Interaction effect of group*image p<0.05	
HRV_SDNN (ms)	HC	28.79±3.81	25.78± 2.51	25.79± 2.50	25.90 ±3.01	25.04± 2.86	Main effect of group p=0.058	Main effect of group p=0.059
	FD	31.52±4.53	32.32± 4.66	27.14± 4.75	32.06 ±5.02	35.43± 5.37		
HRV_HF	HC	383.86± 96.79	236.05 ±42.07	309.05 ±56.23	331.37 ±60.36	235.21 ±42.00	NS	NS
	FD	401.50 ±82.67	338.32 ±109.64	261.48 ±108.06	298.93 ±83.37	459.14 ±143.18		
HRV_LF/HF ratio	HC	1.75± 0.42	1.44± 0.21	1.38± 0.31	1.06± 0.13	1.67± 0.31	Main effect of group p<0.01	Main effect of group p<0.05
	FD	1.02± 0.17	1.21± 0.25	0.48± 0.07*	0.92± 0.21	0.86± 0.17		

Mean±standard error

EMG: electromyography; FD: functional dyspepsia patients; HC: healthy controls; HF: high frequency; HRV: heart rate variability; LF: low frequency; NS: not significant; SCR: skin conductance response; SDNN: standard deviation of all normal RR intervals

*, **, ***: post-hoc analysis, FD vs HC. p>0.05, >0.01, >0.001, respectively

Supplementary Table 3. Anticipated FD symptom rating to food images

		Healthy controls	FD patients	P value (ANOVA)
High fat food	**Abdominal fullness**	5.03±0.44	5.71±0.34	Main effect of group p<0.01 (FD>HC)
	Satiation	4.91±0.51	5.33±0.50	
	Abdominal Pain	2.49±0.32	3.89±0.57*	
	Burning	2.13±0.35	4.13±0.54**	
Low fat food	**Abdominal fullness**	5.02±0.54	5.39±0.46	Not significant
	Satiation	4.82±0.47	5.07±0.58	
	Abdominal Pain	2.96±0.61	3.34±0.55	
	Burning	2.47±0.52	3.36±0.51	

Mean±standard error

ANOVA: analysis of variance; FD: functional dyspepsia; HC: healthy controls

*, **, ***: post-hoc analysis, FD vs HC. p>0.05, >0.01, >0.001, respectively

8. Paper IV. The effect of fat label on gastrointestinal symptoms and brain activity in functional dyspepsia patients: an fMRI study

Author contributions

The material of this chapter was submitted to *Gastroenterology (2017 August)*. All authors designed the study and interpreted the results together. In-Seon Lee acquired and analyzed all the data. Results were discussed with the help of Hubert Preissl, Stephanie Kullmann, and Paul Enck. In-Seon Lee wrote the manuscript and Hubert Preissl, Stephanie Kullmann, and Paul Enck revised the manuscript.

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Fat label versus fat content: Gastrointestinal symptoms and brain activity in functional dyspepsia patients and healthy controls

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Abstract

High fat meals in particular are associated with dyspeptic symptoms in functional dyspepsia (FD) patients. However, it is still unclear what neural processes are involved and how they can be modulated by psychological factors such as expectation. We aimed to investigate brain activity by functional magnetic resonance imaging (fMRI) after the ingestion of high and low fat food with correct and incorrect fat information. Methods: We compared 12 FD patients and 14 age- and BMI-matched healthy controls (5 males in each group). We recorded resting state fMRI on four different days after an overnight fast before and after ingestion of one of four yogurts (200ml, either 10% or 0.1% fat, with 'low fat' or 'high fat' label (2x2 factorial design), sequence-randomized across subjects). The statistical significance level was set at α=0.05 and multiple comparison correction was applied. Results: FD patients showed more pronounced dyspeptic symptoms after consuming high fat-labeled yoghurt than low fat-labeled yoghurt, irrespective of the actual fat content. This is indicative of either a placebo effect of low fat information, or a nocebo effect of high fat information on symptom expression. FD patients showed greater activity than healthy controls in occipital areas before and after ingestion regardless of fat content and label as well as greater activity in the middle frontal gyrus before ingestion. In addition, functional connectivity (FC) from the insula to occipital cortex (I-O) increased after high fat and decreased after low fat ingestion in FD patients. FC from the insula to the precuneus (I-P) was higher in FD patients than in healthy controls after ingestion of yoghurt with a low fat label. In FD patients, I-O functional connectivity negatively correlated with nausea and I-P functional connectivity with FD symptom intensity, food craving, and depression. In summary, our results endorse the importance of psychological perception of food on the incidence of dyspeptic symptoms and on the altered brain activities. Taken together, these findings provide further evidence for the importance of cognitive

components in perception of fat, food craving, depression, and brain functions in pathophysiological mechanisms of FD.

1. Introduction

Functional dyspepsia (FD) is characterized by postprandial fullness, early satiation, epigastric pain, bloating, nausea symptoms after meals, particularly high fat food [1, 2], in the absence of any structural abnormalities in the gastrointestinal tract [3, 4]. The effect of fat in the altered gastrointestinal sensitivity and symptoms is a well-known pathophysiological feature in FD patients. Intra-duodenal infusion of lipids, not glucose nor saline, were shown to induce nausea, bloating, and vomiting symptoms in FD patients [5, 6]. After ingestion of a high fat meal, nausea and pain symptoms were greater than after a high carbohydrate meal [1] and food diaries revealed that FD patients consumed lower amounts of fat and that bloating symptoms were related to the amount of fat ingested [2].

Feinle-Bisset et al. showed that a low fat meal, served to FD patients under the pretense that it was high fat meal, caused more severe fullness and bloating symptoms than a low fat meal served to with the correct fat information. [7]. In addition it has been shown in healthy volunteers that ghrelin levels, as a physiological marker of satiation, varied after ingestion of identical milkshakes when subjects were informed before that this was either a high fat, high calorie or a low fat, low calorie milkshake. These findings suggest that the cognitive perception of fat at the central nervous system level plays a prominent role in the secretion of hormones, altered perceptual response to fat, and symptom reporting. The non-specific improvement (or worsening) of symptoms by an inactive treatment or treatment-unrelated cue – the placebo (or nocebo) effect – is due to the belief or expectation of symptom relief (or exacerbation). It is conceivable that if FD patients were aware of a close association between their symptoms and high-fat diet, the information on the amount of fat (more or less) could have an impact on their dyspeptic symptoms.

