Irritable Bowel Syndrome

Studies of central pathophysiological mechanisms and effects of treatment

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Cover: a processed MRI image of the author's brain.

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Dedicated to my family

Imagination is more important than knowledge.

Albert Einstein

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ABSTRACT

Background and aims

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder characterized by abdominal pain and altered bowel habits. The societal costs of the disorder are significant, as are its negative effects on quality of life. Medical treatment options are limited, but psychological treatments such as hypnotherapy have proven to be effective. Important pathophysiological mechanisms include disturbances in brain processing of visceral sensation and expectation of visceral sensation. Increased sensation of stimuli (hypersensitivity) is present in a subset of IBS patients to distensions in the lower part of the gastrointestinal tract, indicating a probable important pathophysiological mechanism in IBS. The overall aim of the thesis was to further study the central pathophysiological mechanisms involved in IBS. Specifically, we aimed to identify differences in brain response to standardized repeated rectal distensions and expectation of these stimuli between IBS patients (with or without perceptual rectal hypersensitivity), and healthy controls. Furthermore, we aimed to investigate IBS patients' brain responses to standardized rectal distensions and expectation of these stimuli after either a successful course hypnotherapy or educational intervention.

Methods

Functional magnetic resonance imaging (fMRI) data were acquired and analyzed from 15 IBS patients with visceral hypersensitivity, and 18 IBS patients with normal visceral sensitivity (papers I and II). In paper III, fMRI data were analyzed from IBS patients who reported significant symptom reduction after either a course of hypnotherapy, or an educational intervention. FMRI data from IBS patients and healthy controls were also compared.

Results

The findings reported in papers I and II suggest, that the differences in brain response between IBS patients with and without rectal hypersensitivity, can be explained by changes in brain response during the course of the experiment. Even though the brain responses were similar between groups during the early phase of the experiment, they became substantially different during the late phase. The IBS patients with rectal hypersensitivity demonstrated increased brain response in several brain regions and networks involved in visceral sensation and processing. In contrast, IBS patients with normal rectal sensitivity exhibited reduced brain response during the late phase of the experiment. As reported in paper III, similar symptom reduction was achieved for both treatments. The symptomatic improvement was associated with a reduction of response in the anterior insula, indicating an attenuated awareness of the stimuli. The hypnotherapy group had a reduction of response in the posterior insula, indicating less input to the brain, possibly due to changed activity in endogenous pain modulatory systems. In patients who reported significant symptom reduction following treatment, the brain response to rectal distension got more similar to that observed in healthy controls.

Conclusions

The results from papers I and II indicate that a subpopulation of IBS patients lacks the ability to habituate to repeated rectal distensions and expectation of these stimuli. Results from paper III indicate that the abnormal processing of visceral stimuli in IBS can be altered, and that the treatments probably had a normalizing effect on the central processing abnormality of visceral signals in IBS.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Irritable Bowel Syndrome

Studier av centrala sjukdomsmekanismer och effekter av behandling

Irritable bowel syndrome (IBS) är en kronisk sjukdom som kännetecknas av återkommande buksmärtor eller obehag tillsammans med förändrade tarmvanor. IBS är ett vanligt tillstånd med en förekomst på upp till 20 % av befolkningen. IBS för med sig stora samhällskostnader i form av sjukvård och sjukfrånvaro, men framförallt kan tillståndet vara förenat med försämrad livskvalitet för den drabbade individen. Effekten av läkemedelsbehandling vid IBS är begränsad. Dock har psykologiska behandlingar, såsom kognitiv beteendeterapi och hypnosbehandling men även IBS-utbildning visat sig ha god effekt på symtomen.

Sjukdomsmekanismerna vid IBS är ofullständigt kända. De senaste årens forskning har dock visat att ett förändrat samspel mellan hjärna och mag-tarmkanal spelar en viktig roll för buksmärtan och andra IBS-relaterade symtom. Ungefär hälften av IBS-patienterna har nedsatt smärttolerans i tarmen vilket leder till ökad buksmärtor, till exempel vid undersökningar av tarmen eller efter intag av gasbildande föda. Detta fenomen kallas visceral hypersensitivitet och antas vara en viktig del i varför symtom vid IBS uppkommer.

Funktionell magnetresonanstomografi (fMRI) är en teknik som gör det möjligt att studera vilka områden i hjärnan som aktiveras vid olika typer av stimuleringar. I denna avhandling undersöktes vilka områden i hjärnan som aktiveras dels när man blåser upp en ballong i ändtarmen och dessutom när man väntar på att en uppblåsning ska komma. Specifikt studerades hur hjärnans reaktionsmönster skiljer sig mellan friska försökspersoner och IBS-patienter med och utan visceral hypersensitivitet. Uppblåsningarna upprepades många gånger och därför kunde vi jämföra hjärnans aktivering under den tidiga och sena delen av uppblåsningsserien. Dessutom undersöktes hur hjärnans reaktionsmönster påverkas av hypnosbehandling och IBS-utbildning.

Resultaten från delstudie I och II visade att hjärnans reaktionsmönster skiljer sig avsevärt mellan IBS-patienter med och utan visceral hypersensitivitet. Under den första delen av uppblåsningsserien var hjärnans reaktionsmönster mycket lika mellan IBS-grupperna. Under den senare delen av uppblåsningsserien blev det däremot stora skillnader i hjärnaktivitet mellan IBS-grupperna. Hos IBS-patienterna med visceral hypersensitivitet sågs ökad aktivitet i områden och nätverk i hjärnan som är inblandade i förnimmelse och bearbetning av signaler från mag-tarmkanalen medan hjärnaktiviteten i den andra IBS-gruppen minskade. Dessa resultat tyder på att IBS-patienter med visceral hypersensitivitet verkar ha en nedsatt förmåga att vänja sig vid upprepade och förvarnade tarmuppblåsningar.

Resultaten från delstudie III visade att patienternas symptom minskade både efter hypnosbehandling och IBS-utbildning. Symtomförbättringen kunde relateras till en minskning av aktivitet i områden av hjärnan som är inblandade i den känslomässiga upplevelsen av signaler från tarmen. De patienter som genomgick hypnosbehandling fick dessutom minskad aktivitet i hjärnområden som tar emot signaler från tarmen. En möjlig förklaring till detta kan vara att hypnosbehandlingen förändrade hur hjärnan reglerar inkommande signaler. Sammanfattningsvis tyder resultaten på att hypnosbehandling och IBS-utbildning påverkar hjärnans reaktionsmönster vid inkommande signaler från tarmen. Dessutom tyder resultaten på att hjärnans reaktionsmönster efter framgångsrik behandling hos IBS-patienterna blir mer likt de friska försökspersonernas, i samband med tarmuppblåsning.

LIST OF PAPERS

- I. **Brain responses to visceral stimuli reflect visceral sensitivity thresholds in patients with irritable bowel syndrome.**

Larsson MB, Tillisch K, Craig AD, Engström M, Labus J, Naliboff B, Lundberg P, Ström M, Mayer EA, Walter SA.

Gastroenterology 2012;142(3):463-472

- II. **Deficient habituation to repeated rectal distensions in irritable bowel syndrome patients with visceral hypersensitivity.**

Lowen MB, Mayer EA, Tillisch K, Labus J, Naliboff B, Lundberg P, Thorell LH, Ström M, Engström M, Walter SA.

Neurogastroenterology & Motility 2015 May;27(5):646-55

- III. **Effect of hypnotherapy and educational intervention on brain response to visceral stimulus in the Irritable Bowel Syndrome.**

Lowen MB, Mayer EA, Sjöberg M, Tillisch K, Naliboff B, Labus J, Lundberg P, Ström M, Engström M, Walter SA.

Alimentary Pharmacology & Therapeutics 2013;37(12):1184-1197

ABBREVIATIONS

aINS	Anterior insula
aMCC	Anterior midcingulate cortex
AML	Ascending methods of limits
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CMRO2	Cerebral oxygen consumption rate
CT	Computed tomography
dIPFC	Dorsolateral prefrontal cortex
EEG	Electroencephalography
fMRI	Functional magnetic resonance imaging
FWE	Family wise error
GLM	General linear model
HAD	Hospital Anxiety and Depression
Hipp	Hippocampus
IBS	Irritable bowel syndrome
IBS-SSS	Irritable Bowel Syndrome Severity Scoring System
LCC	Locus coeruleus complex
M1	Primary motor cortex
M2	Supplementary motor area
MEG	Magnetoencephalography
mINS	Mid insula
mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NTS	Solitary nucleus
OFC	Orbitofrontal cortex
pACC	Pregenual anterior cingulate cortex
PAG	Periaqueductal gray
PET	Positron emission tomography
pINS	Posterior insula
PPC	Posterior parietal cortex
RF	Radio frequency
ROI	Region of interest
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
sgACC	Subgenual anterior cingulate cortex
TE	Echo time
Thal	Thalamus
TR	Repetition time
vIPFC	Ventrolateral prefrontal cortex
VSI	Visceral sensitivity index

