Brain-Gut Interactions in Irritable Bowel Syndrome (IBS)

Cecilia Grinsvall

Department of Internal Medicine and Clinical Nutrition
Institute of Medicine
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2019

Brain-Gut Interactions in Irritable Bowel Syndrome (IBS)

cecilia.grinsvall@gu.se

ISBN 978-91-7833-207-6 (PRINT)
ISBN 978-91-7833-208-3 (PDF)

Printed in Gothenburg, Sweden 2019
Printed by BrandFactory

"The best driver of neuroplastic change in your brain is your behavior. (....)
Go out and shape the brain you want to have!"

Laura Boyd TEDx Talks Vancouver 2015

Brain-Gut Interactions in Irritable Bowel syndrome (IBS)

Cecilia Grinsvall

Department of Internal Medicine and Clinical Nutrition, Institute of Medicine
Sahlgrenska Academy, University of Gothenburg, Sweden

ABSTRACT

Irritable bowel syndrome (IBS) is a common disorder of gut-brain interaction defined by recurrent and longstanding abdominal pain and disturbed bowel habits. This thesis aims to deepen the knowledge about aberrant visceral sensory processing seen in a large group of patients with IBS, with particular focus on central mechanisms. The methods used were rectal balloon distensions using a barostat to evaluate rectal sensitivity, structural magnetic resonance imaging of the brain to investigate regional gray matter properties, and questionnaires to assess psychosocial factors, gastrointestinal and multiple somatic symptoms (somatization).

Anxiety, depression and somatization were all associated with increased pain intensity ratings in hypersensitive IBS patients. Non-painful intensity ratings were influenced only by anxiety and to the same extent in normo- and hypersensitive IBS patients. Somatization was further associated with several measurements of rectal pain sensitivity, and mediated the effects of depression and GI-specific anxiety on rectal pain perception. Sex, age and sexual abuse in adulthood were also associated with rectal pain sensitivity.

The level of somatization in IBS was related to differences in local gray matter network connectivity, mainly in regions of the prefrontal cortex, insula and cerebellum. The increased importance of prefrontal cortex and decreased importance of insula implies that cognitive aspects are more important than primary viscerosensory aspects in the neurobiological sensitization process in IBS patients with high levels of somatization. Gray matter morphometry differences between IBS and healthy controls in sensorimotor network seem to be related to psychological distress in women, but not in men.

In conclusion, somatization, measured as multiple somatic symptoms, is important for visceral (hyper-) sensitivity in IBS, and associated with altered structural connectivity within the brain.

Keywords: visceral sensitivity, anxiety, depression, somatization, central sensitization, gray matter morphometry

ISBN 978-91-7833-207-6 (PRINT)
ISBN 978-91-7833-208-3 (PDF)

SAMMANFATTNING PÅ SVENSKA

Irritable bowel syndrome (IBS) är ett vanligt tillstånd som karakteriseras av återkommande buksmärta och avföringsrubbning. Orsakerna till IBS är inte helt klarlagda, men störd kommunikation mellan mage-tarm och hjärna, via den så kallade 'brain-gut-axis', anses vara av central betydelse. I denna avhandling fördjupar vi kunskaperna kring bearbetningen av känslosignaler som kommer från tarmen till hjärnan. Hos en stor del av patienter med IBS är denna bearbetning avvikande. Särskilt intresserade är vi av faktorer som involverar centrala nervsystemet.

Med hjälp av ballong-distension av ändtarmen och gradering av symtom har vi utvärderat känslobearbetningen hos personer med IBS. Vi har visat att för personer med IBS som har överkänslig tarm (låg smärttröskel), ökar graden av ångest, depressiva symptom och multipla fysiska symtom (s.k. somatisering) den smärtintensitet som rapporteras vid alla distensionsnivåer. För intensitet av obehag har endast ångestnivån en intensifierande effekt, och då både för personer med och utan överkänslig tarm. Vi har även visat att nivån av somatisering hos personer med IBS är associerat med ett flertal mått på smärtkänslighet i ändtarmen. Somatisering medierar den smärtintensifierande effekt som depressiva symptom och mag- och tarmspecifik ångest har på uppmätt smärtkänslighet.

Med hjälp av anatomisk avbildning av hjärnan med magnetresonanstomografi (MR/MRI) har vi undersökt form och storlek av hjärnans gråsubstans i olika områden. Vi har visat att nätverket av hur lokala gråsubstansvolymer samvarierar skiljer sig mellan IBS-patienter som har hög respektive låg nivå av somatisering. Nätverket skiljer sig även mellan båda dessa IBS-grupper och friska försökspersoner. Vi har utvärderat de områden som är involverade, och på det sätt de skiljer sig i sina kopplingar. Våra resultat tyder på att kognitiva faktorer är viktigare än sensoriska signaler från mag- och tarmkanalen hos personer med IBS som har hög grad av somatisering. Våra resultat indikerar också att skillnader i områden i hjärnan som hanterar direkta känselintryck och motorisk aktivitet är relaterade till psykisk ohälsa hos kvinnor, men vi ser inte samma trend hos män.

Slutsatsen av denna avhandling är att somatisering, mätt som antal och svårighetsgrad av multipla fysiska symtom, är viktig för tarmens känslighet vid IBS. Detta kan ses som en neurobiologisk process med ändrade organiska kopplingar i hjärnområden vars huvudsakliga funktion är inom kognition och bearbetning av känsloinformation från kroppens inre organ.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. C. Grinsvall, H. Törnblom, J. Tack, L. Van Oudenhove[#], M. Simrén[#]; *Psychological factors selectively upregulate rectal pain perception in hypersensitive patients with irritable bowel syndrome*; Neurogastroenterol Motil; 2015 Dec;27(12):1772-82. doi:10.1111/nmo.12689.

II. C. Grinsvall, H. Törnblom, J. Tack, L. Van Oudenhove[#], M. Simrén[#]; *Relationships between psychological state, abuse, somatization and visceral pain sensitivity in irritable bowel syndrome*; United European Gastroenterol J; 2018 Mar;6(2):300-309. doi:10.1177/2050640617715851.

III. C. Grinsvall*, H.J.Ryu*, L. Van Oudenhove, P. Dupont, J.S. Labus, A. Gupta, M. Ljungberg, H. Törnblom, E.A. Mayer[#], M. Simren[#]; *Sensorimotor network gray matter morphometry in irritable bowel syndrome versus healthy controls: sex differences and associations with pain responses*; In manuscript.

IV. C. Grinsvall, L. Van Oudenhove, P. Dupont, M. Ljungberg, H.J. Ryu, J.S. Labus, H. Törnblom, E.A. Mayer[#], M. Simrén[#]; *A somatization brain network in Irritable Bowel Syndrome (IBS) involves altered connectivity in brain regions with cognitive functions*; In manuscript.

[#] Both authors contributed equally as senior authors.
* Both authors contributed equally as first authors.

CONTENT

ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AML	Ascending Methods of Limits
CNS	Central Nervous System
CT	Cortical Thickness
ENS	Enteric Nervous System
FGID	Functional Gastrointestinal Disorder
fMRI	Functional Magnetic Resonance Imaging
GI	Gastrointestinal
GMV	Gray Matter Volume
GSRS	Gastrointestinal Symptom Rating Scale
HADS	Hospital Anxiety and Depression Scale
HC	Healthy Controls
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IBS-SSS	IBS Severity Scoring System
ILGEs	Intraganglionic Laminar Endings
M1	Primary Motor Cortex
M2/SMA	Supplementary Motor Area
MC	Mean Curvature
MDP	Minimal Distending Pressure

mpINS	Mid-posterior Insula
MRI	Magnetic Resonance Imaging
N	Number
OP	Operating Pressure
PFC	Prefrontal Cortex
PHQ	The Patient Health Questionnaire
pINS	Posterior Insula
ROIs	Regions Of Interest
S1	Primary Somatosensory cortex
S2	Secondary Somatosensory cortex
SA	Surface Area
SCL-90R	The Symptom Checklist-90-Revised
sMRI	Structural Magnetic Resonance Imaging
Tha	Thalamus
V	Volume
VAS	Visual Analogue Scale
VBM	Voxel Based Morphometry
WDR	Wide dynamic range
VSI	Visceral Sensitivity Index

DEFINITIONS IN SHORT

Allodynia	Pain in response to a normally non-painful stimulus [1].
Afferent	Input fibers, travelling towards the central nervous system from the periphery.
Efferent	Output fibers, travelling from the central nervous system towards the periphery.
Hyperalgesia	An exaggerated sensitivity and perception of pain in response to nociceptive stimuli [2].
Hypersensitivity	Increased sensation of stimuli [3].
Morphometry	The quantitative analysis of size and shape [4].
Nociception	The neural process of encoding noxious stimuli [5].
Nociceptor	A peripheral sensory receptor that signals actual tissue damage [6].
Noxious stimulus	A stimulus that is damaging or threatens damage to normal tissues [5].
Pain	An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [5].
Perception	The organization, identification, and interpretation of sensory information in order to represent and understand the presented information, or the environment [7].
Plasticity	The ability to change in an activity-dependent manner; it encompasses both structural and functional changes [1].
Viscera	Internal organs [6].