One hypothesis from the early 1990s proposed that abnormalities of the brain-gut axis are one of the key mechanisms governing FD [8]. The presence of the food or nutrient in the

gastrointestinal tract is signaled to the central nervous system which, in turn, modulates gastrointestinal function and eating behavior, and controls the gastrointestinal symptoms [9]. Furthermore, some of the brain's many pathways for controlling the perception of internal and external stimuli might be impaired in FD patients and cause somatic symptoms. The recent development of functional magnetic resonance imaging (fMRI) technique has enabled scientists to characterize the intrinsic brain activities and networks, and its intervention-related changes. A large number functional neuroimaging studies have investigated the brain activities and networks during the resting state (no-tasks) [10-17] and in reaction to the visceral distention [18-20]. They suggest that there is an alteration in the activation of the cognitive and pain processing brain regions (prefrontal cortex, somatosensory cortex, insula, cingulate cortex, thalamus, etc.) in FD patients [21]. However, little is known about how fat or fat information is processed in the brain or how it is mediated by pathological changes such as FD symptoms, decreased quality of life, increased depression and anxiety, food craving, etc., in FD patients.

In the current study, we investigated the effect of fat ingestion and fat label, and pathological factors on the resting state brain activities in FD patients and healthy controls (HC). We hypothesized that i) the resting state brain activity in cognitive and pain processing networks are mainly affected in FD patients, and ii) the functional connectivity (FC) emerging from the middle-posterior insula is associated with the pathological variables in FD patients. Bilateral middle-posterior insula was selected as a seed region since it is involved in signaling interoceptive visceral sensation and homeostatic information. It responds to a wide variety of experimental stimuli including pain/non-painful/salient/emotional stimuli as a region of the homeostatic afferent network [22-27]. Moreover, the insula is activated during baseline condition (compared to healthy controls) and in response to visceral distention (compared to baseline) [19, 20, 28], and correlates with FD symptom intensity [10, 13], disease-related quality of life [29], and disease duration [16]

in FD patients. We tested our hypotheses by measuring resting state fMRI in the fasted and fed state and performing seed-based FC analysis, correlation analysis, and mediation analysis.

2. Methods

2.1. Participants

12 FD patients (5 males, age 46.46±5.64 years, mean±standard error) and 14 for age- and BMI controlled healthy subjects (HCs, 5 males, age 45.79±4.71 years) participated in the study. Right-handed volunteers within the range of 18-75 years of age and with a body mass index (BMI, weight/height^2) of 19-29 kg/m^2 were included. FD patients were diagnosed on the basis of the ROME III criteria [30] as well as an unsuspicious endoscopy documented in their medical records. Volunteers with non-removable metal implants, claustrophobia, severe psychiatric illness, substance dependence and abuse, and any food allergy or intolerance were excluded from the study. The ethics committee of the Medical Faculty, University of Tübingen, Germany (633/2015802) approved the study and all participants gave their informed consent.

2.2. Test food

Two plain yogurts, low fat (0.1% fat, 200ml, 106kcal, 13.8g carbohydrate, 11g protein) and high fat (10% fat, 200ml, 266kcal, 14g carbohydrate, 6g protein, Weihenstephan, Freising, Germany) were used. Congruent or incongruent labels were attached to each yogurt (high fat yogurt with ‘high fat’ label: HH, high fat yogurt with ‘low fat’ label: HL, low fat yogurt with ‘high fat’ label: LH, low fat yogurt with ‘low fat’ label: LL).

2.3. Study design

Each participant was examined in the morning (7-11 a.m.) on four separate occasions following an overnight fast. Smoking and consumption of alcohol, coffee, or tea were prohibited

during the fasting period. Participants completed a visual analogue scale (VAS; 0=no symptoms at all, 10=very severe symptoms) to assess hunger, appetite, abdominal fullness, satiation, nausea, vomiting, abdominal pain and uncomfortable, burning, and bloating (baseline FD symptoms). Identical VAS ratings were assessed again immediately (Post1), 10 minutes (Post2), and 20 minutes (Post3) after the yogurt consumption. Between the pre-yogurt and post-yogurt fMRI sessions, participants were permitted to exit the scanner and were served one of the 4 yogurts (HH, HL, LH, LL) in randomized order. Participants were asked to sit on the MRI table and eat a whole portion of yogurt within 5 minutes. At the end of the study, patients indicated their dyspepsia symptom intensity, and disease-related quality of life was measured using Nepean Dyspepsia Index (NDI) [31]. Depression and anxiety levels were evaluated using Beck Depression Inventory (BDI-II) [32] and State-Trait Anxiety Inventory (STAI) [33]. Furthermore, the Eating Disorders Examination Questionnaire (EDE-Q) [34], Food Craving Questionnaire (FCQ) [35], and Fat Preference Questionnaire (FPQ) [36] were used to evaluate their eating behavior.

2.4. Imaging protocol

All images were obtained with a 3 Tesla scanner (Siemens MAGNETOM Prisma, Erlangen, Germany). On the first day, a high resolution T1-weighted anatomical image (magnetization-prepared rapid gradient-echo) was recorded (repetition time (TR)=2300ms, echo time (TE)=4.18ms, 176 slices, matrix=256×256, voxel size=1×1×1cm^3). Whole brain blood oxygenation level-dependent (BOLD) data were obtained using standard T2*-weighted echo planar sequence (160 volumes, TR=2000ms, TE=30ms, 30 slices, matrix=64×64, flip angle=80°, voxel size=3×3×3.4cm^3) before and after ingestion.

2.5. Imaging processing

Preprocessing of the BOLD signal was performed using Data Processing Assistant for Resting-State fMRI (DPARSF, http://restfmri.net, v2.2) [37] and SPM8 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK). Images for each subject were assessed to identify any excessive movement (>2mm or 2º degree) and the first 4 volumes were discarded for signal equilibrium and adaptation. Slice time-correction and head motion- correction were applied to raw images, and functional images were realigned and co-registered with the structural image. Images were normalized into the Montreal Neurological Institute (MNI) space and smoothed with a Gaussian kernel full width at half maximum 6mm. Following preprocessing, amplitude of low frequency fluctuations (ALFF) analysis within the low frequency band (0.01-0.1Hz) was performed using the DPARSF. The time series data of each voxel was transformed into the frequency domain, and the power spectrum amplitude was calculated. The square root was calculated at each frequency of the power spectrum, and the average square root was then obtained across 0.01-0.1 Hz at each voxel. This average square root was taken as the ALFF and mean ALFF (mALFF) was calculated as the original ALFF value/averaged ALFF across all voxels.

For seed-based FC analysis, 8mm sphere ROIs of the left and right middle-posterior insula were defined by peak coordinates (x=-42, y=-33, z=17; x=36, y=-15, z=13, respectively) of clusters from a resting state ALFF map (family-wise error (FWE) corrected $p<0.05$, cluster dimension $k>10$ voxels). The averaged time course was then obtained from the ROIs and the correlation analysis was performed in a voxel-wise fashion. Finally, the correlation coefficient map was converted into z maps by Fisher's r-to-z transform to improve the normality (zFC). For correlation and mediation analysis, the first eigenvariate of each cluster that survived the threshold from mALFF and zFC maps was extracted.