INTRODUCTION

Irritable Bowel Syndrome

In 1871, Jacob Mendes da Costa published an article in the American Journal of the Medical Sciences in which he described a medical condition he called “mucous colitis.” Da Costa studied anxiety disorders among soldiers in the American Civil War, focusing on a disorder he called “irritable heart.” In these studies, he and others noted the connection between “irritable heart” and symptoms such as diarrhea. In the article, he described seven cases of “mucous colitis” presenting with diarrhea, high incidence of dyspepsia, abdominal pain and related the symptoms to emotional stress.¹ Even though this sort of disorder had been mentioned previously, this is probably the first description in modern medical literature of the cluster of symptoms that PW Brown, in 1950, would label Irritable Bowel Syndrome (IBS).² Since then, IBS, defined as a functional gastrointestinal disorder characterized by recurrent abdominal pain or discomfort associated with altered bowel habits,³ has been studied extensively. It is a highly prevalent disorder although prevalence varies between countries.⁴ In Western countries a prevalence of up to 20% has been reported.⁵ IBS, with its high prevalence, need for diagnostic procedures, job absenteeism, is responsible for considerable health care and societal costs.⁶⁻¹⁰ Even more importantly, IBS is associated with an impaired of quality of life for the affected individuals.^{11 12}

In the absence of generally agreed upon diagnostic tests, diagnosis of IBS relies on symptom reports and, in appropriate circumstances, the exclusion of organic disease. The most recent diagnostic criteria for IBS, ROME III, rely on retrospective symptom reports and require reports of recurrent abdominal pain or discomfort at least three days per month over a three month period, associated with at least two of the following criteria: (1) improvement with defecation; (2) onset associated with a change in frequency of stool; and (3) onset associated with a change in form (appearance) of stool. Onset of symptoms should be at least 6 months prior to diagnosis.³ However, a recent study indicates that existing diagnostic criteria perform modestly in distinguishing IBS from organic disease.¹³ Therefore, development of psychological markers, biomarkers, and diagnostic criteria, most likely used in combination, is necessary to improve the accuracy of IBS diagnosis.

In spite of intensive study, the pathophysiology of IBS is incompletely understood; classical theories include gastrointestinal dysmotility, visceral hypersensitivity, and an altered brain-gut interaction. In recent years, however, other mechanisms have been proposed, such as low-grade inflammation, increased intestinal mucosal permeability, immunologic and genetic factors, altered intestinal microbiota, and dietary factors.¹⁴⁻¹⁷ These theories indicate the heterogeneity of pathophysiological mechanisms in the IBS population.¹⁷ In addition, the importance of psychological and social factors such as social learning, comorbid psychiatric disorders, chronic life stress, and impaired coping is evident in IBS.¹⁸⁻²¹ This has led to the concept of a bio-psycho-social disease model in IBS which takes in account many of the factors leading to IBS symptoms.^{20 21}

The results of medical treatment of IBS are varied and limited. Because of the heterogeneity of the disorder, no single drug is likely to resolve all symptoms. With different degrees of evidence, symptomatic treatments for IBS include dietary fiber or bulking agents, spasmolytics, and antidepressants, which are used for their analgesic effect.²² However, development of new drugs shows promising results in subsets of IBS patients.^{23 24} Psychological treatments, for example, cognitive behavioral treatment and hypnotherapy, as well as educational interventions, have been shown to be effective in relieving the global symptoms of IBS.^{22 25-27}

Hypnotherapy in the treatment of IBS

Hypnosis can be defined as a procedure directed at inducing responses to suggestions for changes in subjective experience, such as alterations in perception, sensation, thought, emotion, and/or behavior.²⁸ In medicine, hypnotherapy was first used as an anesthetic during surgery, and hypnotic suggestion was later shown to be capable of altering several physiological mechanisms thought not to be under voluntary control, such as acid secretion and gastric motility,²⁹ and production of salivary immunoglobulin A.³⁰ Several studies have shown hypnotherapy to have beneficial effect in the treatment of IBS.^{27 31-35} In a recent report, it was found that gut-directed hypnotherapy significantly improved IBS symptoms after 3 months when compared to supportive therapy or waiting list, and the improvement was more

prominent for sensory symptoms, such as pain and bloating, than for bowel habit disturbances.³¹ This finding confirmed earlier study results that also demonstrated that hypnotherapy could improve abdominal pain and overall symptoms in IBS.²⁷⁻³⁶ Several studies have examined the effects that hypnotherapy treatment may have on the experience of experimental visceral stimuli and other physiological factors.³⁷⁻⁴⁰ Despite the fact that hypnotherapy has been used to treat IBS successfully for more than 20 years, the neural mechanisms of pain relief after a course of hypnotherapy still remain unclear. There is evidence, however, that hypnosis, for example, exerts its effect on the pain-processing regions of the central nervous system.⁴¹⁻⁴²

Educational interventions in IBS

Educational interventions in IBS can aim, for example, at increasing understanding of IBS pathophysiology, improving stress management, and decreasing symptom-related anxiety.⁴³ Such structured educational interventions have been proven to successfully reduce IBS symptoms as well as gastrointestinal-specific anxiety, and also improve health-related quality of life in IBS.²⁶⁻⁴⁴⁻⁴⁷ However, the brain mechanisms behind the effects of educational interventions in IBS are largely unknown.

Brain imaging

The human brain is one of the most complex structures in the known universe. This intriguing organ, weighing about 1.5 kg, is composed mainly of water and lipids. The cerebral cortex alone contains up to 33 billion neurons, each connected to numerous other neurons by synapses. The human brain is responsible for all the abilities and qualities that define the human species: from basic functions such as initiation of movement, and interpretation of sensory input to complex motor skills and our intelligence and empathy. It provides the ability to compose “Für Elise” (Ludwig van Beethoven), construct the Eiffel tower (Gustave Eiffel), and decorate the ceiling of the Sistine Chapel (Michelangelo). Throughout history, the function of the human brain has been a fascinating topic of research for scientists and philosophers. Until recently, brain research was limited to studies of loss of function from strokes, injuries or tumors. In this way regions connected to various features of the human mind, such as

feelings, memory, verbal language, and sensations were elucidated. Also, non-invasive methods for collecting information about electrical activity, such as electroencephalography (EEG) were commonly used.⁴⁸ However, in recent decades, technological advancements have facilitated the development of imaging methods to study structure and function of the brain in both health and disease. Examples of these methods are: computed tomography (CT),⁴⁹ positron emission tomography (PET),⁵⁰ magnetoencephalography (MEG),⁵¹ and magnetic resonance imaging (MRI).^{52 53} Furthermore, there is now an atlas of the human brain available, which combines gene expression mapping with neuroanatomical data.⁵⁴

Principles of magnetic resonance imaging and functional magnetic resonance imaging⁵⁵⁻⁶⁰

MRI utilizes the quantum mechanical property called spin to create high-resolution anatomical images. This is achieved by using strong magnetic fields and radio frequency (RF) pulses. By changing certain properties when images are acquired, different aspects of the examined tissue can be highlighted. MRI technology can be used in order to further investigate and localize brain areas involved during experimental events; for example, in the current studies, distension of the gastrointestinal tract. This method is known as functional MRI (fMRI), and utilizes the local inhomogeneity of the magnetic field caused by changes in blood flow and oxygenation. The primary type of fMRI is performed using the blood-oxygen-level-dependent (BOLD) contrast, which utilizes the fact that oxygenated and deoxygenated hemoglobin demonstrate different magnetic properties.^{61 62} In the event of neural activity, the metabolic demand in the affected brain tissue is thought to increase. This leads to the extraction of nutrients and oxygen from blood vessels. In addition, via neurovascular coupling, a regional increase in cerebral blood flow (CBF) occurs. Together, this is called the hemodynamic response. The BOLD response to an experimental event is, in a complex way, correlated to CBF, cerebral blood volume (CBV), and to the cerebral oxygen consumption rate (CMRO₂),^{63 64} which can be linked to neuronal activity⁵⁸ and is likely to reflect changes in pre- and post-synaptic activity rather than spiking output.^{65 66}

Processing of raw BOLD data^{55 56 67}

After the BOLD data is collected it must be processed in order to reduce noise, correct for artifacts and ensure that it is adequately prepared for statistical analysis. fMRI data is very sensitive to the motion artifacts that frequently occur during a scan. Therefore, the data is corrected for motion parameters recorded during the scan. Also, in order to be able to compare brain scans from different people, brain scans can be aligned to a template brain with a standardized atlas space using a standard coordinate system, for example, the Montreal Neurologic Institute (MNI) template. To further compensate for inter individual differences in brain anatomy, and as a necessary step prior to statistical analysis of the data, spatial smoothing is applied.

Statistics^{56 67}

The general linear model (GLM) is the most commonly used model for statistical analysis of fMRI data. In this model the data is treated as a linear combination of predictor variables plus noise. By stating linear conditions and contrasts, brain response to specified tasks can be evaluated. In addition, different conditions can be compared by subtracting contrast. The result is a statistical parametric map that can be illustrated graphically. Since each voxel (the image's smallest element or volume) is analyzed separately, a vast amount of comparisons are performed in every analysis. This produces many false positives and therefore correction for multiple comparisons is necessary when analyzing BOLD data. Different methods can be used to minimize the multiple comparison error, for example, controlling for family wise error (FWE) rate.