1 INTRODUCTION

The brain is a fascinating place, a piece of jelly of about 1.3 kilograms [8, 9] where we experience the world. Within the brain emotions, affections, goals and dreams are created, but it is also the residence for pain.

The gut has an enteric nervous system (ENS) with approximately 100 million neurons with the same embryonic basis as the brain and the spinal cord [10]. The gut with its nervous system has by tradition been closely linked to feelings and emotions, such as having butterflies in ones stomach [11]. Irritable bowel syndrome (IBS) is a disorder hallmarked by pain and disturbed bowel habits [12]. It is also a disorder of gut-brain-interaction [13]. Many patients with IBS suffer from visceral hypersensitivity [14] i.e. “feel their gut too much”, from anxiety, depressed mood and various symptoms from many other regions of the body (a phenomenon called somatization) [15]. Furthermore, some have noted that IBS patients more often than others have experienced abuse [15]. What this does to the brain, having these chronic or recurrent adverse experiences, or why some people experience these symptoms while others do not, is still far from known.

In this thesis, we show that psychological distress in IBS is associated with increased perception of rectal pain and that anxiety, but not depression or somatization, is associated with unpleasant rectal sensations (paper I). We show that the experience of multiple somatic symptoms from different bodily regions (somatization) is related to the upregulation of rectal pain sensitivity in IBS (paper II). Further, we show that possible differences in the brains of IBS patients and healthy controls are influenced by sex and psychological distress (manuscript III). Finally, we found differences in the gray matter connectivity pattern based on different levels of somatization in IBS (manuscript IV).

With this introduction, let the journey begin. A journey into the most intriguing organ in the human body and its relation to the gut, in people suffering from abdominal complaints, where the links are so complex no thesis alone can try to explain it. This is my contribution.

1.1 IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) is a common disorder affecting approximately 11% of the adult population worldwide [16]. It is associated with impaired quality of life [17] and limitations in daily activities [18], without affecting the overall survival [19]. The economic burden of the disease is substantial at the individual, healthcare system and societal levels [20].

IBS is defined by symptom-based diagnostic criteria that have changed over time. The Rome IV criteria [21] were published in 2016 and are the current standard. However, all subject inclusions for the works in this thesis were completed before 2016. Therefore, for this thesis, Rome II [22] and Rome III [23] criteria are relevant. Common for all diagnostic criteria is recurring and longstanding abdominal pain associated with disturbed bowel habits.

Differences between criteria are outlined in Table 1. The differences between Rome II and III is the increased pain frequency, and the clarification in Rome III that the symptoms must have been prevalent the last 3 months and present for at least 6 months prior to the diagnosis.

Table 1. Definition of IBS according to different Rome criteria.

	Rome II	**Rome III**	**Rome IV**
Frequency	≥ 12 weeks/ 12 months	≥ 3 days/ month	≥ 1 day/ week
Duration		The last 3 months	The last 3 months
Symptom onset		≥ 6 months	≥ 6 months
Pain	Abdominal pain or discomfort	Abdominal pain or discomfort	Abdominal pain
Associated with ≥2 of:	(1) Relieved with defecation. (2) Onset associated with a change in frequency of stool. (3) Onset associated with a change in form (appearance) of stool.	(1) Improvement with defecation. (2) Onset associated with a change in frequency of stool. (3) Onset associated with a change in form (appearance) of stool.	(1) Related to defecation. (2) Associated with a change in frequency of stool. (3) Associated with a change in form (appearance) of stool.

In addition to the diagnostic criteria, symptoms of bloating, distension, gas, dyspepsia, urgency and sensation of incomplete evacuation are common in IBS [24]. There is also an increased prevalence of symptoms originating from

outside the gastrointestinal (GI) tract, extraintestinal symptoms, compared to healthy controls as well as compared to patients with organic GI disorders [25].

IBS is considered a multifactorial disorder with many functional alterations described, amongst others: visceral hypersensitivity, functional brain alterations, bowel motility and secretory dysfunctions, gut dysbiosis, altered expression and release of mucosal and immune mediators, and somatic and psychiatric comorbidities [12]. However, a coherent link between particular pathologies and specific IBS symptoms has not yet been established [12]. The disorder might be best understood through the biopsychosocial model [13, 15]. The biopsychosocial model postulate that illness presentation is the product of biological (physiological and pathophysiological), psychological, and social functions interacting at multiple levels [13].

IBS is one of many Functional Gastrointestinal Disorders (FGID). More than a third of subjects with FGIDs, have multiple affected GI regions [26]. Increased number of FGIDs is associated with reduced quality of life (QoL), increased health care consumption and increased level of somatization [26].

IBS and other FGIDs are often considered as chronic; however, there is a considerable turnover, to other FGIDs or complete remission. In a population based longitudinal study of FGIDs, around 20% had the same symptoms at follow-up, 40% had no symptoms, and 40% had different symptoms at follow-up [27]. In a 5-year longitudinal study in our secondary/tertiary outpatient clinic at Sahlgrenska University hospital, 83% of the IBS patients had reduced GI symptom severity over time and 17% had not [28]. The individuals with GI symptoms without improvement had higher GI symptom burden to begin with, and compared to the groups with improvement they were younger, more likely female, had higher visceral sensitivity (lower rectal pain threshold during rectal barostat), and had higher/worse baseline score on all psychological features [28].

With this brief overview, we can conclude that IBS is common, and that a subgroup of IBS patients have multiple somatic symptoms, high levels of psychological symptoms, lower quality of life and worse prognosis than IBS patients with limited GI symptoms. This view has recently been supported by a study from our group using mixture model analysis showing six latent groups based on bowel habits and the presence of high or low co-morbidities [29].

1.1.1 PATHOPHYSIOLOGY

There are several pathophysiological mechanisms that have been identified at the group level, but at the individual level a coherent link between particular

pathologies and specific IBS symptoms has not yet been established [12]. In this section only a few of the pathophysiological mechanisms are described, the ones with the greatest relevance for this thesis; the brain-gut axis, visceral hypersensitivity, psychological distress and somatization.

The **brain-gut axis** is a complex, bi-directional system connecting the enteric nervous system (ENS) and central nervous system (CNS). Disturbances at all levels of the brain-gut axis could cause gastrointestinal symptoms [30, 31]. It has been shown that IBS patients have lowered rectal pain threshold [32, 33] and alterations in brain activation, demonstrated with functional brain imaging techniques, when stimulating the rectum or sigmoid colon [34-37]. Moreover, when simultaneously applying rectal and somatic painful stimuli, there is a greater reduction in rectal pain intensity in healthy controls than in IBS patients. This implies impaired activation of pain inhibitory control mechanisms, termed conditional pain modulation, in IBS [38, 39]. In a recent review and meta-analysis, it was shown that conditional pain modulation indeed was significantly lower in IBS compared to controls with an odds ratio of 4.84 (95% CI: 2.19-10.71) [40]. This strongly supports abnormal descending pathways, most likely in combination with central sensitization, to play an important pathophysiological role in IBS [40]. In the last decades, the understanding of the role of the CNS and brain-gut interactions in IBS has increased. In fact, in Rome IV, the term "Disorders of Gut-Brain interaction" has been decided to be a more suitable term than Functional Gastrointestinal Disorders for denoting this group of disorders [13].

Visceral hypersensitivity is a collective term for increased sensitivity for visceral stimuli. It could be either the perception of normally non-noxious stimuli as painful (analogous to allodynia), or increased intensity of noxious stimuli (analogous to hyperalgesia) [41]. Rectal hypersensitivity, most often defined as reduced thresholds to rectal balloon distensions, is found in 35-60% of IBS patients [42]. Increased visceral sensitivity is associated with increased GI symptom severity, and visceral hypersensitivity is considered an important contributor to GI symptom generation in FGIDs [14].

There has been a longstanding debate on the role of peripheral versus central factors contributing to visceral hypersensitivity in IBS [43]. Both peripheral and central mechanisms are likely involved, but to which degree each of these mechanisms contributes to the overall perception of visceral pain remains unclear [44]. Visceral hypersensitivity can be mediated by a variety of factors acting on peripheral, spinal, and/or central pathways [45]. Peripheral factors might involve epithelial barrier dysfunction, alteration in serotonin signaling, gut microbiota and mucosal immune response, and central factors might

include hypervigilance, stress and symptom-driven anxiety [43]. It is stated that the pathogenesis of visceral hypersensitivity is clearly complex and multifactorial, involving a multitude of mediators, neurotransmitters, cell types, at different locations of the brain-gut axis [46].

An important factor that warrants attention in IBS (and other disorders of gut-brain interaction) is **psychological distress** [47]. Associations between IBS symptoms, psychological factors and psychiatric co-morbidity have been found in several epidemiological studies [48-51]. In the general population about half of the people with IBS have psychiatric symptoms (compared with one third in controls), and in other settings 40-90% of the IBS patients fulfill criteria for a psychiatric disorder [52]. Levels of anxiety and depression are consistently higher in IBS patients compared to healthy controls (regardless of subgroup) [53], as well as compared to patients with inflammatory bowel disease (IBD) [54]. Psychological symptoms and psychiatric disorders such as anxiety, depression, panic, posttraumatic stress and somatization disorders often precede or exacerbate FGID symptoms [55].