2.5 Statistical analysis

All statistical analyses were performed using IBM SPSS statistics 24.0 (IBM Corp. New York, USA). An independent two sample t-test was used to compare sample characteristics between the groups. For VAS ratings of FD symptoms, a two-way repeated ANOVA was conducted with time (pre, post1, post2, post3) and the group (HC, FD) factors. For ALFF and FC maps, SPM second level t-tests between the groups (HC, FD), fat content (high, low), and labels (high, low) were performed. Two-tailed Pearson's partial correlation analysis was also computed between questionnaire variables and the intensity of ALFF and FC while controlling for age and BMI. Mediation analysis was performed as described by Hayes using the PROCESS macro [38]. Age and BMI were included as covariates in the simple mediation model with 1000 bootstrap samples. The statistical significance level was set at α=0.05 while FWE correction for fMRI analysis and Bonferroni correction for the analysis of behavior data were applied to account for multiple comparison.

3. Results

3.1. Sample characteristics

Sample characteristics and questionnaire scores are presented in Table 1. We ascertained no significant differences in age and BMI between the groups ($p>0.5$). FD patients showed a significantly higher NDI_Symptom score and lower NDI_QOL score ($p<0.001$) than HC. FD patients also had significantly higher depression, anxiety state and trait levels ($p<0.01$, $p<0.001$, $p<0.05$, respectively) and higher FCQ-state scores ($p<0.05$) than HC. Among FPQ subscales (FPQ_TASTE: % of high fat food which tastes better than low fat food, FPQ_FREQ: % of high fat food which is eaten more frequently than low fat food, FPQ_DIFF: TASTE-FREQ), only FPQ_TASTE score was significantly higher in FD patients than HC. No significant differences were observed between the groups in EDE-Q total and subscale scores.

Table 1 Baseline characteristics of the study sample

	Healthy controls	FD patients	P value
Gender (m/f)	5/9	5/7	-
Subgroup	-	PDS: 4/6, EPS: 5/4	-
FD duration (month)	-	156±57.24	-
Age (year)	45.79±4.71	46.46±5.64	-
BMI (kg/m^2)	23.79±0.91	22.93±0.63	-
NDI_Symptom	4.5±1.35	64.5±9.10	<0.001
NDI_QOL	49.44±0.25	31.56±3.74	<0.001
EDE-Q Total	1.30±0.26	0.80±0.34	NS
Restraint	1.13±0.18	0.38±0.17	NS
Eating concern	0.44±0.22	0.20±0.13	NS
Weight concern	1.71±0.39	0.86±0.36	NS
Shape concern	1.87±0.49	1.33±0.44	NS
BDI-II	3.07±1.70	13.17±2.57	<0.01
STAI_state	29.64±1.97	43.17±2.78	<0.001
STAI_trait	30.63±2.17	41.92±3.76	<0.05
FCQ_state	31.82±1.72	38.07±1.94	<0.05
FCQ_trait	77.71±8.47	87.17±9.82	NS

FPQ_TASTE	46.48±4.03	67.56±12.24	<0.01
FPQ_FREQ	40.63±4.54	55.67±5.60	NS
FPQ_DIFF	5.85±3.57	11.88±6.52	NS

Mean±standard error

BDI: Beck depression inventory; BMI: body mass index; EDE-Q: Eating disorder examination questionnaire; EPS: epigastric pain syndrome; f: female; FCQ: Food cravings questionnaire; FD: functional dyspepsia; FPQ: Fat preference questionnaire; FPQ_TASTE: % of high fat food which tastes better than low fat food; FPQ_FREQ: % of high fat food which is eaten more frequently than low fat food; FPQ_DIFF: high fat restriction (TASTE-FREQ); m: male; NDI: Nepean dyspepsia index; NS: statistically not significant; PDS: postprandial distress syndrome; QOL: quality of life; STAI: State trait anxiety inventory

P value: independent two sample t-test, FD vs HC

3.2. Food induced FD symptoms

FD symptom ratings are described in Supplementary Table 1. The significant main effect of time was found in appetite, hunger (decreased after ingestion and later increased), and satiation ratings (increased after ingestion and later decreased, $p<0.001$). The significant main effects of group were found in nausea, vomiting, and bloating symptoms (FD>HC, $p<0.001$). Both significant main effects of group (FD>HC, $p<0.01$) and time (increased after ingestion and decreased later in both groups, $p<0.001$) were found in fullness rating. Both significant main effects of group and label were found in abdominal pain, discomfort, and burning symptoms. FD patients reported more severe symptoms than HC ($p<0.001$, $p<0.001$, $p<0.01$, respectively) and the high fat labeled yogurt resulted in more pronounced symptoms than the low fat labeled yogurt ($p<0.05$, $p<0.01$, $p<0.05$, respectively). Interaction of the group and the label was found in the symptom of discomfort ($p<0.05$) and the main effect of time was also found in the symptom of burning (which decreased after yogurt eating and later increased, $p<0.05$). No adverse events were recorded.

3.3. Resting state brain activity

3.3.1. Baseline ALFF (pre-yogurt session)

FD patients showed a significantly greater ALFF than HC in the bilateral middle frontal gyrus, left middle and right inferior occipital gyrus and lower ALFF in the left superior frontal gyrus and left middle cingulate gyrus (all $p<0.001$, FWE corrected, Table 2).

3.3.2. Changes of ALFF (post-yogurt vs pre-yogurt session)

After yogurt ingestion, significant group differences of changes of ALFF were observed in the left middle occipital gyrus and right cerebellum. ALFF increased in FD patients regardless of the type of yogurt consumed but decreased in HC compared to baseline. ALFF of the left middle occipital gyrus is significantly higher in FD patients than in HC, particularly in HH state (all $p<0.05$, FWE corrected, Table 2).