Advantages and limitations of fMRI^{55 57}

fMRI is an established method for gaining insights into brain mechanisms. However, there are both advantages and disadvantages to its use. The main advantages of using fMRI compared to other modalities when studying brain activity are that the spatial resolution is good, and there is no exposure to ionizing radiation. The temporal resolution is sufficient to examine brain response to experimental stimuli, though it is better in some of the other modalities. The fMRI environment can be perceived as stressful, which must be taken into consideration

when interpreting results. Also, the hemodynamic response to an experimental stimulus is not a direct measure of neuronal activity, but rather a substitute signal. In turn, the hemodynamic response is influenced by several factors, such as imprecision of the biological control of cerebral blood flow, variations in the structure of the cerebral vasculature, or changes in excitation-inhibition balance. Furthermore, as with most research methods, the experimental design and data analysis can influence the results, making it difficult to compare study results and to draw general conclusions.

Central aspects of visceral sensation

Research in recent years on the communication between the gut and the brain using neuroimaging, both in healthy subjects as well as in patients with gastrointestinal diseases such as IBS, has led to a deeper understanding of these complex processes. In recent reviews, Mayer *et al.* describe the different brain regions and networks related to visceral sensation and IBS symptoms.^{68 69} A schematic overview of these brain regions and networks is presented in Figure 1. However, regions can have multiple functions, which is why distinct borders between networks can be difficult to define.

Figure 1. Brain regions and networks related to visceral sensation and IBS.

Amyg amygdala, *aINS* anterior insula, *aMCC* anterior midcingulate cortex, *BG* basal ganglia, *dlPFC* dorsolateral prefrontal cortex, *Hipp* hippocampus, *Hypo* hypothalamus, *LCC* locus coeruleus complex, *M1* primary motor cortex, *M2* supplementary motor cortex, *mPFC* medial prefrontal cortex, *NTS* solitary nucleus, *OFC* orbitofrontal cortex, *PAG* periaqueductal gray, *pgACC* pregenual anterior cingulate cortex, *pINS* posterior insula, *PPC* posterior parietal cortex, *sgACC* subgenual anterior cingulate cortex, *Thal* thalamus, *vIPFC* ventrolateral prefrontal cortex.

Adopted from Mayer *et al.*, 2015.

Overview of networks implicated in visceral sensation and IBS

The sensorimotor network is involved in receiving afferent input from the periphery, with the thalamus acting as a relay station for incoming signals, and the posterior insula as the primary interoceptive cortex.⁷⁰⁻⁷⁴ The salience network responds to subjective salience of stimuli, including visceral sensation and the expectation of such stimuli.^{75 76} The core regions in this network are the anterior insula and the anterior midcingulate cortex.^{55 68} The dorsal part of the anterior insula is influenced by prefrontal regions, and the ventral part is closely connected to the amygdala and the emotional arousal network. The emotional arousal network is responsible for the changes in brain response in a situation (actual or perceived), where the homeostasis of the organism is challenged.⁷⁷⁻⁷⁹ This is achieved in close connection with the salience and central autonomic networks, with an appropriate, or sometimes inappropriate, response aimed at maintaining homeostasis. There are many studies reporting an increased reactivity in the emotional arousal network to rectal distensions and, also expectation of such distensions, in IBS.⁷⁹ Core regions of the emotional arousal network include amygdala, hippocampus, anterior cingulate cortex, and prefrontal cortices. The central autonomic network is responsible for the central control of the autonomic nervous system, which includes gastrointestinal activity during, for example, visceral sensation.^{74 80-82} Central regions in this network include the amygdala, anterior insula, anterior cingulate cortex, prefrontal regions, hypothalamus, and periaqueductal gray. The central executive network is active during tasks involving attention, planning, and selection of response, often in close connection to activity in the salience network.^{75 77 83} Core regions in the central executive network include the lateral prefrontal cortex and the posterior parietal cortex. Outputs from the above described networks include descending pain modulation and activity in the autonomic nervous system. Alterations in these functions have been shown to be important in IBS.^{82 84-87}

Perception of visceral sensations in IBS

There is no linear connection between the subjective experience of visceral discomfort or pain and the intensity of incoming signals from the gastrointestinal tract. Perception of visceral stimuli is a complex process influenced by a number of variables, including emotional and cognitive factors, memories of previous experiences, and prediction of coming experiences.⁶⁸

⁷³ For instance, the emotional state of the individual has been shown to be important,⁸⁸ eg, a negative emotional environment has been shown to modulate anxiety levels, discomfort, and brain response to visceral stimuli.⁸⁹⁻⁹⁰ Attention to gastrointestinal stimuli is another important process, leading to modulation of incoming signals from the gut.⁹¹ In IBS, hypervigilance, selectively attending to gastrointestinal sensations, and prediction error have been demonstrated.⁹²⁻⁹⁴ The expected severity of stimuli and previous experience have been shown to be of great significance in the perception of pain, and form an established concept in the somatic pain literature.⁹⁵⁻⁹⁹ Expected intensity has an essential effect on both reported pain and the brain response signature to pain. In a similar manner, the impact of expectations on perception and brain response has been shown to be central in the field of visceral pain.¹⁰⁰⁻¹⁰⁵ Expectation and involved learning processes (such as conditioning) can be linked to placebo/nocebo responses, which have been proven to be important factors in the processing of signals from the gastrointestinal tract, both in healthy subjects and in IBS patients.¹⁰⁴⁻¹⁰⁶⁻¹¹⁰ The importance of habituation and sensitization, ie, increasing and decreasing response to repeated stimuli has been implicated in several chronic pain conditions,¹¹¹⁻¹¹³ but data for IBS patients are limited.⁷⁶⁻¹¹⁴

Hypersensitivity in IBS

Hypersensitivity can be defined as increased sensitivity to stimuli, and is present in a subset of IBS patients in response to distensions in the lower part of the gastrointestinal tract compared to healthy controls,¹¹⁵⁻¹¹⁷ indicating a probable important pathophysiological mechanism in IBS. The mechanism behind visceral hypersensitivity is not clear, and there are several proposed causes¹¹⁸ (by themselves or in combination), such as: (1) mechanical or chemical sensitization of receptors in the rectal mucosa¹¹⁹⁻¹²¹; (2) sensitization of the dorsal horn in the spinal cord¹²²⁻¹²³; (3) dysfunction in more central modulating mechanisms such as pain inhibitory/facilitatory networks, the emotional arousal network, and/or networks involved in afferent processing.⁷³⁻¹²⁴⁻¹²⁵ However, visceral hypersensitivity can be difficult to measure, and response bias to experimental sensations in IBS patients, for example, has been proposed as an important factor.⁹³⁻¹²⁶⁻¹²⁷ Experimental sensations from the gastrointestinal tract have traditionally been provoked by rectal balloon distensions. This method provides a

safe, relevant, and reliable experimental model in the study of visceral pain and sensations.¹²⁸⁻¹³⁰ The device commonly used when examining visceral sensitivity is called a barostat. The barostat delivers computer-controlled distensions with precise and static pressure.

Brain responses to treatment and placebo in IBS

A few studies have examined the brain mechanisms of treatment in IBS. In an fMRI study, brain responses to rectal distensions were compared in female IBS patients treated with amitriptyline or placebo.¹³¹ Amitriptyline, a tricyclic antidepressant with antinociceptive properties, is widely used to treat diseases related to chronic pain. The main finding of the study was that a low dose of amitriptyline significantly reduced the BOLD response in the pregenual anterior cingulate cortex and left posterior cortex. However, the reduction was only seen when subjects were exposed to additional stress in the form of stressful sounds.

In another study, the effects on brain response to rectal distensions after a course of cognitive behavioral therapy was examined using PET.²⁵ Even though no changes in brain response during the distensions were observed, the authors demonstrated that treatment effect was correlated with reduced resting activity in the limbic system, including the amygdala and subregions of the anterior cingulate cortex, regions involved in the perception of pain.

An emerging field in IBS research is the mechanisms underlying placebo effects observed in treatment and experimental studies.^{107 132-135} Brain imaging studies have revealed mechanisms involved in the placebo response in both healthy individuals and IBS patients.^{106 108-110 136-139} Several brain regions implicated in pain-related processing of visceral input have been shown to be affected by successful placebo therapy, including the somatosensory cortices, thalamus, anterior cingulate cortex, prefrontal cortex, and insula. Also, the importance of expectation was evident as notable placebo effects were observed during the expectation of visceral pain.^{104 138} These studies prove that placebo effects not merely lie in response bias but have distinct brain mechanisms. Also, placebo has the potential to maximize treatment effects when used in an ethical manner.¹⁴⁰

Summary

The current thesis focuses on the importance of brain-gut interaction in the pathophysiological mechanisms of IBS, and the effects of treatment on the brain. Numerous brain imaging studies have demonstrated that IBS patients show abnormal brain activity during rectal distensions, but also during the expectation of rectal stimuli.^{84 86 102 141-145} An altered brain-gut interaction is thought to play an important role in the cardinal symptoms of IBS, particularly in the case of abdominal pain.^{146 147} Increased knowledge about how the brain receives and processes signals from the gastrointestinal tract is important in order to understand the basic pathophysiological mechanisms of IBS. Specifically, there is a need for further knowledge about how subgroups of IBS patients differ. In our studies we examined how IBS patients with or without rectal hypersensitivity differed in their brain response to the delivery and the expectation of standardized rectal distensions. Deeper understanding of pathophysiological mechanisms will most certainly provide the opportunity for more effective treatments in the future.¹⁴⁸ To further elucidate the central pathophysiological mechanisms in IBS, we investigated the brain responses to rectal distensions and expectation of these stimuli, after a successful course of hypnotherapy or educational intervention.

AIMS

The overall aim of the thesis was to further study the central pathophysiological mechanisms involved in IBS.