Somatization is a concept that can be defined and operationalized in different ways. It can be viewed as a tendency to express psychological distress or psychiatric illness as bodily symptoms [56]. The view of somatization as secondary to psychological distress is sometimes referred to as "presenting somatization" [57]. Alternatively, it can be viewed as the primary presence of multiple medically unexplained symptoms, possibly reflecting an altered functioning of the CNS [58]. This phenomenon is sometimes referred to as "functional somatization" [57]. For research purposes, the number and severity of somatic symptoms can be measured using questionnaires, with the sum of the scores indicating level of somatization [59]. About two-thirds of FGID patients experience symptoms of other functional somatic syndromes such as interstitial cystitis, chronic pelvic pain, headaches, and fibromyalgia, at least partly independent of psychiatric comorbidity [15]. The majority of the excess health care costs in IBS is from medical care not directly related to lower GI problems [60].

However, there has been some debate about the nosology. 'Multisystem symptoms' or 'multiple somatic symptoms' might be a more appropriate nomenclature, since somatization unfortunately is conceived as a patient-blaming term, not fully related to the underlying neurobiological mechanisms [61]. Another suggestion for the terminology has been 'multiple physical symptoms' [62]. In this thesis, the term somatization is consistently used, as the questionnaires used to measure multiple somatic symptoms are known as questionnaires of somatization. It is my personal view that somatization is a

neurobiological process that makes some individuals more susceptible to perceive stimuli (in particular painful stimuli), both external and from within the body, in association with psychological and behavioral attributes. This will be brought up in the Results and Discussion subchapter 4.5.1 'Somatization or central sensitization?'. Somatization in this sense is most likely only present in a subgroup of IBS subjects, and probably represents the ones who are affected worst by their disorder [26].

In a large 5-cohort, 3-country study of visceral hypersensitivity, the association between visceral sensitivity and GI symptom severity remained significant after controlling for psychological distress as well as after controlling for non-GI symptom severity [14]. This suggests that visceral hypersensitivity, psychological factors and somatization have independent, and possible additive effects, in the pathophysiology of IBS.

1.2 SENSORY TRANSMISSION OF THE GI TRACT

The GI tract has many functions necessary for digesting food, absorbing nutrients and expelling waste. In paper I, II and manuscript III, rectal mechanosensory function was investigated in IBS. In order to put the results in a broader context, a brief overview of the transmission of sensory stimuli, especially focusing on pain, is presented in this section.

The innervation of the GI tract can be divided into extrinsic and intrinsic, and only a minority of the sensory information from the GI tract induces conscious perception [63]. The intrinsic innervation, i.e. the ENS, is a part of the autonomic nervous system [64]. It consists mostly of regulatory loops controlling motility, mucosal secretion and absorption, local blood flow and immune function in the gut [65]. The relevance of the ENS related to pain is thought to be primarily based on excitation of extrinsic afferents by neuropeptides [65].

The extrinsic innervation conveys information between the GI tract and the CNS, and consists of vagal and spinal (splanchnic and pelvic nerves) innervation [6]. The vagus nerve and pelvic nerves are mostly involved in physiological sensations; the vagus for the upper GI tract (hunger, satiety, emesis etc) and the pelvic nerves for the lower GI tract (urgency, desire to defecate, etc), even though they have some implications for painful sensations as well [65]. The major players in colorectal pain transmission are the splanchnic nerves. The splanchnic nerves have cell bodies in thoracolumbar

dorsal root ganglia, and secondary neurons in the spinal cord dorsal horn. The ascending pathways takes one of five tracts to the brain. Some paths have mainly subcortical regulatory functions not reaching consciousness and some conveys information via thalamus to the cortex were it is consciously perceived and interpreted [65]. There are two parallel streams from the thalamus, one reaching the posterior insula (pIns) and somatosensory cortex (S1), and one reaching the anterior cingulate cortex (ACC) and prefrontal cortex (PFC) [66]. The pIns-S1 stream is thought to primarily encode sensory-discriminatory aspects of visceral sensory perception. The ACC-PFC stream is thought to primarily encode motivational and cognitive aspects of visceral sensory processing [66].

There are several different ways to characterize the sensory afferents of the GI tract. To simplify this, Brookes et al have suggested five types of sensory neurons; intraganglionic laminar endings (IGLEs), mucosal afferents, muscular-mucosal afferents, intramuscular endings, and vascular afferents [67]. Of these, vagal IGLEs and mucosal afferents likely only generate limited conscious perception. Rectal intraganglionic laminar endings (rIGLEs), on the other hand, are low-threshold slow adapting mechanoreceptors with a wide dynamic range (WDR) of responses to distension, also into the noxious range [67]. Vagal intramuscular endings responds to higher thresholds than the IGLEs, have a wide dynamic range into the noxious range of intraluminal pressures and contain capsaicin-sensitive TrpV1 channels, as is the case for many nociceptors [67]. Spinal/rectal intramuscular endings exist, but their physiological function is less well studied than their vagal counterparts. Vascular afferents seem to be present only in the spinal innervation, not the vagal. They are thought to be nociceptors, responding to strong compression or probing, changes in perfusion rate, and are sometimes called mesenteric or serosal afferents based on their anatomical location [67].

Pain pathways could probably be activated by rectal distension through pelvic nerve activation induced by rIGLEs, rectal muscular and muscular–mucosal endings, and vascular afferents. The muscular and muscular-mucosal afferents are thought to have low-threshold, WDR mechanoreceptors in the colorectum. Vascular afferents have high thresholds to distension, show slow firing rates and have other characteristics frequently associated specifically with nociceptors [67].

A special class of afferents have also been implicated in visceral hypersensitivity, so called silent nociceptors or mechanically insensitive afferents [2]. These are normally insensitive to mechanical stimulation but

become active and mechanosensitive in pathophysiological conditions where they contribute to the development of hyperalgesia [2].

1.3 RECTAL SENSITIVITY TESTING USING RECTAL BAROSTAT

Rectal barostat testing for evaluating the visceral sensitivity status in IBS is widely used for research and, to a lesser extent, clinical purposes. “The principle of the barostat is to maintain a constant pressure within an air-filled flaccid bag positioned in the lumen of the organ to be studied” [68].

Several techniques can be used to assess visceral responses to colorectal distension. Nociceptive responses can be quantified by pain ratings, neurophysiological readouts, inhibition of the RIII reflex, autonomic responses or brain imaging [46]. Perceptual responses to rectal barostat testing refers to the participant defining thresholds or intensity ratings for sensations (usually of first sensation, urge to defecate, discomfort and pain), or viscerosomatic referral. Perceptual responses to rectal distension have shown moderate correlations with IBS symptoms, are highly reproducible and are relatively easy to assess [46].

If choosing perceptual responses to quantify visceral sensitivity in rectal barostat testing, various other considerations need to be made which might influence the results. The exact location of the distension (sigmiodeum, colon, rectum); distensions based on pressure or volume; the speed of inflation; returning to baseline pressure between distensions or not; ascending methods of limits (AML) or (double) random staircase methods; how to define visceral hypersensitivity, etc.

The discriminatory properties of rectal barostat to identify IBS subjects were investigated in a study using AML distension. Pain threshold was defined as the first time subjects rated the sensation as painful, with sensation of pain scoring at least VAS 3 out of 10 [32]. With a cut-off level for pain threshold $\leq$ 40 mmHg, the sensitivity of the rectal barostat to identify IBS patients from healthy controls and non-IBS patients with abdominal pain was 95.5% and its specificity was 71.8%. The positive and negative predictive value was 85.4% and 90.2% respectively [32].

The definitions of visceral hypersensitivity have varied in different studies. It could for example be based on pressure thresholds, or VAS cut-off values [69]. By calculating receiver operating characteristic curves of pain perception, the

optimal cut-off for defining visceral hypersensitivity was in one study detected to be a VAS pain ratings ≥20 mm at ≤ 26 mmHg rectal distension [69]. The author points out that this cut-off, however, is for their specific barostat protocol.

Although there are several considerations to be made, rectal barostat testing can reliably give valuable information about the visceral sensitivity status in an individual. However, direct comparisons between studies can be difficult based on the many different ways of perform rectal barostat testing, as highlighted above.

1.4 NEUROPLASTICITY

Neuroplasticity, or neuronal plasticity, refers to structural and functional changes in neural circuits in response to experience [70, 71]. Plasticity can be addressed at different levels: from the level of synapses with changes in synaptic strength or formation of new synapses and removal of existing synapses [70] to changes in the human adult brain in response to changes in the external environment or the internal milieu [71]. These adaptive changes can occur in response to new experiences throughout life [70], and there is strong evidence of correlation between learning and brain changes [71].

In a review of 33 longitudinal studies in healthy volunteers using MRI-based techniques to measure morphological changes in response to learning, the results were mixed [72]. In most visuo-motor tasks, such as juggling, there were increased regional gray matter volumes whilst the results for cognitive tasks were less consistent (both increase, decrease and no change in gray matter volume were found) [72].