Table 2 Brain regions showing amplitude of low frequency fluctuation (ALFF) differences at baseline (pre-yogurt) and changes of ALFF (post-pre yogurt) between groups

Regions	Z scores of peak voxel	Coordinates of peak voxel in MNI space	P value
pre-yogurt FD>HC			
Left mid. frontal gyrus	6.12	-48, 30, 38	P<0.001
Right mid. frontal gyrus	5.68	36, 15, 60	
Right inf. occipital gyrus	5.48	39, -93, -13	
Left mid. occipital gyrus	5.23	-36, -75, 8	
pre-yogurt HC>FD			
Left sup. frontal gyrus	6.14	-12, 15, 72	P<0.001
Left mid. cingulate cortex	5.45	-9, 18, 34	
post-yogurt vs pre-yogurt FD>HC			
Left mid. occipital gyrus	4.36	-33, -81, 34	P<0.05
Right cerebellum	4.06	48, -60, -30	
post-yogurt vs pre-yogurt FD>HC, HH			
Left mid. occipital gyrus	4.43	-30, -81, 38	P<0.05

Family-wise error-corrected p value, cluster dimension k>10 voxels

FD: functional dyspepsia; HC: healthy controls; inf.: inferior; mid.: middle; MNI: Montreal Neurological Institute; sup.: superior

3.4. Functional connectivity

In FD patients, functional connectivity of the left insula to the right insula and the left inferior occipital gyrus increased significantly after eating high fat yogurt (HH, HL) and decreased after eating low fat yogurt (LH, LL) regardless of the label ($p<0.05$). There were no significant differences of changes in FC in HC.

In comparison to HC, FD patients showed increased FC between the right insula and the bilateral precuneus while FC decreased in HC compared to baseline after they had eaten low fat labeled yogurt ($p<0.05$, Table 3).

Table 3 Changes of functional connectivity (post-pre yogurt) within and between groups

Condition	Seed region	Regions of significant FC changes	Z scores of peak voxel	Coordinates of peak voxel in MNI space	P value
High fat>low fat in FD	**Left insula**	**Right insula**	5.09	39, 18, -4	<0.05
		Left inf. Occipital gyrus	4.30	-33, -87, -4	
FD>HC low fat label yogurt	**Right insula**	**Left precuneus**	3.71	-6, -57, 13	<0.05
		Right precuneus	3.71	21, -51, 21	

Family-wise error corrected p value, cluster dimension k>10 voxels

FC: functional connectivity; FD: functional dyspepsia; HC: healthy controls; inf.: inferior; MNI: Montreal Neurological Institute

3.5. Pearson's correlation analysis

Significant negative correlations were established between the intensity of FD symptom and disease-related quality of life ($r=-0.85$, $p<0.01$), and positive correlations between the intensity of FD symptom and state depression level ($r=0.52$, $p<0.05$) in FD patients. Baseline resting state

brain activity (ALFF) in the left middle frontal gyrus negatively correlated with the intensity of FD symptom (r=-0.77), food craving score (r=-0.78, p<0.01), and depression (r=-0.73, p<0.001) and positively correlated with QOL (r=0.73, p<0.05) in FD patients. FC intensity before ingestion (pre-yogurt session) between the right insula and right precuneus negatively correlated with the FD symptom intensity, food craving (p<0.01), quality of life, and depression level (p<0.05) and positively correlated with QOL (p<0.05) in FD patients. The FC intensity of the post-yogurt session between the left insula and left inferior occipital gyrus negatively correlated with nausea symptom rating in FD patients (p<0.05, Table 4).

Table 4 Pearson's partial correlation analysis

	NDI_QOL	STAI_state	pre-yogurt ALFF left mid. frontal gyrus	pre-yogurt FC right insula-right precuneus	post-yogurt FC left insula-left inf. occipital gyrus
NDI_symptom	-0.85**	NS	-0.77**	-0.70**	NS
FCQ_state	NS	0.72*	-0.78**	-0.69**	NS
BDI-II	NS	NS	-0.73***	-0.64*	NS
Nausea (Post3)	NS	NS	NS	NS	-0.64*

Correlation coefficients with p values (*, **, ***: p<0.05, <0.01, <0.001, respectively)

Age, sex, BMI controlled and multiple comparison-corrected

ALFF: amplitude of low frequency fluctuations; BDI: Beck depression inventory; FC: functional connectivity; FCQ: Food cravings questionnaire; inf.: inferior; mid.: middle; NDI: Nepean dyspepsia index; NS: statistically not significant; QOL: quality of life; STAI: State trait anxiety inventory

3.6. Mediation analysis

To assess the relationship of FD-related psychological symptoms, mediation analysis was performed. The models and the investigated variables are described in Figure 3. The total effect of the quality of life on depression was significant (path c $p<0.05$), and was fully mediated by FD symptom (path a $p<0.001$; path b $p<0.05$; path c' not significant; standardized indirect effect=-0.51, 95% confidence interval [-1.07, -0.25]) in FD patients (Figure 3.B., Model 1). The total effect of depression on the quality of life was also significant (path c $p<0.01$) and fully mediated by FD symptom (path a $p<0.01$; path b $p<0.001$; path c' not significant; standardized indirect effect=-0.43, 95% confidence interval [-0.59, -0.26]) in FD patients (Figure 3.B., Model 2). We also found that the total effect of food craving on the baseline resting state brain activity in the left middle frontal gyrus (path c $p<0.001$) is fully mediated by depression (path a $p<0.01$; path b $p<0.01$; path c' not significant; standardized indirect effect=-0.17, 95% confidence interval [-0.29, -0.07]) in FD patients (Figure 3.B., Model 3).

Discussion

Our data demonstrate I) an expectancy effect of the information about the fat content on symptom severity, either in high fat or low fat yogurt condition, II) the altered resting state brain activities in the prefrontal, occipital, cingulate, and cerebellum cortices, III) high fat-induced changes in the FC of the insula-inferior occipital gyrus (vs low fat) and the group difference of the changes in FC between the insula-precuneus in response to low fat label, IV) the negative correlations between FD symptom, food craving, depression and the middle frontal gyrus activity, nausea and the FC amplitude of the insula- inferior occipital gyrus, and V) the mediation effect of depression on the influence of food craving to the middle frontal gyrus activity in FD patients.

Psychological factors in FD patients

Among the many psychological factors in functional dyspepsia, anxiety and depression have been most frequently studied. In general, anxiety and depression are more severe in FD patients than in healthy controls and correlate with various dyspeptic symptoms [39-42]. In this study, anxiety, depressive, and also food craving state were more intense in FD patients than in healthy controls. In a bid to understand the psychological processes in FD patients, mediation analysis was performed. This enabled us to determine which independent variable affects another (dependent variable) and which variable mediates it. We found that the bidirectional effect between depression and disease-related QOL scores is mediated by FD symptom severity. This indicates that increased depression, symptoms and decreased QOL in FD patients are influenced by each other and that the role of dyspeptic symptoms is crucial in these psychological interactions. Moreover, the inhibitory effect of craving for food on the amplitude of prefrontal brain activity is also mediated by depression, leading to the plausible hypothesis that food craving enhances depression and suppresses the brain activity involved in executive control in FD patients.