Specifically, we aimed to identify differences in brain response to standardized repeated rectal distensions and expectation of these stimuli between IBS patients with or without perceptual rectal hypersensitivity, and healthy controls.

Furthermore, we aimed to investigate IBS patients' brain responses to standardized rectal distensions and the expectation of these stimuli after either a successful course of hypnotherapy or educational intervention.

METHODS

Subjects

To recruit patients, information about the study was given to general practitioners in the catchment area of the Department of Gastroenterology, Linköping University Hospital, Sweden. Previously referred IBS patients attending the Department of Gastroenterology were also asked to participate. Twenty healthy, right-handed women were recruited by advertisement. Healthy controls were monetarily compensated for participating. Patients and healthy controls interested in participating received written and oral information about the study. If patients and healthy controls fulfilled basic criteria, an appointment with a physician (Mats Lowén or Susanna Walter) at the Department of Gastroenterology was completed. At this appointment, questionnaires were filled out and inclusion and exclusion criteria were reviewed. Inclusion criteria for participants included female sex, right handed, and for patients, fulfilling Rome III criteria. Exclusion criteria included: organic gastrointestinal disease; metabolic, neurologic, or psychiatric disorders; nicotine intake; centrally acting medication; pacemaker; metal implants in the brain; and claustrophobia. Additional exclusion criterion for healthy controls was a medical history of gastrointestinal symptoms or complaints. If necessary, organic gastrointestinal disease was excluded in patients by standard diagnostic procedures such as blood and fecal samples and/or endoscopic investigations. In total, 44 women with IBS and 20 healthy controls were included in the studies. An overview of the studies is presented in Figure 2. In papers I and II, 11 patients and 2 healthy controls were excluded from data analysis due to: incomplete data collection ($n = 2$); balloon leakage ($n = 1$); excess motion ($n = 4$); major scanner artifacts ($n = 2$); and inability to tolerate the procedure ($n = 4$). In total, data from 18 healthy controls and 33 IBS patients were analyzed in these papers. In Paper III, 25 patients were assigned to hypnotherapy treatment and 16 to educational intervention. The treatment assigned depended on the availability of the hypnotherapist, and was made in weekly blocks. Eighteen patients completed the hypnotherapy. Reasons for withdrawal were: start of centrally acting medication ($n=1$); noncompliance with the study protocol ($n=5$); panic attacks during hypnotherapy ($n=1$). In the hypnotherapy group, two fMRI data sets were excluded from analysis due to exceeding predefined motion parameters ($n=1$) and major scanner artifact ($n=1$). Thirteen patients

completed the educational intervention. Reasons for discontinuation were: pregnancy (n=1); start of centrally acting medication (n=1); unrelated disease (n=1). In the educational intervention group, four fMRI data sets were excluded from analysis due to exceeding predefined motion parameters (n=3) and major scanner artifact (n=1). In total, there were complete data sets from 16 patients in the hypnotherapy group, 9 patients in the educational intervention group, and 18 healthy controls.

Figure 2. Flow chart summarizing the progress of patients and healthy controls during the course of the studies. Data used for each paper are indicated.

Questionnaires

The *IBS severity scoring system (IBS-SSS)* is used to measure IBS symptom burden.¹⁴⁹ Five items are included: abdominal pain severity, pain frequency, bowel distension, bowel habit dysfunction, and quality of life. Total maximum score is 500. Mild, moderate, and severe symptoms are indicated by scores of 75–175, 175–300 and >300 respectively. Treatment responders were defined a priori as a pre–post treatment reduction of at least 50 points in IBS-SSS (Paper III).¹⁴⁹

The *Visceral sensitivity index (VSI)* consists of 15 items graded on a 6-point scale, and measures gastrointestinal symptom-specific anxiety by assessing the cognitive, affective, and behavioral responses to fear of gastrointestinal sensations, symptoms, and the context in which sensations and symptoms occur.^{150 151}

The *Hospital Anxiety and Depression Scale (HAD)* is a self-assessment scale developed for detecting states of depression and anxiety in medical outpatient settings.¹⁵² The scale consists of 14 items (7 relating to anxiety and 7 relating to depression), which are graded on a 4-point scale.

The *Gastrointestinal symptom diary* is composed of validated diary cards used by subjects to record gastrointestinal symptoms during 2 weeks.¹⁵³ Along a 24-hour time axis, subjects recorded episodes of abdominal pain and graded the pain intensity as light, moderate, or intense. The diary was filled out before and after treatment. Data reported in Paper III.

Ratings of present intensity and unpleasantness of gastrointestinal symptoms is a scale ranging from 0 to 10 that was used to assess: (1) the subject's current intensity of gastrointestinal symptoms; and (2) abdominal unpleasantness during the fMRI session protocol.

Hypnotherapy

The subjects assigned to the hypnotherapy treatment group were treated by an experienced hypnotherapist with a standard course consisting of seven 1-hour sessions of individual hypnotherapy at a rate of approximately one session per week. The gut-directed hypnotherapy script has been in clinical use for numerous years. During the first session, the hypnotherapist established a working alliance with the patient and explained the hypnotherapy treatment. The following six self-hypnosis training sessions consisted of inducing the hypnotic state and delivering hypnotic suggestions, with the goal of reducing threat perception and gut symptoms, and increasing overall physical relaxation. Subjects received a pre-recorded compact disc with the same content as in the clinical sessions. Subjects were instructed to practice at home on a daily basis.

Educational intervention

The subjects assigned to the educational intervention group received seven individual sessions, with tutorials covering gastrointestinal anatomy and physiology, IBS symptoms, diet and the theory behind different IBS treatments. The sessions consisted of 20 minutes studying the material covering the session topic, followed by a 25-minute discussion. Tutors included gastroenterologists and experienced physiotherapists specialized in functional bowel and pelvic floor disorders.

fMRI experimental protocol

An overview of the fMRI experimental protocol is shown in Figure 3. Dates of sessions did not coincide with menses. The subjects were instructed to fast at least four hours before arriving at the experiment site. A highly compliant rectal balloon was installed by an experienced assistant nurse. Subjects were then placed in the fMRI scanner and equipped with high-resolution MR goggles (Resonance Technology, Inc., Los Angeles, CA, USA) to enable presentation of experimental visual cues, and headphones allowing two-way communication. Superlab Pro 4 (Cedrus Corp, San Pedro, CA, USA) was used for experimental design and

information presentation. After a 5-minute rest and acclimatization phase, resting state fMRI data was collected over a 10-minute period (data not reported here).

Figure 3. Overview of the fMRI session protocol. After 5-min rest and collection of resting-state data (data not reported), rectal sensitivity thresholds were determined using ascending method of limits: 0 = no sensation, 1 = sensation, 2 = urgency and 3 = maximum tolerable pressure. Twenty visually-cued high- and 18 low-intensity rectal distensions were pseudorandomly delivered with 18 rest periods. Ratings of current gastrointestinal symptoms and unpleasantness are indicated by *. Rating of last high- and low-intensity rectal distension is indicated by +.

Determination of perception thresholds

Subjects underwent a thresholding procedure using a barostat (Dual Drive Barostat, Distender series II; G&J Electronics, Inc, Toronto, Canada). Perceptual visceral sensitivity was tested using the ascending method of limits (AML) with intermittent phasic isobaric rectal distensions lasting 30 seconds and with pressure increments of 5 mmHg. The interval between distensions was 60 seconds. After each distension, subjects rated sensation on a 4-point scale: 0, no sensation; 1, first sensation/some sensation; 2, urge to defecate; and 3, maximum tolerable distension. When subjects reported “3” the protocol was ended.

Expectation and visceral stimuli fMRI paradigm

Twenty high- (45 mm Hg) and 18 low- (15 mm Hg) intensity rectal distensions with duration of 15 seconds were delivered in a pseudorandomized order, divided in two identical runs. Each distension was preceded by a visual cue (duration 3 seconds) predicting the intensity of the distension (certain expectation). The high- and low-intensity distensions were signaled by an orange and blue cue respectively. The time between the cue and the beginning of the inflation was jittered by 2, 4, or 6 seconds. Between distensions, the subjects had 18 rest periods (safety baseline) of 14, 16 or 18 seconds' duration, signaled by a gray cue (3 seconds), in pseudorandomized order. The total duration of the visceral stimuli paradigm was 24 minutes. Subjects rated the present intensity and unpleasantness of GI symptoms before and after thresholding and at the end of the experiment. Directly completing the fMRI distension protocol, subjects rated the most recent low- and high-intensity distensions. Following that, high-resolution anatomical images were acquired. For paper II, the expectation and visceral stimuli fMRI paradigm was divided into two identical phases (Figure 4).