Plasticity could be maladaptive, leading to behavioral loss or development of disease symptoms [71]. Maladaptive plasticity is considered an established factor in pain chronicity [1]. In a meta-analysis of chronic pain, decreased gray matter volumes were seen in several regions, some involved in pain perception (such as insula, putamen, ACC, precentral gyrus and thalamus), as well as regions not commonly regarded as pain-processing areas (such as inferior and middle frontal gyrus and superior temporal gyrus) [73].

Longitudinal studies suggest that the pain state drives the morphological changes, as opposed to morphologic alterations being predisposing for disease [74-77]. In some instances, longitudinal studies have shown disease-related changes that normalize after treatment [76, 78]. Further, the association of transient gray matter changes in relation to pain has been shown in patients

with episodic tension-type headache [79], and patients who develop chronic headache after traumatic whiplash injury [77]. Interestingly, in healthy subjects who sensitized to repeated noxious stimuli, there were regional gray matter reductions similar to those seen in chronic pain states (including cingulate, insular and frontal regions, and amygdala), whereas subjects who habituated did not show any changes after repeated heath stimuli [80].

The cellular correlates that underlie the macroscopically detected gray matter plastic changes are so far incompletely understood. They are thought to be composed of a combination of adult neurogenesis (for certain regions such as the hippocampus), synaptic changes (such as changes in dendritic length and branching or in the number of dendritic spines per neuron), and changes in the number and morphology of glial cells [81]. It could also reflect a change in cell sizes, as well as changes in blood flow or interstitial fluid. [71].

It has been suggested that individual brain structure can potentially be used to predict subsequent performance and plasticity [82], and that inter-individual variability can give information about the neural basis of human behavior and cognition [83]. For example, the size of the amygdala correlates with inter-individual differences in memory, social phobia and social network size [83]. There is also a possibility that experience-induced plasticity differ between health and disease.

With structural brain MRI, regional changes in gray matter have been seen in response to learning, as well as in disease. To fully understand these changes, the underlying mechanisms must be determined. The results and discussion in this thesis will be unsatisfactory in this regard, since mapping the cellular changes of regional gray matter in relation to disease is not being studied herein. Instead, we will increase our knowledge of regional gray matter properties in IBS, and how regional gray matter volumes are linked depending on level of somatization.

1.5 FUNCTIONAL NEUROANATOMY

This section gives a brief overview of the known or postulated functions of brain regions of relevance for this thesis. The descriptions are incomplete but are meant to serve as a dictionary when reading this thesis, and will mostly highlight functions that might be of relevance for IBS. However, it is crucial to also have the basic understanding that the relationship between information encoded in primary afferents of the GI-tract and the conscious perception of such information is far from linear [37].

The brain regions below are included in manuscript III or IV, or both, which is displayed in brackets and the regions with descriptions in this brief overview are written in *italic*. The regions are displayed alphabetically and includes: *Amygdala* (IV), *Basal ganglia* (III and IV), *Cerebellum* (IV), *Cingulate* (IV), *Hippocampus* (IV), Hypothalamus (IV), *Inferior parietal lobe* (IV), *Insula* (III and IV), *Prefrontal cortex* (IV), *Primary motor cortex* (III and IV), *Primary somatosensory cortex* (III and IV), *Secondary somatosensory cortex* (III), Superior frontal gyrus (III and IV), Superior temporal gyrus (IV), *Supplementary motor area* (III and IV), and *Thalamus* (III and IV).

The Amygdala (IV) is a subcortical structure in the bilateral temporal lobes, which has been well conserved through evolution [84]. It has a crucial role in fear responsivity and the acquisition of experience-dependent fear and threat memories. Amygdala contributes to human emotional behavior in general [85]. Amygdala is a rapid detector of aversive environmental stimuli and situations, leading to affective or behavioral responses to potential threats [84].

The Basal ganglia is a collection of subcortical gray matter nuclei: putamen (III and IV), caudate nucleus (III), nucleus accumbens (III) (collectively also known as the striatum) and globus pallidus (III) [85]. The striatum receives input from all cortical areas through the thalamus, and projects principally to frontal lobe areas [86]. There are interactions between the regions, but there seems to be regional specializations with the ventral striatum being more associated with reward and reinforcement, the caudate nucleus with cognition, and the putamen with motor control and automated movements that require little cognitive effort [87]. The basal ganglia and frontal cortex cooperate to learn optimal behavior and to execute goal directed behaviors [87]. The dorsal striatum is implicated in habit memory and stimulus-response learning [88]. There is evidence indicating that stress and glucocorticoids may, in addition to influencing the hippocampus-dependent memory system, also impact memory processes in the dorsal striatum, and may induce a shift from hippocampal to dorsal striatal control of learning [88].

The Cerebellum (IV) contains almost 80% of the total brain neurons. It consists anatomically of two hemispheres and a narrow midline zone (vermis), and is functionally divided into the flocculonodular lobe, the medial (vermis), intermediate (paravermis) lobes –together called the spinocerebellum, and the lateral zone regarded as the cereberocerebellum [89]. The cerebrocerebellum is the largest and functionally most important part, and is reciprocally connected with the cerebral cortex [89].

The cerebellum is believed to constantly compare planned motor commands and the actual somatosensory feedback to adjust motor output if necessary. In functional brain imaging studies, the cerebellum is also activated during cognitive tasks involving working memory, language, time perception, executive functioning and emotional processing [89]. The cerebellum is critical to motor and cognitive automation and adaptation, situations in which operations become skilled and automatic. [90].

Further, the cerebellum is involved in emotions, social cognition, autonomic functions, perception and pain [91]. Perceptual optimization and prediction of incoming information have been shown to rely on cerebellar processing in virtually all sensory domains, and cerebellum is one of the brain structures that is most consistently responsive to pain [91].

The Cingulate Cortex is situated on the medial side of the hemispheres and can be subdivided into anterior cingulate cortex/ **ACC** (IV), middle cingulate cortex/ **MCC** (IV), and posterior cingulate cortex/ PCC. The middle cingulate has relatively recently been acknowledged as separate from ACC and PCC in terms of structure and function, and has two divisions, anterior (aMCC) and posterior (pMCC) middle cingulate cotex [92]. Sometimes the term dorsal ACC has been used for aMCC [92].

The cingulate cortex receives afferent signals from anterior nuclei of the thalamus, sends efferent projections to parahippocampus and then hippocampus, and has many bilateral connections with frontal, temporal and occipital cortices [93].

The **ACC** is assumed to have a role in memory functioning and filtering of irrelevant information in order to protect memory processes from interfering stimuli [93]. It is activated by a range of emotions, and seems to facilitate prefrontal influences in corticolimbic inhibition, including emotional and cognitive modulation of pain [94]. The ACC is involved in emotional awareness and has a role in central autonomic regulation [92]. The ACC evaluates the outcome of planned actions, and if confident we can reach our goal, the ACC informs the motor system about the best action, whereas if the outcome is uncertain, the ACC initiates a stress response through amygdala [95]. The ACC is involved in the affective aspects of pain processing [73].

The **MCC** has a role in skeletomotor functioning, cognitive information processing, and pain [92]. The aMCC is frequently activated during fear but not during non-emotional conditions, and generates avoidance responses to fear, which is postulated to be implicit premotor signals, not conscious

emotions [92]. The aMCC is also frequently activated in human pain studies [92]. The pMCC is involved in reflexive orientation of the body in space to sensory stimuli, including noxious stimuli. The pMCC has almost no evoked emotional activity, and is activated in response to pain that is perceived as controllable rather than uncontrollable [92].

Hippocampus (IV) is one of the most studied parts of the brain, with associations to various memory functions, spatial navigation/discrimination, and creative thinking and flexible cognitions, amongst others [96]. Hippocampus is a key structure for spatial and declarative memory formation, and therefore important for plasticity and adaptive brain functions [97]. The hippocampus encodes both spatial and temporal information, and is essential for remembering information about temporal and situational contexts, as well as sequences [98].

Further, the hippocampus, and adjacent parahippocampal regions, have vast and complex bidirectional interconnections with amygdala. These interactions play a critical role in emotion, learning, memory and complex behavior [99].

There is adult neurogenesis (formation of new neurons) in the hippocampus, which is highly regulated by experience as well as environmental and biological factors [100]. The adult neurogenesis has been implicated in learning, memory, but also in regulating emotional status, such as anxiety and depression, and cognitive flexibility. Further, adult neurogenesis is required for some of the beneficial effects of antidepressants through 5HT1A receptors [100].

Insula can be roughly subdivided into anterior (IV) and posterior (III and IV) sections, and each section has different cytoarchitectonic features, connectivity, and functions. The insular cortex might be the main cortical target of the spinothalamic system [101]. Insula has been related to sensory, gustative, language and auditory functions, but also viscerosensation, vomiting, autonomic control and vestibular processing [101]. Insular seizures are commonly associated with viscerosensory and gastromotor symptoms [102].