Expectancy effect of fat label on FD symptom

The effect of high fat food on symptom aggravation was not established in this study, albeit high fat-labeled food induced more severe symptoms (abdominal pain, discomfort, and burning) than low fat-labeled food. This result provides new knowledge on the pathophysiology of dyspeptic symptoms since it demonstrates an expectancy effect of the information about fat content; these may be called placebo or nocebo effects [43]. While other dyspeptic symptoms, including fullness, nausea, vomiting, and bloating symptoms were higher in FD patients than in HC, these remained unchanged for the different yogurts. This may indicate that some, but not all of the visceral symptoms are subjective and can be modulated by cognitive factors. In particular, pain and burning symptoms are mainly observed in patients with epigastric pain syndrome, a subtype of functional dyspepsia known to be not exclusively meal-related. This may suggest that patients in different subgroups of functional dyspepsia may have other underlying mechanisms of peripheral and cognitive responses to food.

The behavior results are inconsistent with the previous study in which both a high fat content and an information of high fat (HH, LH) caused higher fullness and bloating ratings than low fat-labeled low fat yogurt (LL) in FD patients [7]. Furthermore, the effect of label was for both high and low fat yogurt in our study while previous findings did not demonstrate the effect of low fat label for high fat yogurt (no differences between HH and HL). This might be due to the total fat amount in the high fat yogurt used in our study (18g vs 23.6g) and different sample characteristics. The high fat yogurt used in this study may not suffice to provoke high fat effect on the symptom reporting. The threshold of fat amount and varieties of symptoms which are affected by psychological factors together with the role of expectation and previous experience of food in the placebo/nocebo effect on visceral symptoms in FD patients will require further investigation.

Functional connectivity between the insula and the precuneus

Functional connectivity of the insula-precuneus negatively correlated to the FD symptom, food craving, and depression in hunger state. This is enhanced in response to low fat-labeled yogurt in FD patients compared to HC. The precuneus and insula are known to be functionally connected during resting [44] and activated in response to smoking cues in smokers [45]. Insula is the core region of the visceral sensory [25, 26] and interoceptive networks [22-24], and is believed to be involved in ingestive behavior [46]. The precuneus is related to the episodic memory retrieval and processing of self [47, 48], appetite control [49, 50], reward of food receipt [51], reappraisal of benefits of eating the food [52], and comprises the default mode network [53]. Taken together, this connection may be affected by visceral symptoms and psychological factors and strengthened by the food signal processing in reward context (low fat label) by retrieving previous memories of food.

Food craving

We isolated two hyper-sensitized brain regions; the middle frontal gyrus in the prefrontal cortex (PFC) and the inferior occipital gyrus; which probably subserve different functions. We found a higher craving for food in FD patients than in HC in a hunger state. Furthermore, food craving influenced the middle frontal gyrus activity indirectly via depression. Food craving and depression affect each other reciprocally and FD symptom mediates the influence. Food craving, an intense urge to eat a particular food, is more related to the restraint or deprivation of food and calories [54, 55] or negative emotional state [56] than to hunger. Although the role of the food craving in obesity and eating disorders has been well established [57], it has not yet received sufficient attention in FD patients. The PFC is well known for the executive functions (decision-making, reward evaluation, associative learning, and control of eating behavior) and the inhibitory

regulation of craving for drug [58], smoking [59], and food [46]. In terms of craving, PFC has been used for transcranial direct current stimulation to reduce food craving and calorie intake [60, 61]. Its activity increased more dramatically in the bulimia nervosa patients than in either healthy controls or binge eating disorder patients [62]. With mediation analysis results, it is plausible that the long-term experience of FD symptoms and consequent dietary restriction lead to higher food craving, and that craving disrupts the functional demands of the PFC indirectly, with depression as a mediator.

Nausea and the occipital cortex

The amplitude of functional connectivity between the insula and the inferior occipital gyrus negatively correlated with the nausea ratings after food ingestion. FD patients suffered from higher nausea symptom than HC and they reported more pronounced nausea after ingestion of high fat yogurt than of low fat yogurt (statistically not significant). The occipital cortex is one of the most frequently reported brain areas in other functional neuroimaging studies in FD patients [21]. However, the underlying cause of the functional change in the occipital cortex in patients remains unclear. Previous studies showed that a visually induced nausea correlated with the occipital gyrus activity [63] and that a gastric electrical stimulation with an anti-emesis effect increased the brain activity in the occipital cortex [64]. The occipital gyrus is presumably affected by the food-induced nausea as well as by visually induced nausea. A study on the food-induced nausea and the occipital cortex activity would provide insight into the central mechanisms of nausea in patients.

In summary, our results showed the placebo/nocebo effect of fat information, the reward cue- related change of functional connectivity of the insula-precuneus, the food craving-induced activity in the PFC, and nausea-related functional connectivity of the insula-occipital cortex. These results provide further important information about the underlying mechanism of brain activities concerned with somatic symptoms and psychological factors in FD patients. Limitations are a

relatively small sample size and the food used in the study. Various food items were avoided or preferred by FD patients and the unusual environment of MRI restricted the choice of the test meal. Yogurt was selected since it had already proved successful in inducing FD symptoms in patients in an earlier study, and because its fat composition is familiar to the participants and easily modulated. However, patients suffering from lactose intolerance were unable to participate. Larger sized studies are required to comprehend the central mechanisms of responses to food in FD patients.

Conclusion

Individuals with FD have latent impairments in their cognitive perception of high fat food, altered activity of the PFC, occipital cortex, and impaired connectivity between the insula and occipital cortex, precuneus. Intensity of intrinsic FD symptom, food craving and depression, food-induced nausea symptom correlated with abnormal brain activities in patients. Cognitive perception of fat, food craving, depression, and altered brain functions as well as the somatic symptoms should be deemed important pathological characteristics of FD.

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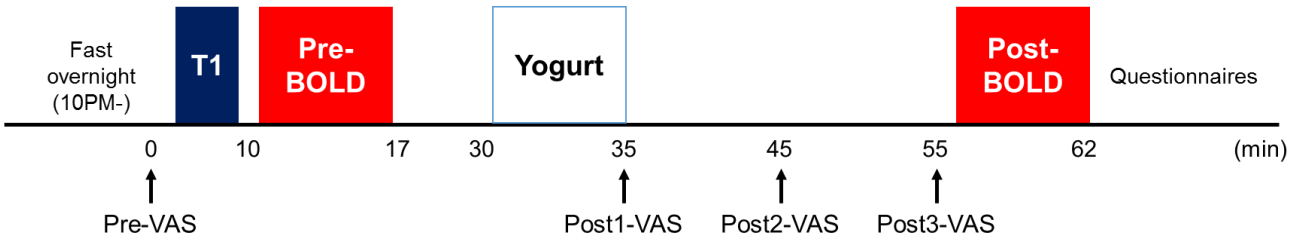
Figures and figure legends