Figure 4. For paper II the expectation and visceral stimuli fMRI paradigm was divided into two identical phases consisting of 10 high-intensity and 9 low-intensity cued distensions.

fMRI data acquisition

A 1.5 T MR scanner (Philips Achieva; Philips, Best, The Netherlands) was used to collect MRI and fMRI images. Functional brain images were acquired using a blood oxygen level-dependent (BOLD) sensitive gradient echo sequence, using the following acquisition parameters: repetition time (TR) 3 seconds, echo time (TE) 40 milliseconds, flip angle 90°, voxel size 3 x 3 x 3 mm. A total of 35 slices were acquired in interleaved mode with a 0.5 mm slice gap.

fMRI data analysis

Statistical parametric mapping 8 (SPM8) (Wellcome Trust Centre for Neuroimaging, London, UK, <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) was used for the preprocessing and statistical analysis of the BOLD fMRI data. The 482 volumes acquired were realigned to the first image of the time series to correct for movement during scanning. The images were normalized to a standard brain atlas in Montreal Neurological Institute (MNI) space to allow for voxel-wise statistical testing between subjects. Finally, the images were smoothed using an 8-mm full width half maximum Gaussian kernel to reduce image noise and to ameliorate differences in intersubject localization. Subjects were excluded from further analysis if the BOLD fMRI images exceeded the predefined movement threshold of > 3 mm or contained scanner artifacts when visually inspected. To estimate the correlation between the time series of the measured BOLD response and the evoked rectal stimuli, we applied a general linear model (GLM) with 4 regressors representing the different conditions of the stimuli, 1 regressor representing the safety baseline, and 6 regressors representing movements during scanning. The 4 conditions during the fMRI experiment were as follows: (1) expectation of high-intensity distension; (2) high-intensity distension (45 mmHg); (3) expectation of low-intensity distension; and (4) low-intensity distension (15 mmHg). These conditions were compared to the safety baseline in a first-level analysis of fixed effects in each subject.

In the second-level analysis a region of interest (ROI) approach was applied. A priori defined ROIs included the following regions: the amygdala, hippocampus, pregenual and subgenual anterior cingulate cortex (pACC and sgACC), anterior midcingulate cortex (aMCC), periaqueductal gray (PAG), thalamus, ventrolateral and dorsolateral prefrontal cortex (vLPFC and dLPFC), and ventral and dorsal anterior insula (aINS), mid insula (mINS) and posterior insula (pINS). Brain regions studied in the current thesis are presented in Figure 5.

Figure 5. Brain regions studied in the current thesis.

adINS anterior dorsal insula, *aMCC* anterior midcingulate cortex, *avINS* anterior ventral insula, *dLPFC* dorsolateral prefrontal cortex, *mINS* mid insula, *pACC* pregenual anterior cingulate cortex, *pINS* posterior insula, *sgACC* subgenual anterior cingulate cortex, *vLPFC* ventrolateral prefrontal cortex. Amygdala, hippocampus, periaqueductal gray and thalamus are not illustrated.

ROIs were constructed using WFU Pick Atlas implemented in SPM8 except for subregions of the insula, which were drawn by hand.¹⁵⁴⁻¹⁵⁶ Contrast maps were initially thresholded at $p < 0.01$, uncorrected. Results were considered to be significant if the peak voxel P-value in ROIs was less than 0.05 corrected for multiple comparisons using family-wise error (FWE) correction.

Paper I

An ANOVA confirmed differences between groups. Two-sample t-tests were used to test for group differences in brain activity during the distension and expectation conditions.

Paper II

An ANOVA confirmed differences between groups. For comparison of early and late phase within groups, paired t-tests were performed. Between-group differences were evaluated by two-sample t-tests.

Paper III

Separate one-sample t-tests were performed to evaluate brain response in the hypnotherapy and educational intervention groups. To evaluate treatment effects within the two treatment groups, paired t-tests were used. To compare treatment effects, difference images of activity between, before, and after were created in SPM8 and entered in two-group t-tests. Correlation analysis was performed between significant pre–post treatment changes in symptoms of all therapy responders and, correspondingly, significant pre–post treatment changes of BOLD response in ROIs. For the correlation analysis, changes in symptoms were entered as a covariate in SPM8 and inclusively masked by the significantly changed cluster as estimated by the analysis of treatment effects. Eigenvariates in peak voxels were extracted as measures of the correlation. The eigenvariates represent the β -values in the regression model. The correlation analysis between the eigenvariates and the pre–post treatment effects in symptoms (Pearson's r) was performed in GraphPad Prism 4 (GraphPad Software, Inc, La Jolla, CA, USA). The alpha level for significance was set at 0.05.

Ethical approval

The study protocol was approved by the Regional Ethical Review Board, Linköping, Sweden (DNR M71-09). Written and oral informed consent was obtained from all participants.

RESULTS

Classification of visceral sensitivity and clinical characterization of IBS patients

Classification of the IBS patients’ visceral sensitivity was based on the data from the healthy controls: there was, by definition, no overlap in maximum tolerable rectal distension pressure between hypersensitive IBS patients and healthy controls (Figure 6). Healthy controls (n = 18) had a median maximum tolerable rectal pressure of 55 mmHg (range 40–70). Eighteen IBS patients had a maximum tolerable pressure of 40 mmHg or higher (median 45, range 40–65), and were therefore considered to be normosensitive to visceral stimuli. Fifteen IBS patients had a maximum tolerable rectal pressure of less than 40 mmHg (median 30, range 25–35), and were considered to be hypersensitive to visceral stimuli. The hypersensitive IBS patients had significantly lower thresholds for first sensation and urgency than the normosensitive IBS patients and the healthy controls.

Figure 6. Maximum tolerable rectal pressure in hypersensitive IBS, normosensitive IBS, and healthy controls. There was no statistical difference in maximum tolerable pressure between normosensitive IBS and healthy controls. Median and range are shown. *NS* not significant.

The baseline clinical data for IBS patients and healthy controls are presented in Table 1. The normosensitive and hypersensitive IBS patients were similar in terms of IBS symptom severity, IBS duration, anxiety and depression symptoms, and gastrointestinal symptom-related anxiety. According to the IBS-SSS, 11 hypersensitive and 10 normosensitive subjects had severe symptoms. IBS patients as a group had significantly higher anxiety and depression scores than healthy controls. There were no significant differences between the group that received hypnotherapy and the group that received educational intervention regarding IBS symptom severity, anxiety and depression symptoms, gastrointestinal symptom-related anxiety, or perceptual rectal distension pressure thresholds.

Table 1. Age, evaluation of anxiety and depression, IBS duration, IBS symptom burden, and gastrointestinal symptom-specific anxiety in hypersensitive IBS, normosensitive IBS, and healthy controls.

	Hypersensitive IBS (n=15)	Normosensitive IBS (n=18)	Healthy controls (n=18)	p-value ^A	p-value ^B	p-value ^C
Mean age, years (range)	40.3 (21-60)	32.5 (20-60)	32.5 (21-54)	0.054	0.046	0.987
Mean HAD anxiety (range)	7.4 (2-17)	8.2 (0-17)	3.0 (0-11)	0.764	0.005	0.002
Mean HAD depression (range)	3.3 (0-8)	3.7 (1-10)	1.3 (0-3)	0.850	0.011	0.009
Mean duration, years (range)	13.8 (2-44)	13.2 (1.5-35)		0.885		
Mean IBS-SSS (range)	362.3 (271-484)	319 (156-455)		0.099		
Mean VSI (range)	44.7 (16–63)	44.3 (8–68)		0.950		

Results calculated by using unpaired t-tests.

HAD Hospital Anxiety and Depression; IBS-SSS IBS Severity Scoring System; VSI Visceral Sensitivity Index.

^A Comparison between hypersensitive IBS patients and normosensitive IBS patients.

^B Comparison between hypersensitive IBS patients and healthy controls.

^C Comparison between normosensitive IBS patients and healthy controls.

Brain responses to rectal distension and expectation of rectal distension

Results presented stem from ROI analyses. Figure 7 presents an overview of brain regions and networks related to visceral sensation and IBS with findings in the current thesis.

Figure 7. Brain regions and networks related to visceral sensation and IBS with findings in the current thesis. *pACC* pregenual anterior cingulate cortex, *aINS* anterior insula, *pINS* posterior insula, *aMCC* anterior midcingulate cortex, *PAG* periaqueductal gray, *dlPFC* dorsolateral prefrontal cortex, *vIPFC* ventrolateral prefrontal cortex.

IBS patients compared to healthy controls

Complete experiment results demonstrated that the IBS patients as a group had greater BOLD signals than healthy controls in the left vIPFC in response to the high-intensity distension and in the left mINS during the low-intensity distension. During expectation of the high-intensity distension, IBS patients had more activation in the right ventral aINS, right mINS, and right hippocampus than healthy controls. There were no regions with significantly greater BOLD response in healthy controls than in IBS patients.

Normosensitive IBS patients compared to healthy controls

Complete experiment results demonstrated that the normosensitive IBS patients and healthy controls did not differ significantly in their BOLD response to the high- or low-intensity distensions or expectation of low intensity distension. During expectation of the high-intensity distension, the normosensitive IBS group had more BOLD response than the healthy controls in the right hippocampus. Healthy controls had more activation than the normosensitive IBS group during the low-intensity distension in the right aINS. During the early and late phase, normosensitive IBS patients and healthy controls had a similar BOLD response, though healthy controls has greater BOLD response in the right dIPFC, vIPFC, and left thalamus during the low-intensity distension in the late phase.

Hypersensitive IBS patients compared to healthy controls

Complete experiment results demonstrated that the hypersensitive IBS patients had greater BOLD response compared with healthy controls during the high-intensity rectal distension in the left pINS, left pACC and left thalamus. During the late phase, hypersensitive IBS patients had greater BOLD response in multiple brain regions and networks associated with visceral sensation during high- and low-intensity distensions compared with healthy controls. In addition, a similar pattern was seen during expectation of low-intensity distension.