Interceptive information reaches the **posterior insula** by ascending sensory inputs from spinal and brainstem pathways via specific thalamic relays [103]. From posterior insula, the information is projected onto the **anterior insula**, where it is integrated with emotional, cognitive, and motivational signals from an array of cortical and subcortical regions [103]. In a small study of intracranial stimulation of human insula, stimulations that produced visceral

responses were located in midposterior insula [104]. Anterior insula is associated with the affective dimension of pain processing and expectation of pain, and posterior insula is associated with sensory-discriminative aspects of pain processing, including somatotopy [73].

The Inferior parietal lobe /IPL (IV) is one of the most densely interconnected cortical regions in the human brain [105], and has connections with inferior frontal, posterior temporal and insular regions [106]. The inferior parietal cortex is generally considered to intergrade various modalities (eg somatosensory, visual and auditory), and plays an important role in various higher cognitive functions [107]. IPL, in conjunction with prefrontal areas, is argued to have an important role in how self relates to other, as well as in the sense of agency [108]. IPL has been suggested being a part of the neuronal basis for empathy; specifically the emotional part of empathy [109].

Prefrontal cortex /PFC (IV) is an important brain structure for performing executive functions. Executive function is a product of the coordinated operation of various neural systems, is essential for achieving a particular goal in a flexible manner, and is essential for most cognitive functions [110]. PFC functions includes attentional set, temporal organization of behavior, planning of complex tasks to accomplish future goals, access and manipulate information stored in long-term memory, working memory and error monitoring [110, 111].

PFC exerts top-down modulation on various cortical and subcortical structures [110]. During working memory, PFC is activated in conjunction to other regions dependent of the nature of the memory task. Regardless of memory task, orbital PFC (OFC) is activated after the choice, reflecting the reward value of the outcome of that choice [111].

Different parts of PFC have some specific characteristics. Mental simulation of an outcome, especially if it is pleasant, activates medial prefrontal areas (mPFC). The ventrolateral prefrontal regions (vlPFC) is engaged during complex pain modulation, leading to a change or reappraisal of the emotional significance of pain [112]. The vlPFC has been shown to mediate the hyperalgesic effects of negative cognitions in patients with fibromyalgia [113]. Further, vlPFC contribute to long-term memory formation, and the degree of activity in the vlPFC during encoding predicts the probability of the successful recall of memorized materials [110]. The anterior prefrontal regions are involved in memory retrieval, and hemispheric differences in prefrontal contribution to long-term memory processes have been reported. [110]. The

venteromedial prefrontal cortex (vmPFC) is closely associated with visceral and emotional functions, and has strong connections to the hypothalamus, amygdala, and hippocampus [87]. The orbitofrontal cortex (OFC) links sensory representations of stimuli to outcomes [87].

Primary motor cortex/ M1/ Precentral gyrus (III and IV) is by tradition known to consist of 'upper motor neurons', but the evidence of its involvement in cognitive motor processing and motor learning is increasing [114, 115]. Intracortical recordings in human patients with epilepsy have shown activation in M1 in response to both non-noxious and especially noxious cutaneous stimuli. The noxious response in M1 had similar latencies as in S1, which suggests parallel processing, and indicates spinothalamic input directly to M1 [116]. Also with MEG technique in healthy subjects, evidence for M1 excitation by noxious stimuli have been found [117].

Primary somatosensory cortex/ S1/ Postcentral gyrus (III and IV) is associated with coding innocuous tactile somatosensory information in a somatotopic fashion, thereby generating somatic sensations [118, 119]. Evidence supports that S1 is also involved in pain processing, in particular the localization of the stimuli [118]. It has been argued that neuronal activity in S1 participates in producing awareness of the sensory/discriminative aspects of pain, but not the awareness of its unpleasantness [119]. There are also indications of S1 being involved in modulation of the affective and attentional component of pain [120]. Visceral stimuli, both innocuous and noxious, have in many (but not all) studies been show to elicit activation in S1 in HC, as well as in IBS subjects [37].

Secondary somatosensory cortex/ S2 (III) is involved in the processing of both nociceptive and non-nociceptive information [121], with a predominant role of S2 is in the sensory–discriminative dimension of pain [120]. There exists parallel pathways from thalamus to S1 and thalamus to S2, as well as intrinsic connectivity between S1 and S2 [122]. The quality of the pain elicited by direct stimulation of S2 are very similar to those elicited by stimulation to (adjacent) insula. [121]. The activity in S2 measured by MEG were tightly time-locked to the painful esophageal stimulus, showing it has a role also in visceral sensory characterization and intensity coding [120].

Superior frontal gyrus (III –included in SMA, IV –included in PFC but specified as superior frontal gyrus (and sulcus)).

Supplementary motor area/ M2/ SMA (III, IV) has traditionally been considered a key region for motor planning and execution. SMA has been

implied in timing, spatial processing, numerical cognition and working memory. The diversity of the functions in which SMA are involved in has given rise to the notion that the unifying cause of these associations is a role of SMA in sequence processing [123]. SMA has an abstract role, subserving sequential processes required to create a representation of time, likely dependent on other brain regions being co-activated [123].

Superior temporal gyrus (IV) might be involved in the aspect of pain processing responsible for monitoring mismatches between predicted and actual sensations [73].

The Thalamus (III and IV) is a subcortical gray matter structure in the midline, deep within the brain. The thalamus is a critical hub, consists of several nuclei, and relays sensory information from the periphery, or subcortical structures, to the cortex. In fact, all cortical regions receive projections from the thalamus. [124]. It is implied that the human thalamus is an integrative hub for functional brain networks, not only a passive relay station, and that it is engaged in multiple cognitive functions. [124]. In supraliminal rectal distension studies, thalamus is consistently activated in HC and IBS, but to a larger spatial extent in IBS patients [36].

1.6 BRAIN IMAGING IN IBS

Brain imaging, or neuroimaging, is a collective term for the use of various techniques to directly or indirectly visualize the structure or function of the CNS. There are different modalities of brain imaging, such as magnetic resonance imaging (MRI), positron emission tomography (PET), electroencephalography (EEG) and magnetoencephalography (MEG) [37](suppl.). The most commonly used modality of brain imaging in IBS is MRI, which can be either structural or functional. MRI modalities are further divided into structural gray matter (as is used in manuscript III and IV) or structural white matter (for instance with diffusion tensor imaging/DTI) analyses, and functional MRI (fMRI) using an evoked paradigm or resting state recording of spontaneous blood oxygenation level dependent (BOLD) fluctuations [125]. The BOLD fluctuations in fMRI are indirect measures of neuronal activity and can be used to identify which brain regions are more or less active during a certain experimental paradigm, such as painful rectal stimulation [125].

The MRI technique uses magnetic fields, radio waves and field gradients [96]. “A strong electromagnetic field align the hydrogen atoms of water molecules in the brain tissue. Radio frequency fields are used to systematically change the alignment of the magnetization. This results in a rotating magnetic field

created by the hydrogen atoms as they return to baseline that can be detected by the MRI scanner. The resulting signal can be used to construct an image of the brain because different tissues (e.g., gray and white matter) have different magnetic properties" [81]. T1-weighted images creates large differences between white and gray matter and are therefore commonly used for measures of gray matter morphology [81]. Structural MRI scans are high resolution anatomical scans, and time course for changes of regional gray matter is not completely established but seems to take days to weeks to occur [126].

There are several studies on FGIDs, especially IBS, using different brain imaging modalities. In this background, I will not be able review the entire literature, but will highlight the current knowledge by referring to a few influential papers published on this topic.

First, the Rome Working Team Report from 2009 [37] covered published fMRI studies using acute visceral stimulation of the esophagus, stomach and colon in healthy controls (36 studies) and patients with FGIDs (18 studies, 16 of which were in IBS) from 1997 and later. They found that the insula and anterior cingulate cortex (ACC) were the most commonly reported regions activated by visceral stimuli both in patients and in healthy controls. Overall, patients showed similar regions of activation as healthy controls, but greater activation in ACC, insula, hypothalamus, infragenual cingulate cortex and amygdala, and decreased activation in dorsal pons [37].

Next, a meta-analysis from 2011 by Tillisch et al [36] aggregated brain imaging data (fMRI and PET) from 18 supraliminal rectal distension studies. They found regional overlap in IBS and healthy controls in the thalamus, aMCC, pACC, and anterior insula, though greater spatial extent of activation in IBS was seen in thalamus and aMCC. The insular activation differed between groups, with IBS having greater extension posteriorly to middle insula, and healthy controls greater activity in anterior insula. IBS showed greater activation in right pACC, left amygdala, right superior frontal cortex and the midbrain. Healthy controls had greater activity in the putamen, pre- and postcentral gyrus and some parts of the prefrontal cortex. Further, Brodmann area 40 (inferior parietal lobe) were activated in healthy controls but not in IBS. The authors conclude that IBS patients have greater engagement of regions associated with emotional arousal and endogenous pain modulation, similar activation of regions involved in processing of visceral afferent information, and controls have greater engagement of cognitive modulatory regions [36].