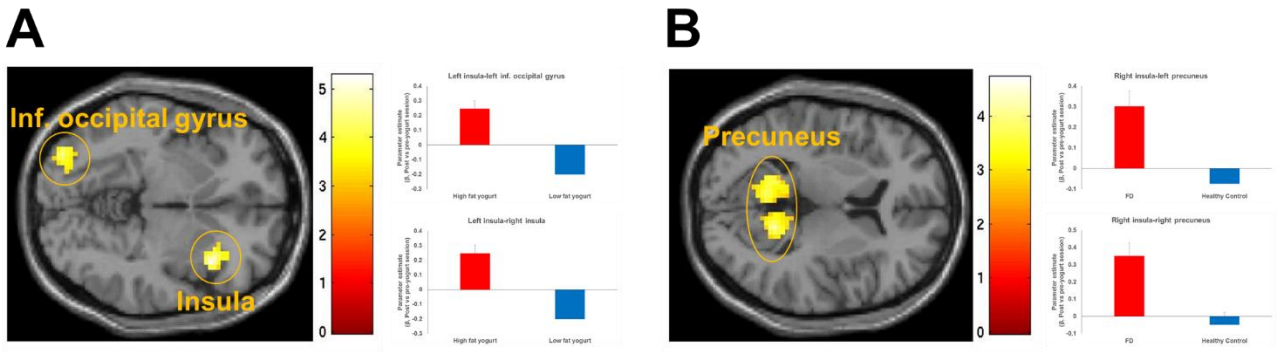
Figure 1 Study procedure



Schematic illustration of the study procedure with timeline. Following an overnight fast the study commenced in the morning (7-11AM). Baseline (Pre-VAS) and three subsequent dyspeptic symptoms after ingestion (Post1, 2, 3-VAS) were assessed every 10 minutes using visual analogue scale.

BOLD: Blood oxygen level-dependent contrast imaging; T1: T1-weighted image for structure imaging; min: minutes; Post-: after yogurt ingestion; Pre-: before yogurt ingestion; VAS: visual analogue scale

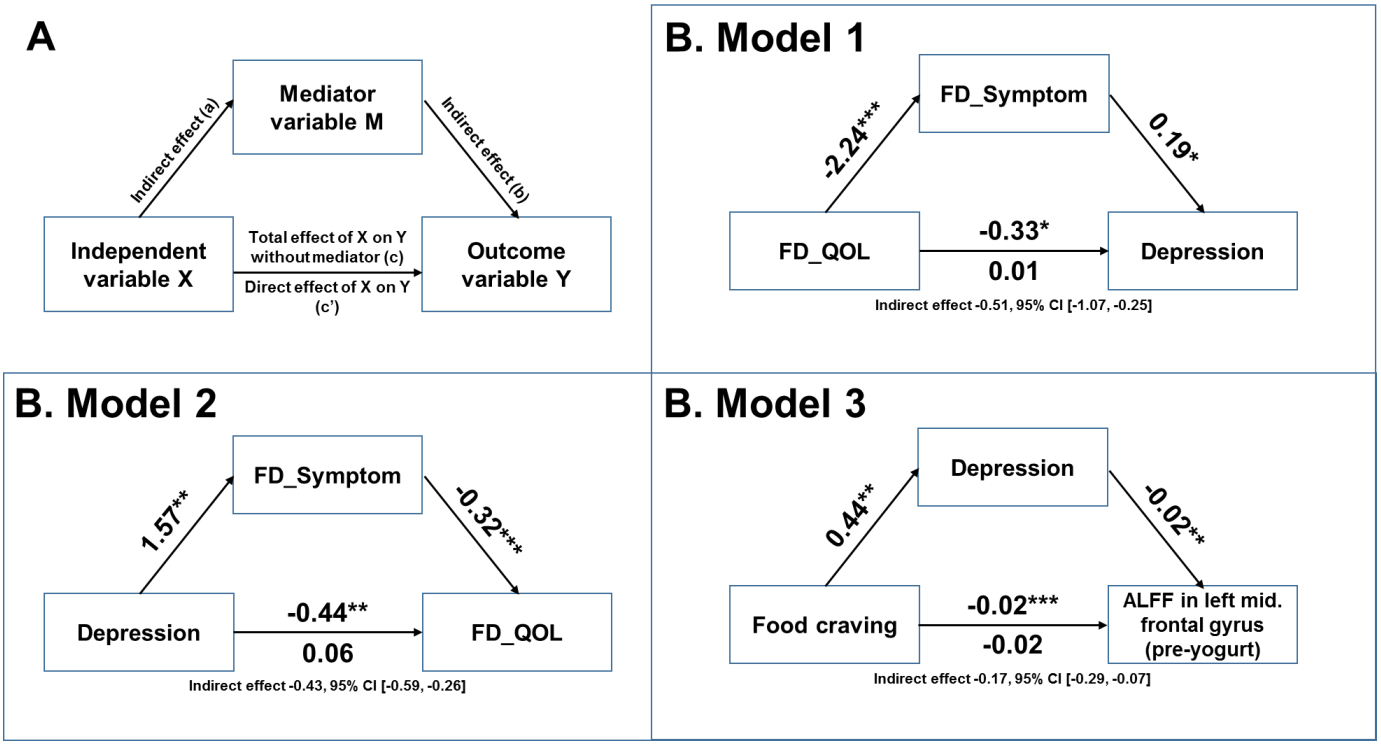
Figure 2 Seed-based FC analysis



Effects of fat and fat information on functional connectivity between the left insula and the right insula, left inferior occipital gyrus, and between the right insula and the bilateral precuneus. (A) High fat yogurts (HH, HL) increased the functional connectivity of the left insula to the left inferior occipital gyrus and the right insula, while low fat yogurts (LH, LL) reduced the strength of identical connections after ingestion ($p<0.05$) in FD patients. (B) Low fat-labeled yogurts (HL, LL) increased the functional connectivity of the right insula to the bilateral precuneus in FD patients, while the identical connections decreased in healthy controls ($p<0.05$). Family-wise error-corrected, cluster dimension $k>10$ voxels.

inf.: inferior; FD: functional dyspepsia; HC: healthy controls; HH: high fat yogurt with high fat label; HL: high fat yogurt with low fat label; LH: low fat yogurt with high fat label; LL: low fat yogurt with low fat label; post-: after yogurt ingestion; pre-: before yogurt ingestion;

Figure 3 Conceptual diagram of mediation analysis



A. Conceptual diagram of mediation analysis model with one mediator as used in this study. Total effect of X on Y (c) = indirect effect of X on Y through M (ab) + direct effect of X on Y (c'). B. Model 1: FD_QOL (X), FD_Symptom (M), depression (Y); Model 2: depression (X), FD_Symptom (M), FD_QOL (Y); Model 3: food craving (X), resting state brain activity in left middle frontal gyrus before eating yogurt (Y), depression (M). Path coefficients with p values (*, **, ***: $p<0.05$, <0.01, <0.001, respectively).