Hypersensitive IBS patients compared to normosensitive IBS patients: distensions

Figure 8 presents brain regions and networks in which hypersensitive IBS patients had greater BOLD response than normosensitive IBS patients during the early and late phases of the experiment and the complete experiment during high- and low-intensity distensions. The two IBS groups had a similar brain response during the early phase for both distensions. During the high-intensity distension hypersensitive IBS patients had a greater BOLD response in several brain regions and networks, both during the complete experiment and the late phase. Regions with significant findings included: the pACC, subregions of the insula, aMCC, and dIPFC. Differences in BOLD response during the low-intensity distension became apparent only during the late phase. Regions with significant findings included: subregions of the insula, right aMCC, prefrontal cortices, and left hippocampus.

Brain regions and networks with greater BOLD response in hypersensitive IBS (n=15) compared with normosensitive IBS (n=18): distensions				
		Early phase	Late phase	Complete
High-intensity distension	Central executive	NS	Central executive R dlPFC	Central executive R dlPFC
	Salience	NS	Salience L + R aMCC R ventral aINS	Salience R aMCC
	Emotional arousal	NS	Emotional arousal L + R pACC	Emotional arousal L + R pACC
	Central autonomic	NS	Central autonomic L + R aMCC R ventral aINS	Central autonomic R aMCC
	Sensori-motor	NS	NS	Sensorimotor L pINS
Low-intensity distension	Central executive	NS	Central executive R dlPFC	NS
	Salience	NS	Salience R dorsal aINS R + L ventral aINS R aMCC	NS
	Emotional arousal	NS	Emotional arousal L Hippocampus L + R vlPFC	NS
	Central autonomic	NS	Central autonomic R dorsal aINS R + L ventral aINS R aMCC	NS
	Sensori-motor	NS	Sensorimotor L pINS	NS

Figure 8. Brain regions and networks where hypersensitive IBS patients had significantly more blood oxygen level dependent response during *rectal distensions* than normosensitive IBS patients during early and late phases of the experiment and complete experiment. Results calculated using two-sample t-tests and thresholded at $p \leq 0.05$, corrected for multiple comparisons (FWE) at peak level. *pACC* pregenual anterior cingulate cortex, *aINS* anterior insula, *pINS* posterior insula, *aMCC* anterior midcingulate cortex, *dlPFC* dorsolateral prefrontal cortex, *vIPFC* ventrolateral prefrontal cortex. L left, R right. NS no significant findings.

Hypersensitive IBS patients compared to normosensitive IBS patients: expectations

Figure 9 presents brain regions and networks in which hypersensitive IBS patients had greater BOLD response than normosensitive IBS patients during the early and late phases of the experiment and complete experiment during expectation of high- and low-intensity distensions. During the early phase the BOLD response was similar in both hypersensitive IBS patients and normosensitive IBS patients. In the late phase, during expectation of high-intensity distension, hypersensitive IBS patients showed more BOLD response than normosensitive IBS patients in the aINS as well as the pINS and dlPFC. During the late phase, expectation of low-intensity rectal distension led to a greater BOLD response in the subregions of the insula, right aMCC, and vlPFC. Complete experiment results demonstrated that the expectation of high-intensity distension led to greater BOLD response in the right pINS, and thalamus, and expectation of low-intensity distension led to greater BOLD signal in the right aINS in hypersensitive IBS patients compared to normosensitive IBS patients.

Brain regions and networks with greater BOLD response in hypersensitive IBS (n=15) compared with normosensitive IBS (n=18): expectations				
		Early phase	Late phase	Complete
Expectation of high-intensity distension	Central executive	NS	Central executive R dlPFC	NS
	Saliency	NS	Saliency R ventral aINS	NS
	Emotional arousal	NS	NS	NS
	Central autonomic	NS	Central autonomic R ventral aINS	NS
	Sensori-motor	NS	Sensorimotor L pINS	Sensorimotor R pINS R Thalamus
Expectation of low-intensity distension	Central executive	NS	NS	NS
	Saliency	NS	Saliency L + R dorsal aINS L + R ventral aINS	Saliency R ventral aINS
	Emotional arousal	NS	Emotional arousal L Hippocampus R vlPFC	NS
	Central autonomic	NS	Central autonomic L + R dorsal aINS L + R ventral aINS	Central autonomic R ventral aINS
	Sensori-motor	NS	NS	NS

Figure 9. Brain regions and networks where hypersensitive IBS patients had significantly more blood oxygen level dependent response during *expectation of rectal distensions* than normosensitive IBS patients during early and late phases of the experiment and complete experiment. Results calculated using two-sample t-tests and thresholded at $p \leq 0.05$, corrected for multiple comparisons (FWE) at peak level. *aINS* anterior insula, *pINS* posterior insula, *dlPFC* dorsolateral prefrontal cortex, *vIPFC* ventrolateral prefrontal cortex. L left, R right. NS no significant findings.

Early vs late phase

Figure 10 and Figure 11 present brain regions where hypersensitive IBS patients and normosensitive IBS patients demonstrated significantly increased or decreased BOLD response during the late phase of the experiment. Overall, hypersensitive IBS patients showed increased regional BOLD response over time, while normosensitive IBS patients showed reduced BOLD response during both high- and low-intensity stimulus and expectation of low-intensity stimulus.

Hypersensitive IBS patients

In hypersensitive IBS patients, significant BOLD increase was observed in the left pACC during high-intensity distension, while during low-intensity distension, increases were seen in the left and right aINS, left mINS, and right aMCC. During expectation of high-intensity distension, BOLD increase was observed in the left hippocampus, while during expectation of low-intensity distension there was a significant increase in the left dorsal aINS, right ventral aINS, and, left and right aMCC.

Normosensitive IBS patients

In normosensitive IBS patients, BOLD reductions in the right amygdala were seen during low-intensity distension expectation, while no significant BOLD reductions were observed during the expectation of high-intensity distension. During high-intensity distension, BOLD decreases were seen in the right dorsal and ventral aINS, left pINS, right dIPFC, and right vIPFC. During low-intensity distension, reductions were seen in the right amygdala, left and right dorsal aINS, right mINS and left pINS.

Brain regions and networks with increased or decreased BOLD response during the late phase compared with the early phase: distensions					
		Hypersensitive IBS (n=15)		Normosensitive IBS (n=18)	
		Increase	Decrease	Increase	Decrease
High-intensity distension	Central executive	NS	NS	NS	Central executive R dlPFC
	Saliency	NS	NS	NS	Saliency R dorsal aINS R ventral aINS
	Emotional arousal	Emotional arousal L pACC	NS	NS	Emotional arousal R vlPFC
	Central autonomic	NS	NS	NS	Central autonomic R dorsal aINS R ventral aINS
	Sensori-motor	NS	NS	NS	Sensorimotor L pINS
Low-intensity distension	Central executive	NS	NS	NS	NS
	Saliency	Saliency L + R ventral aINS R aMCC	NS	NS	Saliency R Amygdala L + R dorsal aINS
	Emotional arousal	NS	NS	NS	Emotional arousal R Amygdala
	Central autonomic	Central autonomic L + R ventral aINS R aMCC	NS	NS	Central autonomic R Amygdala L + R dorsal aINS
	Sensori-motor	NS	NS	NS	Sensorimotor L pINS

Figure 10. Brain regions and networks where hypersensitive IBS patients and normosensitive IBS patients had significantly increased or decreased blood oxygen level dependent response during the late phase of the experiment during *rectal distensions*. Results calculated using paired t-tests and thresholded at $p \leq 0.05$, corrected for multiple comparisons (FWE) at peak level. *pACC* pregenual anterior cingulate cortex, *aINS* anterior insula, *pINS* posterior insula, *aMCC* anterior mid cingulate cortex, *dlPFC* dorsolateral prefrontal cortex, *vlPFC* ventrolateral prefrontal cortex. L left, R right. NS no significant findings.

Brain regions and networks with increased or decreased BOLD response during the late phase compared with the early phase: expectations					
		Hypersensitive IBS (n=15)		Normosensitive IBS (n=18)	
		Increase	Decrease	Increase	Decrease
Expectation of high-intensity distension	Central executive	NS	NS	NS	NS
	Salience	NS	NS	NS	NS
	Emotional arousal	Emotional arousal L Hippocampus	NS	NS	NS
	Central autonomic	NS	NS	NS	NS
	Sensori-motor	NS	NS	NS	NS
Expectation of low-intensity distension	Central executive	NS	NS	NS	NS
	Salience	Salience L dorsal aINS R ventral aINS L + R aMCC	NS	NS	Salience R Amygdala
	Emotional arousal	NS	NS	NS	Emotional arousal R Amygdala
	Central autonomic	Central autonomic L dorsal aINS R ventral aINS L + R aMCC	NS	NS	Central autonomic R Amygdala
	Sensori-motor	NS	NS	NS	NS

Figure 11. Brain regions and networks where hypersensitive IBS patients and normosensitive IBS patients had significantly increased or decreased blood oxygen level dependent response during the late phase of the experiment during *expectation of rectal distensions*. Results calculated using paired t-tests and thresholded at $p \leq 0.05$, corrected for multiple comparisons (FWE) at peak level. *aINS* anterior insula, *aMCC* anterior midcingulate cortex. *L* left, *R* right. *NS* no significant findings.