Lastly, a review by Weaver at al [125] was published in 2016, covering 27 articles (6 structural gray matter MRI studies, 2 structural white matter connectivity analysis/DTI studies, 9 rectal distension fMRI studies, 4 resting state fMRI studies, 4 other fMRI studies, and 2 studies using other brain imaging modalities) from the years 2009 to 2015. The two most consistent findings using gray matter structural MRI was: reduced cortical thickness of anterior insula in IBS, and in women increased gray matter (cortical thickness and volume respectively) in left primary somatosensory cortex. In the two white matter (DTI) studies included in this review, no consistent results were reported [125]. Differences in functional responsiveness during rectal distension showed consistently greater activation in IBS than healthy controls in ACC, MCC, amygdala, anterior and posterior insula and PFC. [125]. They also conclude that the results of brain imaging studies in IBS are greatly influenced by confounding factors such as sex, anxiety, depression, traumatic experiences, pain catastrophizing and level of gastrointestinal symptoms [125].

To date (November 1st 2018), there are 13 published papers available on PubMed on gray matter morphometry in IBS [127-139], the first one published in 2008 [127]. Eleven studies compared IBS and healthy controls, one study compared IBS with healthy controls and ulcerative colitis [133] and one study compared IBS with asymptomatic diverticular disorder, low somatization diverticular disorder and high somatization diverticular disorder [139]. Eleven studies investigated correlations between gray matter and clinical parameters (two did not investigate group differences, only looked at correlations between brain and Catechol-O-methyltransferase (COMT) genotype [134] and microbiota and brain [138] respectively), and one study only investigated group differences and not correlations with clinical variables [127]. Study populations ranged from N=IBS/HC: 9/11 [127] to 121/209 [134]. Six studies were done in females only, and seven was done in, or in collaboration with the group at UCLA, Los Angeles, USA.

There are several different methodologies, regions of interest (ROIs), research questions etc., which precludes from direct comparisons between studies. There are a number of inconsistent findings, but the overall picture is summarized below. Please note that I have not done a formal structured review or meta-analysis supporting these statements.

1) In all instances but one (right posterior cingulate cortex in an adolescent male/female cohort) where IBS had greater gray matter metrics than healthy controls, the studies were made only in females or this group difference were only seen in the female subgroup.

2) Regions larger in (female) IBS than healthy controls were mainly seen in primary sensory- and motor- cortices (e.g. pre- and postcentral gyrus, central sulcus, paracentral lobe).
3) There were more instances where healthy controls had greater gray matter metrics than IBS, these were seen both in studies in females only, as well as in mixed-sex-studies.
4) Regions larger in healthy controls than IBS were more frequently seen in *subcortical regions* (thalamus, ventral striatum, amygdala, hippocampus, brainstem, putamen), *insula* (anterior, middle and posterior), *ACC* (especially subgenual ACC were shown repeatedly), *PFC* (middle frontal gyrus, middle orbitofrontal gyrus, lateral and medial orbitofrontal gyrus, dorsomedial and dorsolateral PFC), and *posterior parietal cortex*.
5) Group differences and correlations does not have to occur in the same regions within the studies.
6) Due to different clinical measurements used, gray matter metrics, other methodological differences and inconsistent findings, there is hard to see an overall picture for the correlational analyses.

2 AIMS

At the start of this PhD project, it was clear that abnormal CNS function is present in at least subgroups of IBS patients, and probably important in the etiology and pathogenesis of IBS. It was not clear if these abnormalities are restricted to visceral sensations, or represent a generalized phenomenon of altered CNS function. It was also not well known if the heightened visceral sensitivity in IBS is due to local factors in the colon, such as receptor abnormalities, dysfunction in the gut-brain-gut-communication, or abnormal processing or modulation of the signals that reaches the brain.

The overall aim of this PhD project was to deepen the knowledge of the abnormal visceral sensory processing in IBS patients, especially regarding the involvement of central factors such as psychological distress and somatization in this pathophysiologic mechanism of IBS.

Specific aims and hypotheses

Paper I: The aim was to characterize the differences in perception of painful and non-painful sensations during rectal distension, and explore the impact of psychological factors on this perception in hypersensitive and normosensitive IBS patients. We hypothesized that we would be able to demonstrate the type of afferent pathways that are upregulated in visceral hypersensitivity: high-threshold afferent pathways, or wide dynamic range (WDR) afferent pathways. This concept comes from the theory that the sensory intensity ratings at different distension levels can give information about the type of afferent pathways are involved [140], see Figure 1.

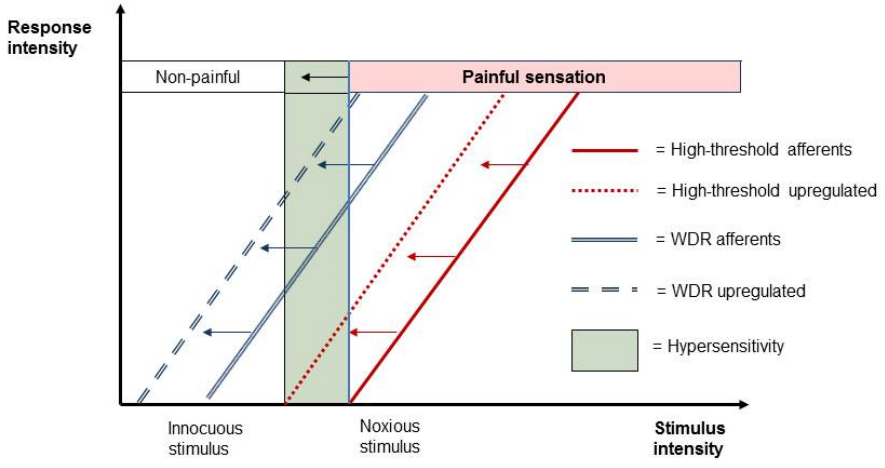

Figure 1. Hypothesis of separate upregulation of pain-specific (high-threshold) afferent pathways and wide dynamic range (WDR) afferent pathways (first presented by Vandenberghe et al [140]). Upregulation of pain-specific pathways should only increase the response to higher levels of distension pressures. Upregulation of WDR afferent pathways, on the other hand, should increase the response to all pressure levels.

Paper II: The aim was to explore how psychological factors, abuse and somatization relate to visceral sensitivity. The main hypothesis was that somatization mediates the relationship between abuse and/or psychological factors and visceral sensitivity.

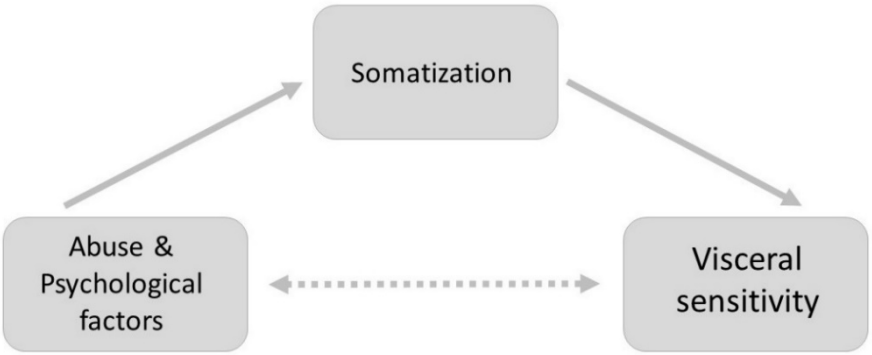

Figure 2. Hypothesis of paper II, somatization mediates the relationship between abuse and/or psychological factors and visceral sensitivity.

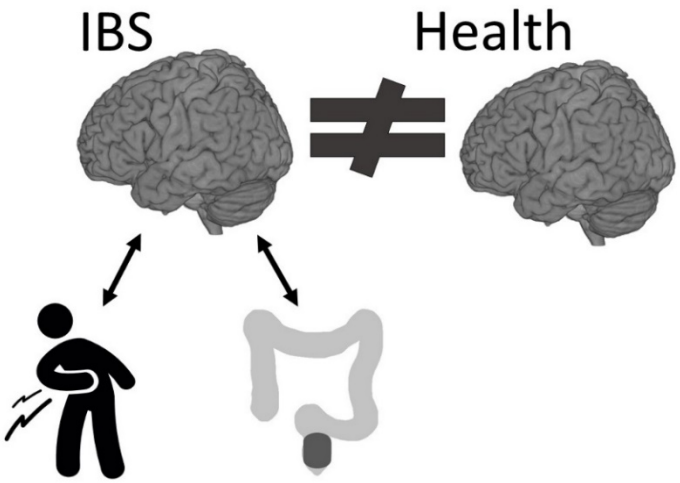

Figure 3. Hypothesis of manuscript III, gray matter morphometry of the sensorimotor network differs between patients with IBS and healthy controls. In the IBS group, different parts of the sensorimotor network correlates with reported symptoms of abdominal pain and bloating, than the regions correlating with symptoms evoked by rectal barostat distension.

Manuscript III: The aim was to explore the sensorimotor brain network morphometries and the relation to psychological factors, symptoms and visceral sensitivity in IBS. The hypotheses were that there would be differences between IBS and HC in regions of the sensorimotor network, that these differences would be influenced by psychological factors, abuse and sex, and that there would be correlations between clinical measurements of visceral sensitivity and gray matter morphometry in IBS.