ALFF: amplitude of low frequency fluctuations; FD: functional dyspepsia; QOL: quality of life

Supplementary Table 1 FD symptom ratings before (baseline) and after (Post) yogurts ingestion

			Baseline	Post1	Post2	Post3	P value (ANOVA)
Hunger	HC	HH	3.86±0.78	2.11±0.58	1.79±0.39	2.39±0.55	Main effect of time ($p<0.001$)
		HL	3.07±0.71	1.83±0.59	2.23±0.54	2.37±0.54	
		LH	3.29±0.79	1.89±0.44	1.96±0.46	2.18±0.48	
		LL	3.03±0.81	1.83±0.66	2.23±0.71	2.70±0.73	
	FD	HH	5.85±0.98	2.50±0.63	2.69±0.76	3.71±0.75	
		HL	4.50±0.92	2.45±0.74	3.23±1.01	3.09±0.95	
		LH	4.79±0.85	2.96±0.61	3.33±0.82	4.29±0.82	
		LL	4.38±0.98	2.77±0.83	2.88±0.79	3.46±0.80	
Appetite	HC	HH	3.86±0.68	3.29±0.64	2.18±0.39	2.18±0.45	Main effect of time ($p<0.01$)
		HL	2.83±0.66	2.17±0.55	2.03±0.48	2.13±0.45	
		LH	2.96±0.81	1.46±0.41	1.65±0.42	2.00±0.42	
		LL	3.70±0.81	2.40±0.71	2.64±0.72	2.67±0.66	
	FD	HH	5.15±0.88	3.27±0.66	2.77±0.75	3.42±0.80	
		HL	3.41±0.70	2.77±0.72	3.14±1.00	3.59±0.96	
		LH	3.54±0.91	3.54±0.81	3.63±0.93	4.42±0.99	
		LL	4.62±0.93	3.88±0.92	3.15±0.81	4.17±0.76	
Fullness	HC	HH	0.61±0.27	1.64±0.63	1.89±0.69	1.68±0.71	Main effect of group (FD>HC, $p<0.01$), time ($p<0.001$)
		HL	0.43±0.33	1.80±0.58	1.80±0.56	1.50±0.45	
		LH	0.75±0.39	1.89±0.68	1.75±0.57	1.39±0.50	
		LL	0.83±0.36	1.47±0.58	1.20±0.49	1.30±0.40	
	FD	HH	2.27±0.68	3.00±0.59	2.69±0.67	2.38±0.47	
		HL	1.64±0.60	4.09±0.85	2.82±0.72	2.95±0.74	
		LH	1.79±0.72	3.63±0.89	4.54±0.81	4.25±0.87	
		LL	1.42±0.45	3.73±0.77	2.77±0.70	2.54±0.46	
Satiation	HC	HH	1.50±0.41	3.14±0.85	2.93±0.80	3.39±0.93	Main effect of time ($p<0.001$)
		HL	2.20±0.91	3.87±0.80	3.27±0.93	3.20±0.88	
		LH	1.86±0.76	3.14±0.94	2.79±0.80	2.54±0.72	
		LL	1.77±0.69	3.10±0.77	2.60±0.77	2.47±0.71	
	FD	HH	2.31±0.83	3.62±0.57	3.58±0.70	3.21±0.84	
		HL	1.36±0.51	3.73±0.89	2.86±0.71	2.91±0.75	
		LH	1.46±0.43	4.71±0.82	4.38±0.72	4.88±0.85	
		LL	1.88±0.66	4.04±0.90	3.77±0.97	2.63±0.61	
Abdominal pain	HC	HH	0.25±0.10	0.21±0.09	0.18±0.07	0.21±0.10	Main effect of group (FD>HC, $p<0.001$), label (high>low, $p<0.05$)
		HL	0.13±0.06	0.07±0.05	0.10±0.05	0.07±0.05	
		LH	0.18±0.08	0.21±0.11	0.18±0.08	0.11±0.06	
		LL	0.13±0.08	0.13±0.06	0.17±0.06	0.13±0.08	
	FD	HH	2.35±0.95	1.15±0.36	1.65±0.45	1.17±0.34	
		HL	1.36±057	0.91±0.41	1.27±0.61	1.45±0.62	
		LH	0.88±044	1.08±0.44	1.17±0.32	1.58±0.31	
		LL	1.42±0.76	1.35±0.74	1.31±0.75	0.54±0.24	
	HC	HH	0.25±0.09	0.32±0.15	0.36±0.17	0.39±0.20	