Behavioral responses to treatment

Subjects who completed hypnotherapy ($n = 18$) reduced their IBS-SSS score from 342 (SD 65) to 233 (SD 89) ($p < 0.0001$), and their VSI score from 48 (SD 18) to 34 (SD 18) ($p < 0.0001$). Subjects who completed educational intervention ($n = 13$) reduced their IBS-SSS score from 340 (SD 77) to 256 (SD 94) ($p = 0.02$), and their VSI score from 48 (SD 15) to 36 (SD 13) ($p = 0.005$). There was no statistical difference in improvement in IBS-SSS or VSI between the two treatment groups. Thirteen subjects in the hypnotherapy group and 7 subjects in the educational intervention group responded to therapy measured as a decrease in IBS-SSS score of 50 points or more. Combined responders from both treatment groups ($n = 20$) demonstrated a significant decrease in VSI score from mean 47 (SD 17) to 33 (SD 17) ($p < 0.0001$). There were no significant changes in rectal sensitivity.

Brain responses to successful treatment

Figure 12 and Figure 13 present intragroup pre-post treatment BOLD response of hypnotherapy and educational intervention responders.

Hypnotherapy responders

Hypnotherapy responders ($n=13$) demonstrated a significant pre-post treatment BOLD attenuation in the left aINS during both the expectation and delivery of high-intensity distensions, and also showed a reduction in then left pINS during the high-intensity distension. In addition, hypnotherapy responders reduced their BOLD response in the left hippocampus, left pINS, and right thalamus, but showed increased BOLD response in the right amygdala, right hippocampus, and left PAG during expectation of low-intensity distension. Decreased BOLD response in the left pINS was seen during low-intensity distension.

Educational intervention responders

Educational intervention responders ($n=7$) demonstrated a decrease in BOLD response in the left aINS only seen during the high-intensity distension, as well as a decrease in the left vIPFC. Educational intervention responders exhibited increased activity in the right hippocampus during expectation of low-intensity distension.

Hypnotherapy responders compared to educational intervention responders

Significantly more pronounced pre–post reductions in BOLD response were observed in the hypnotherapy responders compared with the educational intervention group responders during low-intensity distension, and included the right aINS, right mINS and right aMCC.

Brain regions and networks with increased or decreased BOLD response after successful treatment: distensions					
		Hypnotherapy (n=13)		Educational intervention (n=7)	
		Increase	Decrease	Increase	Decrease
High-intensity distension	Central executive	NS	NS	NS	NS
	Salience	NS	Salience L ventral aINS	NS	Salience L dorsal aINS L ventral aINS
	Emotional arousal	NS	NS	NS	Emotional arousal L vIPFC
	Central autonomic	NS	Central autonomic L ventral aINS	NS	Central autonomic L dorsal aINS L ventral aINS
	Sensori-motor	NS	Sensorimotor L pINS	NS	NS
Low-intensity distension	Central executive	NS	NS	NS	NS
	Salience	NS	NS	NS	NS
	Emotional arousal	NS	NS	NS	NS
	Central autonomic	NS	NS	NS	NS
	Sensori-motor	NS	Sensorimotor L pINS	NS	NS

Figure 12. Brain regions and networks significantly affected by a successful course of hypnotherapy or educational intervention during *rectal distensions*. Results calculated using paired t-tests and thresholded at $p \leq 0.05$, corrected for multiple comparisons (FWE) at peak level. *aINS* anterior insula, *pINS* posterior insula, *vIPFC* ventrolateral prefrontal cortex. *L* left, *R* right. *NS* no significant findings.

Brain regions and networks with increased or decreased BOLD response after successful treatment: expectations					
		Hypnotherapy (n=13)		Educational intervention (n=7)	
		Increase	Decrease	Increase	Decrease
Expectation of high-intensity distension	Central executive	NS	NS	NS	NS
	Saliency	NS	Saliency L dorsal aINS L ventral aINS	NS	NS
	Emotional arousal	NS	NS	NS	NS
	Central autonomic	NS	Central autonomic L dorsal aINS L ventral aINS	NS	NS
	Sensori-motor	NS	NS	NS	NS
Expectation of low-intensity distension	Central executive	NS	NS	NS	NS
	Saliency	Saliency R Amygdala	NS	NS	NS
	Emotional arousal	Emotional arousal R Amygdala R Hippocampus	Emotional arousal L Hippocampus	Emotional arousal R Hippocampus	NS
	Central autonomic	Central autonomic R Amygdala L PAG	NS	NS	NS
	Sensori-motor	NS	Sensorimotor L pINS R Thalamus	NS	NS

Figure 13. Brain regions and networks significantly affected by a successful course of hypnotherapy or educational intervention during *expectation of rectal distensions*. Results calculated using paired t-tests and thresholded at $p \leq 0.05$, corrected for multiple comparisons (FWE) at peak level. *aINS* anterior insula, *pINS* posterior insula, *PAG* periaqueductal gray. L left, R right. NS no significant findings.

Figure 14 shows BOLD response during high-intensity rectal distension before and after a successful course of hypnotherapy in IBS patients. BOLD response to the same stimuli in healthy controls is also presented. Before treatment, hypnotherapy responders had statistically significantly greater BOLD response than healthy controls during high-intensity distension in the left mINS, left and right pINS and left vIPFC. These differences were no longer seen after hypnotherapy.

Figure 14. Blood oxygen level dependent response during *high-intensity rectal distension* before (top panel) and after (middle panel) a course of successful hypnotherapy in IBS patients. Blood oxygen level dependent response to the same stimuli in healthy controls is shown in the bottom panel. Images are thresholded at $p < 0.01$, uncorrected. Red color indicates increased and blue color decreased blood oxygen level dependent response. Numbers indicate slice level. L left, R right.

GENERAL DISCUSSION

Difference in brain response between hypersensitive and normosensitive IBS patients

The differences in brain response to repeated, expected, and standardized rectal distensions between female IBS patients with similar self-reported symptom burden, general depression or anxiety, and gastrointestinal-specific anxiety but with or without perceptual visceral hypersensitivity (pre-experimentally determined by AML) can be explained by differences in how these IBS patients responded to repeated experimental stimuli during the course of the experiment. Even though the brain responses were similar between the IBS patients during the early phase of the experiment, they became substantially different during the late phase. The hypersensitive IBS patients demonstrated increased brain response in several brain regions and networks involved in visceral sensation and processing, both during high- and low-intensity rectal stimuli and expectation of these stimuli. In contrast, normosensitive IBS patients exhibited a reduction in BOLD response in several brain regions and networks, especially during rectal distensions. In summary, the results indicate that a subpopulation of IBS patients have a deficiency in the ability to habituate to repeated expected rectal distensions.

In healthy subjects, inhibitory activity in cortical pain processing and areas involved in descending pain modulation have been implicated in habituation to noxious stimuli.^{157 158} In chronic pain- and IBS-related diseases, habituation and sensitization to painful stimuli have been shown to differ when compared to healthy controls.^{111 159-161} Previous studies have demonstrated that the cingulate and insular cortical regions are involved in central pain sensitization.¹⁶²⁻¹⁶⁵ In the present thesis, during the high-intensity distension, hypersensitive IBS patients consequently exhibited more activation of the pACC, a region associated with the emotional arousal network and endogenous pain control,¹⁶⁶ relative to normosensitive IBS patients and healthy controls (Figure 8). When the brain activation pattern was examined, the difference was a non-occurring or attenuated deactivation of the pACC in the hypersensitive IBS patients compared to normosensitive IBS patients. The same pattern was seen in the dlPFC, a brain region involved in the central executive network and cognitive modulation of pain^{97 167} and the aMCC, a brain region implicated in the salience and central autonomic

network as well as in homeostasis.^{79 166} These deactivation patterns were seen in the healthy controls but were even more pronounced compared to normosensitive IBS patients in the brain regions described above. One interpretation of this might be that hypersensitive IBS patients engage pain inhibitory systems but that these systems do not seem to work in an appropriate way. Another indication of this is that during the late phase of the experiment, the hypersensitive IBS patients increased their BOLD response to high-intensity distension in the pACC (Figure 10). On the other hand, normosensitive IBS patients decreased the BOLD response during the rectal distension in the late phase in several regions and networks, including the pINS. Given the role of the primary interoceptive cortex, this decrease could be a consequence of activity in pain inhibitory systems, leading to a decreased inflow of peripheral signals by influence on, for example, the dorsal horn in the spinal cord.⁷³

In our studies, one might speculate that the dysfunctional activation of pain modulatory regions could in part cause sensitization to the low-intensity stimuli in hypersensitive IBS patients, expressed as an increased BOLD response in the aMCC and aINS (Figure 10 and Figure 11). Since brain response was increased both during expectation and delivery of a low-intensity rectal distension and there were no differences in the sensorimotor network, this indicates that a central rather than a peripheral sensitization occurred, though alternative explanations are possible. The aINS is a core region in the salience and central autonomic network, and has been shown to be activated in visceral pain studies and is a brain region implicated with several functions, including integration of afferent inputs, affective response including awareness of feelings, and regulation of physiological response.^{156 168-172} Since activity in the aINS was increased in hypersensitive IBS patients during the expectation of a low-intensity distension, one might presume that there is an increased awareness of the stimuli, and since activity in the aINS during the expectation of pain has previously been demonstrated,⁹⁵ there was a subsequent effect on the brain response to the coming stimuli. In the current studies we used a paradigm designed to dissociate the brain response to the expectation of rectal distension from the actual distension. This was done by using a pseudorandomized expectation period in accordance with other studies in the pain research field.^{105 173 174} Still, expectation of a coming stimulus greatly influences the brain response to

the actual stimulus,^{105 175} and it is probable that the response to the actual stimuli are influenced by the expectation period.