Manuscript IV: The aim was to identify "a somatization brain network" in IBS. The hypothesis was that the brain connectivity in IBS with high level of somatization would differ from those with IBS low somatization, and that both IBS groups should differ from healthy controls, but IBS high somatization should differ greater compared to healthy controls than IBS low somatization. This would indicate that the underlying pathophysiology differs between IBS high and low somatization, with IBS high somatization having more contributing components of central nature.

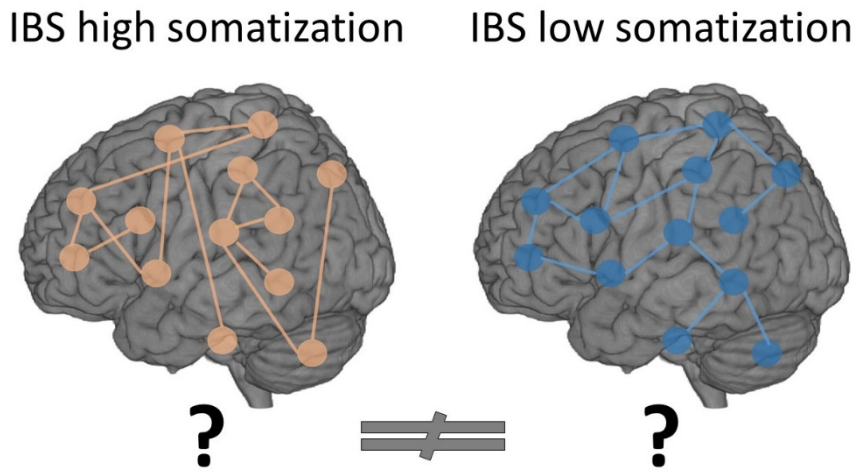

Figure 4. Hypothesis IV. The concept of using graph analysis to compare connectivity between two groups. Please note that the nodes and edges are completely unrelated to brain structures and hypothesized links, but are meant only for illustrative purposes of the concept.

3 SUBJECT COHORTS AND METHODS

All four articles are based on analyses of two different study cohorts, collected in large studies with the overall aim to evaluate different aspects of the pathophysiology of IBS. Both cohorts were recruited from the outpatient clinic for functional GI disorders (Mag- och tarmlaboratorium) at Sahlgrenska University Hospital, Gothenburg, Sweden, which has a mixed secondary – tertiary care function. The patients came for their IBS through self-referral or referral from another physician, mainly in primary health care. The diagnosis was based on Rome criteria, a typical clinical presentation and additional investigations if considered necessary. Exclusion criteria for both cohorts were: other GI disease(s) explaining the symptoms, severe disease(s) such as malignancy, severe heart disease, kidney disease or neurological disease, severe psychiatric disease or pregnancy.

Table 2. Overview of the subject cohorts used in the analyses for the different papers.

	Paper I	Paper II	Paper III	Paper IV
Total subjects	N= 138	N= 372	N= 98	N= 97
IBS Females, N (%) Males, N (%)	N= 138 105 (76%) 33 (24%)	Cohort 1 (C1) N= 231 Cohort 2 (C2) N= 141 C1/C2: 181 (78)/ I00 (71) C1/C2: 50 (22)/ 41 (29)	N= 67 48 (72%) 19 (28%)	N= 66 48 (72%) 18 (27%)
Healthy controls Females, N (%) Males, N (%)	N=0 (previous study for reference, N=34)	N= 0	N= 31 20 (65%) 11 (35%)	N= 31 20 (65%) 11 (35%)
Age range (accepted in study)	18-75	C1: 18-75 C2: 18-65	18-65	18-65
Age mean +/- sd IBS HC	36±12	C1/C2: 36±12/ 35±12	33±10 32±9	33±10 32±9
Included year	2003-2007	C1: 2003-2007 C2: 2010-2014	2011-2014	2011-2014
Rome criteria	Rome II	C1: Rome II C2: Rome III	Rome III	Rome III

Cohort 1 was enrolled between 2003 and 2007, aged 18 to 75, and diagnosed with IBS according to Rome II criteria [22]. The second cohort was enrolled between 2010 and 2014, aged 18 to 65 and had IBS according to Rome III criteria [23]. Cohort 1 is used in article II and (a subgroup) in article I. Cohort

2 is used in article II, III, and IV. Cohort 2 is almost the same in paper III and IV (apart from one male IBS patient who had not completed the PHQ questionnaire), and the subjects in paper III/IV is a subset of the individuals in cohort 2 in paper II.

Table 3. Overview of the questionnaire data and physiological measurements used in the different papers.

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Anxiety	SCL-90R	HADS subscore	-	-
Depression	SCL-90R	HADS subscore	-	-
Psychological distress	-	-	HADS total score	HADS total score
Somatization	SCL-90R	C1: SCL-90R (N=124), PHQ-15 (N=107) C2: PHQ-15	-	PHQ-14
Abuse data	-	C1 N=124 C2 N=141 4 subcategories.	N= 96 (missing= 2, both IBS) Overall Yes/No	-
Other questionnaires		VSI (GI-specific anxiety)	IBS-SSS GSRS-IBS -Abdominal pain -Bloating	IBS-SSS
Rectal barostat, distension protocol	Protocol A	Protocol A Protocol B	Protocol B	-
Rectal barostat measurements	-Thresholds: *First sensation *Urge to defecate *Discomfort *Pain -Intensity ratings, (several distension levels): *Unpleasantness *Pain	-Pain threshold -Pain referral area -Pain intensity rating (36 mmHg rectal distension).	-Pain threshold -Pain intensity ratings at 24 mmHg	-
Brain imaging Structural MRI -Measurments -Region of interest (ROIs)	-	-	-Volumes -Cortical thickness -Surface area - Mean curvature -Sensorimotor network	-Volumes -ROIs from literature [36] [125] associated with IBS

3.1 QUESTIONNAIRES

IBS symptoms

Two of the most widely used questionnaires to assess the severity of IBS symptoms are the IBS severity scoring system (**IBS-SSS**) and the Gastrointestinal Symptom Rating Scale (GSRS), which has been developed into an IBS-specific version (**GSRS-IBS**) [59].

IBS-SSS is a validated questionnaire to assess symptom severity consisting of five questions: pain severity, frequency of pain, severity of abdominal distension, bowel habits dissatisfaction and how much IBS interferes with life in general [141]. Each question generates a score between 0-100, and the total questionnaire is scored 0-500 where 0 is no symptoms and 500 is the maximal symptom severity [141].

The GSRS-IBS and IBS-SSS differs in several ways. Most importantly, in GSRS-IBS it is possible to separately determine the perceived severity of diarrhea and constipation, which is not possible with IBS-SSS, as this questionnaire only asks for dissatisfaction with bowel habits [59]. The GSRS-IBS contains 13 items, arranged into 5 domains: satiety, abdominal pain, diarrhea, constipation and bloating [24].

Psychological symptoms

The Hospital Anxiety and Depression scale (**HADS**) is a self-report questionnaire consisting of 14 questions to assess emotional and cognitive aspects of depression and anxiety [142]. The score is traditionally calculated for the 7 anxiety items and 7 depression items separately, resulting in 2 scores; one for anxiety (0-21) and one for depression (0-21) with high scores reflecting high symptom burden [143]. In recent years, the latent structure of HADS has been questioned with the suggestion to use the total score as a measure of emotional/psychological distress [144].

The Visceral Sensitivity Index (**VSI**) is a validated questionnaire to measure GI-specific anxiety [145, 146] consisting of 15 statements covering five dimensions of GI-related cognitions and behaviors; worry, fear, vigilance, sensitivity and avoidance. After conversion, the total scores range from 0 to 75 and reflects the level of GI-specific anxiety with a high VSI score indicating a high level of GI-specific anxiety. The Visceral Sensitivity Index is the only validated instrument to measure GI-specific anxiety, with good psychometric properties [59].

The Symptom Checklist-90-Revised (**SCL-90R**) [147] is a questionnaire developed to measure psychological symptom patterns of psychiatric and medical patients. It consists of nine primary symptom dimensions and three global indices of distress. Of these, we have used depression and anxiety in paper I, and somatization in paper I and cohort 1 in paper II.

Somatic symptom severity / somatization

The **PHQ-15** consists of 15 questions about the most prevalent somatic symptoms across different bodily systems; nausea, abdominal pain, altered bowel habit, back pain, limb pain, headaches, chest pain, dizziness, fainting spells, palpitations, breathlessness, menstrual cramps, dyspareunia, insomnia, and lethargy. The total score ranges from 0 to 30, with increased scores denoting increased somatic symptom severity, or somatization [148]. The 15 somatic symptom account for more than 90% of symptoms seen in primary care [59]. Three of the questions are gastrointestinal questions ('stomach pain'; 'constipation, loose bowels, or diarrhea'; and 'nausea, gas, or indigestion'), and one question is regarding menstrual pain. In paper IV, the menstrual question was removed, and we called this questionnaire **PHQ-14**.

In primary care setting, the psychometric properties of nine questionnaires of somatization were tested, including PHQ-15 and SCL-90R. In that setting, PHQ-15 was one of the top two questionnaires, whereas SCL-90R had some promising results, but had too little evidence for higher ranking [149]. For large-scale studies, the SCL-90 somatization subscale and PHQ-15 has been recommended in a review of 40 questionnaires, due to their well-established psychometric properties, inclusion of relevant symptoms, and relatively short length [150].