Abdominal discomfort		HL	0.20±0.11	0.23±0.14	0.20±0.14	0.20±0.14	Main effect of group (FD>HC, p<0.001), label (high fat>low fat, p<0.01), interaction of group*label (p<0.05)
		LH	0.25±0.11	0.32±0.17	0.21±0.10	0.25±0.15	
		LL	0.30±0.14	0.30±0.14	0.20±0.08	0.17±0.11	
	FD	HH	3.38±0.92	2.77±0.60	2.73±0.84	1.75±0.53	
		HL	3.09±0.93	1.73±0.60	2.09±0.79	1.95±0.82	
		LH	2.42±0.76	2.50±0.78	2.21±0.51	2.96±0.62	
		LL	2.38±0.86	1.62±0.79	1.85±0.79	1.17±0.45	
Burning	HC	HH	0.32±0.11	0.25±0.09	0.14±0.06	0.18±0.08	Main effect of group (FD>HC, p<0.001), label (high fat>low fat), time (p<0.05)
		HL	0.13±0.06	0.07±0.05	0.10±0.05	0.10±0.05	
		LH	0.32±012	0.29±0.13	0.18±0.08	0.18±0.08	
		LL	0.30±0.14	0.23±0.10	0.20±0.08	0.13±0.08	
	FD	HH	1.46±0.60	1.08±0.55	1.15±0.50	1.63±0.70	
		HL	2.18±0.95	1.27±0.60	1.18±0.62	1.36±0.73	
		LH	2.46±0.90	1.46±0.60	1.67±0.52	1.92±0.63	
		LL	2.15±1.02	1.12±0.74	1.31±0.73	0.50±0.16	
Bloating	HC	HH	0.29±0.09	0.29±0.11	0.25±0.10	0.29±0.13	Main effect of group (FD>HC, p<0.001)
		HL	0.13±0.06	0.07±0.05	0.10±0.05	0.13±0.08	
		LH	0.36±0.13	0.29±0.13	0.25±0.10	0.29±0.10	
		LL	0.37±0.14	0.27±0.11	0.23±0.08	0.20±0.10	
	FD	HH	1.88±0.68	1.85±0.54	2.23±0.74	2.17±0.79	
		HL	1.91±0.56	1.91±0.51	1.73±0.57	1.82±0.53	
		LH	1.58±0.68	1.46±0.47	1.29±0.36	2.04±0.68	
		LL	1.96±0.88	1.96±0.82	1.69±0.86	0.96±0.43	
Nausea	HC	HH	0.21±0.09	0.25±0.11	0.18±0.08	0.18±0.08	Main effect of group (FD>HC, p<0.001)
		HL	0.10±0.05	0.07±0.05	0.13±0.10	0.07±0.05	
		LH	0.21±0.43	0.25±0.11	0.18±0.08	0.07±0.05	
		LL	0.13±0.08	0.60±0.53	0.53±0.43	0.10±0.05	
	FD	HH	2.81±0.99	1.31±0.54	1.69±0.80	0.79±0.24	
		HL	1.64±0.76	1.14±0.64	1.00±0.50	1.09±0.63	
		LH	0.83±0.37	0.42±0.12	1.00±0.32	0.75±0.29	
		LL	1.08±0.75	1.50±0.74	1.46±0.79	1.58±0.48	
Vomiting	HC	HH	0.18±0.08	0.21±0.09	0.18±0.08	0.18±0.08	Main effect of group (FD>HC, p<0.001)
		HL	0.13±0.06	0.07±0.05	0.07±0.05	0.07±0.05	
		LH	0.21±0.09	0.18±0.08	0.18±0.08	0.11±0.06	
		LL	0.13±0.08	0.13±0.06	0.10±0.05	0.11±0.06	
	FD	HH	1.23±0.78	1.38±0.64	1.54±0.81	0.71±0.30	
		HL	1.23±0.76	1.00±0.64	1.00±0.48	0.86±0.52	
		LH	0.50±0.19	0.50±0.21	0.79±0.23	0.54±0.16	
		LL	1.62±0.83	1.38±0.80	1.46±0.78	0.92±0.41	

Mean±standard error

ANOVA: analysis of variance; Baseline: baseline VAS rating before ingestion; FD: functional dyspepsia patients; HC: healthy controls; HH: high fat yogurt with high fat label; HL: high fat yogurt with low fat label; LH: low fat yogurt with high fat label; LL: low fat yogurt with low fat

label; Post1: VAS rating immediately after ingestion; Post2: VAS rating 10 minutes after ingestion; Post3: VAS rating 20 minutes after ingestion

9. Conclusion and future direction

In functional dyspepsia, we observed 1) the absence of previous studies on neural mechanisms of food-related tasks or food-related psychological factors, 2) higher food craving scores but a reduced amount of food intake from standard breakfast compared to healthy controls, 3) lower pleasantness and total visual attention time to food images compared to healthy controls, 4) placebo/nocebo effects of fat label on dyspeptic symptoms, in both high fat or low fat food ingestion sessions, and 5) altered resting state brain activities and functional connectivity in the prefrontal cortex, occipital cortex, insula, and precuneus and their associations with dyspeptic symptoms and psychological factors. The effectiveness of conventional treatments and basic researches for functional dyspepsia might be improved by dietary consultation and modification of their distorted perception of food. This will require the expansion of our conventional perspective of functional dyspepsia, from the peripheral gastrointestinal tracts to the mental process of and behavioral response to food.

Future studies

We propose future studies according to the following categories: studies on 1) the new knowledge of basic mechanisms, 2) the understanding of psychology and placebo/nocebo effects, and 3) clinical diagnosis and therapy in functional dyspepsia patients.

Although we did not find that the effect of high fat food triggered the dyspeptic symptom in patients, the effect of nutritional factors on the dyspeptic symptom development needs to be tested with further food types. Since symptoms related to different types of food or nutrients may be different in each patient, individualized items should be tested in future studies to gain results that are closer to the reality. To achieve this goal, we will require well-structured interviews and validated questionnaires for clinicians and researchers. Future studies on the neuronal mechanisms

on the evaluation, perception, processing of food and behavioral responses to food are necessary to unravel the pathophysiology of functional dyspepsia. How patients perceive, encode, store, and recall the value of food and how negative experience of previous food-induced suffering influences their food decision and eating behavior could be investigated using functional neuroimaging techniques and physiological measurements during food-related tasks.

It would also be worthwhile to study the effect of the food consumption on the composition of microbiota and their contribution to the gastric symptoms. The role of microbiota in the abnormal function of the brain-gut axis is still unclear despite the wide use of probiotics or antibiotics in irritable bowel syndrome patients. Gastric microbiota and fecal microbiota transplantation have been investigated in functional dyspepsia only in the last few years [152, 153]. Various approaches including studies on microbiomics [154], efficacy and mechanisms of probiotics, antibiotics, and fecal microbiota transplantation, changes of emotional, cognitive, and behavioral response to food or other external stimuli (social stress, pain), individualized screening and medication using microbiota may be a revolution in the functional dyspepsia research.

Another valuable approach is to investigate the efficacy and brain mechanisms of the placebo treatment using low fat label on food to relieve the dyspeptic symptoms in patients. Apart from the fact that there is no standard treatment guideline for functional dyspepsia patients, the only intervention whose neuronal mechanism has been studied so far is acupuncture [155, 156]. If the placebo treatment really works in functional dyspepsia patients, its peripheral and neuronal mechanisms may promote the development of new treatment for functional dyspepsia.

We also discovered a new psychological factor which might be important in the pathophysiology of functional dyspepsia: craving for food. Despite increased food craving when fasting, functional dyspepsia patients ate smaller amounts of food compared to healthy controls 4. Furthermore, food craving significantly affected the resting state activity in the middle frontal gyrus

in functional dyspepsia patients, but not in healthy controls. Besides the abnormal responses to external painful stimuli, (e.g., barostat distension) and the effects of anxiety and depression, the physiological cause and effect of increased food craving score in functional dyspepsia patients need to be replicated and further investigated in large-scale studies.

Objective outcomes from functional neuroimaging studies, screening of the composition of microbiota, well-structured interview about eating behavior and cognitive processes of food may improve the current diagnosis. To improve existing treatments for functional dyspepsia, the efficacy, safety, and protocol of psychotherapies with the manipulation of eating behavior, consultation of food choice, and modification of negative response to food should be defined. Moreover, placebo treatments using symptom relief cues, e.g., low fat label, symptom-independent nutrients, elimination or supplement of microbiota, or placebo tools (which are similar or identical to the treatment appliances without any actual effects) need to be tested.

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