These studies were not specifically designed to study the significance of learning and conditioning to expected rectal distensions, but these factors have been demonstrated to affect the brain response in IBS patients in several studies.¹⁷⁶⁻¹⁷⁸ An indication that these processes were important in our studies were that during the course of the experiment, there was an increased activation of the hippocampus during expectation of high-intensity distension in hypersensitive IBS patients, and a decrease in activation of the amygdala during both low-intensity conditions in normosensitive IBS patients (Figure 10 and Figure 11). These regions have been shown to be important in, for example, formation and retrieval of episodic memory, Pavlovian fear learning, and emotional modulation of memory formation, as well as attention.¹⁷⁹⁻¹⁸¹

Brain responses to successful treatment

In our study, a course of hypnotherapy or educational intervention associated with reduction of self-reported IBS symptoms, altered the brain response to cued rectal distension in IBS patients with similar symptom burden, gastrointestinal-related anxiety, general anxiety and depression symptoms, and similar sensory rectal pressures thresholds. Brain regions significantly affected by successful treatment are presented in Figure 12 and Figure 13.

The educational intervention responders had a decreased brain response in the vIPFC indicating a prefrontal cortex mechanism was involved in the treatment effect. Treatment response in both groups was associated with attenuated brain response to rectal stimuli in subregions of the insula. Specifically, there was a reduction of activity in the aINS during the high-intensity rectal distension in both treatment groups. This finding indicates an ability to reduce the affective importance of the incoming signals. This is reinforced by the fact that the reduction in the aINS was correlated with reduction in gastrointestinal-specific anxiety.

Only the group which received successful hypnotherapy decreased activation of the pINS during high- and low-intensity rectal distensions. Since the pINS is considered to be the primary interoceptive cortex, it is conceivable that this represented a decrease in afferent signaling to the brain, although there are other possible explanations.⁷³ Also, when examining the brain responses of successful treatment during the expectation conditions, there are several interesting findings indicating hypnotherapy-induced effects on endogenous pain modulatory systems. The hypnotherapy responders had reduced brain response in the aINS during the expectation of the high-intensity distension, indicating that diminished affective importance was given to input relating to the coming stimulus. Or, perhaps more plausibly, this indicate an ability to attenuate interoceptive signals as the pINS was less activated in the subsequent distension. The increased response in the amygdala and PAG during the expectation of low-intensity distension, and the subsequent decrease in the pINS is consistent with attenuating visceral signals by engagement of endogenous pain modulatory circuits.¹⁸² Again, altered activation of the hippocampus and amygdala in treatment responders is an indication that factors such as learning and conditioning must be taken into account when interpreting the results. As shown in Figure 14, IBS patients seem to be more similar to healthy controls in their brain responses to high-intensity rectal distension, after a successful course of hypnotherapy.

CONCLUSIONS

In this thesis, the central pathophysiological mechanisms involved in IBS were studied. In papers I and II, differences in brain response to standardized repeated rectal distensions and expectation of these stimuli were identified between IBS patients with or without perceptual rectal hypersensitivity. In paper III, the effects on brain response after a successful course of hypnotherapy and educational intervention in IBS patients, were identified.

The results from papers I and II demonstrate that, a subpopulation of IBS patients has a deficiency in the ability to habituate to repeated rectal distensions and expectation of these stimuli, possibly due to disturbances in the pain inhibitory systems. One could speculate that, deficient habituation to signals from the gut could be an important pathophysiological factor of visceral hypersensitivity in IBS.

The results from paper III demonstrate that, successful treatment of IBS with hypnotherapy and educational intervention is accompanied by alterations in brain response to standardized rectal distensions and expectation of these stimuli. The findings of this thesis indicate that, processing of visceral input is modulated by psychological treatment and educational intervention. We also showed that, successful treatment may have a normalizing effect on the central processing abnormality of visceral signals in IBS. For subgroups of IBS patients with predominant disturbances in central processing of visceral signals, psychological treatment will certainly decrease symptom burden, and most probably improve quality of life.

METHODOLOGICAL CONSIDERATIONS

There are different methods of determining visceral sensory thresholds for pain and/or perception. In our studies, the ascending method of limits was used to test visceral sensitivity.¹²⁸ In this method, phasic rectal distensions with increasing pressure are delivered to determine the perception of the distensions. This method may be exposed to response biases such as fear of pain, due to the progressively increasing pressure of the distensions, and hypervigilance.^{127 183} However, the fact that we had a control group of healthy subjects who received the distensions in the same way, the ascending method of limits can be used to detect differences in perception levels (even though these differences can be affected by response biases).¹²⁸ Other distension protocols, such as double random staircase distensions, and tracking technique, may reduce response bias by introducing randomness of distension pressures. However, these protocols are complex and require more distensions and could lead to bias when factors such as fatigue become relevant.¹⁸⁴

Our studies may be limited by the fact that the data of several subjects had to be excluded, which reduced the number of complete data sets. One problem was that several subjects exceeded motion thresholds during data collection in the scanner as a result of intense rectal stimulus, preventing them from lying still.

In accordance with a number of previous fMRI studies of IBS patients, the experimental stimuli were equal for all subjects.^{101 102 143 185} The rationale of using this design was to obtain standardized peripheral visceral afferent input from the gut, and the standardized pressure was selected based on previous studies. However, if we had used individualized distension pressures our results might have been different. In our studies, some subjects received distensions exceeding their reported maximal tolerable rectal pressure threshold during the fMRI paradigm. This renders ethical questions. The pressure levels were based on previous studies and patients were thoroughly informed that they could, at any time, abort the distension protocol by using a panic button; however, no patient used the panic button to abort the experiment due to intolerable rectal pressure. The difference in tolerable rectal pressure between the thresholding procedure and the fMRI distension protocol, may be due to the fact that there are many factors influencing the perception of visceral stimuli.¹⁸⁶ For

example, we can suppose that there was maximal attention to the visceral stimuli during the thresholding procedure. In contrast, during fMRI data collection, attention to visceral stimuli may have been affected by, for example, the noisy environment. Yet another important factor could be differences in habituation or sensitization to the rectal stimulus between IBS subgroups during the visceral sensitivity testing.

In paper III, we compared the effect on brain response in IBS patients before and after gut-directed hypnotherapy and educational intervention. To control for effects that occur simply by repeating the fMRI examination, a group of IBS patients who did not undergo any intervention or regular supportive care could have been included. In the treatment study we did not divide the patients into subgroups in terms of regarding sensitivity or examined the dynamics of BOLD response during the course of the experiments. However, the rectal sensory threshold did not differ between the two treatment groups, and furthermore, there were no differences in rectal sensitivity thresholds pre-post treatment in either group. In addition, the data were not analyzed during the early and late phases.

The generalizability of our findings is limited by the fact that comparable studies used different study designs, for example, methods of analyzing fMRI data and distension protocols.

FUTURE DIRECTIONS

To overcome some of the limitations of fMRI studies, and to further characterize the origin of the alterations in brain activity in IBS, there are several approaches to adopt in the future. For example, task-free resting state fMRI allows analysis of brain function during rest.^{71 83 187 188} Employing a task-free resting state simplifies data collection, and permits multicenter studies with large numbers of subjects for more robust and generalizable results and conclusions. However, there are still limitations to this approach, in regards, for example, to the definition of resting state networks, and analysis strategies.⁸³ Another feasible direction is the use of functional connectivity analysis, where the different regions involved in the perception of visceral stimuli can be analyzed in relation to each other in a more dynamic approach.^{77 187} Studies that combine investigation of brain activity and peripheral mechanisms such as mucosal immune activation, gut microbiota, and/or increased gut mucosal permeability is

potential way to further explore the balance between peripheral and central mechanisms in IBS.¹⁸⁹⁻¹⁹³

Another approach to measuring differences in brain activity is magnetic resonance spectroscopy (MRS). This non-invasive technique is unique in that it is able to identify and measure metabolites and signal substances in brain regions involved in, for example, visceral perception and thus shed further light on the pathophysiological mechanisms involved in IBS.¹⁹⁴ Our results demonstrates that differences in brain response between the examined groups in some part are caused by negative BOLD response (or deactivation). Negative BOLD responses have been coupled to be mediated by inhibitory neurotransmitters such as GABA.¹⁹⁵ Further analysis of the data regarding, for example, regarding levels of GABA in the pACC in the different groups is another way forward.

In our studies we used gut-directed hypnotherapy as treatment for IBS. Unfortunately, hypnotherapy is not very widely available, and could therefore be hard to generally implement in clinical settings. However, studying the brain mechanisms involved in effective emerging therapies for IBS that are more accessible, such as internet-based cognitive behavioral therapy,^{196 197} would be highly interesting.

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ERRATA

In paper I, under Methods (Data analysis) the sentence "An ANOVA confirmed differences between groups." should be added.

In paper II, page 653 the continued Table 2 heading should be "Healthy controls > Hypersensitive IBS" and "Healthy controls < Hypersensitive IBS" respectively.

In paper III, page 1186 the sentence "Twelve patients completed..." should be "Thirteen patients completed...".

In paper III, page 1194 second column row 4, "Table 5" should be "Table S3" and row 13 "Figure 4a" should be "Figure 5a".

Papers

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