History of abuse

The abuse questionnaire by Leserman and Drossman [151] was used to obtain information about abuse history. Four different domains were investigated: childhood physical, childhood sexual, adult physical and adult sexual abuse. In paper II, the presence or absence of abuse history was dichotomized for the four domains, and in manuscript III, subjects were dichotomized as having experienced abuse overall or not. The division between having experienced abuse (or not) was for the yes/no questions of the questionnaire defined as any 'yes', and for the frequency parts of the questionnaire as: 'seldom', sometimes', or 'often'.

3.2 RECTAL BAROSTAT PROTOCOLS

A seminal consensus-paper on rectal barostat testing from 1997 [68] states that subjects should be fasted; only tap water enema should be used for rectal cleansing to minimize rectal irritation; sensory thresholds should be reported as pressures rather than volumes since pressures show less measurement error; and sensations should be reported on a graduated scales (not only yes/no). All these recommendations have been followed in the work presented in this thesis.

In paper I, visceral hypersensitivity was defined as pain threshold ≤ 31 mmHg; this value represents the mean pain threshold for healthy controls minus 2 SD using the same protocol. In paper II and III we did not divide the IBS patients into normo- or hypersensitive. Instead, we used the pain threshold as a continuous variable. Therefore, no threshold defining visceral hypersensitivity was required.

The exact protocols of the barostat procedures can be found in paper I, II (including supplementary information) and III. A summary of protocol A (used in paper I and II), and protocol B (used in paper II and III) is represented in Figure 5 and 6 respectively. The preparations for both protocols were similar; subjects came after an overnight fast and received a rectal cleansing tap water enema (500-800 mL). A polyethylene balloon attached to a double-lumen polyvinyl tube (Salem Sump Tube, 18F; Sherwood Medical, Tullamore, Ireland) was inserted into the rectum, leaving the distal attachment site 5 cm from the anal verge. Distension to a maximal volume (650 mL) resulted in a spherical balloon shape. The catheter was connected to a computer-driven electronic barostat (Dual Drive Barostat, Distender Series II; G&J Electronics Inc, Toronto, Ontario, Canada). The initial inflation sequence to unfold the balloon and familiarize the subjects with the barostat differed between the protocols. For protocol A, two distensions at 25 mmHg each were performed. For protocol B, one distension sequence increasing in steps of 4 mmHg from 0 to 20 mmHg was used. The operating pressure (OP) was set to 2 mmHg above the minimal distending pressure (MDP) necessary to record respiratory variations in the balloon volume, and these definitions were the same in both protocols. The protocols from here on forward differed substantially.

In **protocol A**, an AML rectal distension protocol [33] was performed (Figure 5), with each phasic isobaric distension step (inflation speed 45mL/s) lasting 30 seconds and followed by 30 seconds at OP. Starting at OP, in every distension step the intra-balloon pressure was increased by 5 mmHg until pain was reported, or until a pressure of 70 mmHg was reached. During the last 10

seconds of each distension step, subjects were asked to rate their perceived rectal sensation as either no sensation, rectal fullness, urge to defecate, discomfort or pain. Following each pressure step, all subjects rated the perceived intensity of pain on a 100-mm visual analog scale (VAS), from 'no pain' to 'worst imaginable pain', and the perceived overall intensity of non-painful sensations ('unpleasantness') on a 100-mm VAS ranging from 'no unpleasantness' to 'worst imaginable unpleasantness'.

After the distension protocol, patients were asked to mark the location of their painful sensations on a schematic body map (scale 1:4) to assess the viscerosomatic referral area for pain, considered to reflect processing of sensory information at the level of the spinal cord [152]. Pain threshold in paper I and paper II cohort 1 was defined as the distension pressure above OP at which the subject first reported pain.

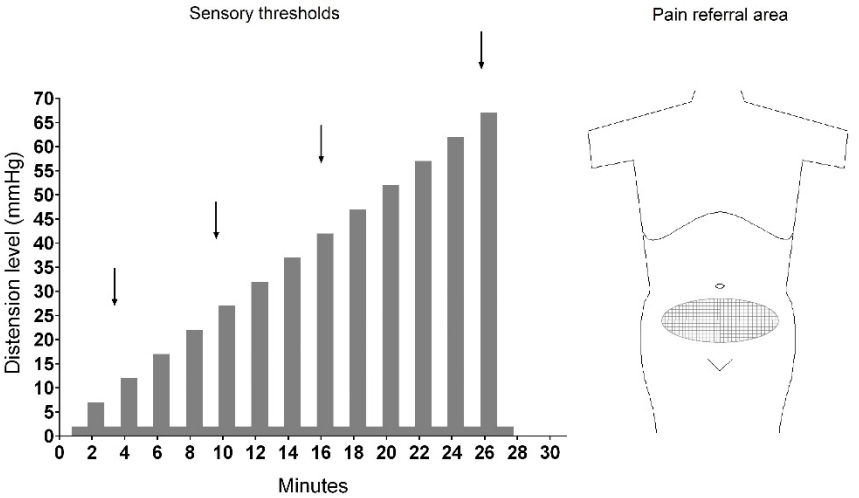

Figure 5. Rectal barostat protocol A [153]. See text for further information.

In **protocol B**, another AML rectal distension protocol [154] was used, with ramp inflation increasing with steps of 4 mmHg (inflation speed 45mL/s) without returning to OP between different steps. Starting at 0 mmHg, the distensions progressed with 4 mmHg increments every 60 seconds until pain was reported or until a pressure of 60 mmHg was reached. Thresholds for first sensation, desire to defecate, urgency, discomfort and pain were assessed (Figure 6).

After this AML protocol, the balloon pressure returned to OP before the second distension paradigm, where the subjects received four fixed phasic distensions at 12, 24, 36 and 48 mmHg above OP in random order. The distensions lasted for 60 seconds with an inter-stimulus interval of 2 minutes with the balloon pressure at OP. During the last 30 seconds of distension, the participants were asked to complete VAS ratings for urge to defecate, gas, discomfort and pain. The maximum pressure used for the random phasic distension was limited by the pain threshold from the previous AML; only one distension level above the AML pain threshold was delivered (e.g. if pain threshold in the AML was 32 mmHg; distensions of 12, 24 and 36 mmHg above OP were delivered, but not 48 mmHg above OP).

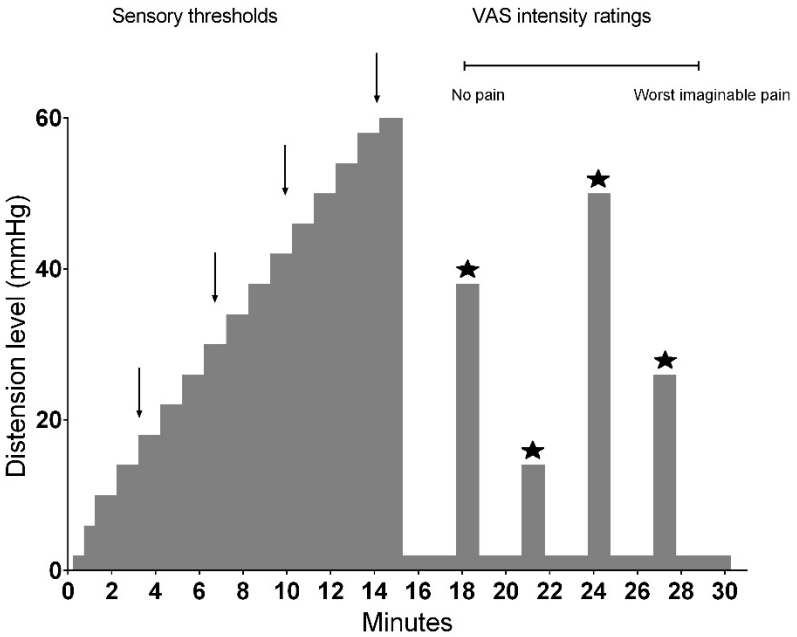

Figure 6. Rectal barostat protocol B [153]. See text for further information.

Few subjects completed all four distensions, therefore we choose to analyze the 36 mmHg distension with last value carried forward if the 36 mmHg distension was not performed in paper II, whereas the distension of 24 mmHg was used for paper III. The different approaches were due to amount of information lost when using the 36 mmHg, last distension carried forward approach.

3.3 GRAY MATTER MORPHOMETRY AND FREESURFER

The gray matter is where the neuronal cell bodies are located, and can in the human brain can be found as the outer surface of the brain called the cortex, and collections of grouped nuclei deep within the brain: the basal ganglia, thalamus and brainstem. Gray matter morphometry refers to the quantitative analysis of gray matter form (size and shape).

The first study describing subtle anatomical changes using morphometry in patients was published in 1999 [71], using voxel-based morphometry (VBM) and studying idiopathic headache [155]. The two most used method to study gray matter morphometry are VBM and surface-based analysis (of which FreeSurfer is the most commonly used segmentation tool) [156]. A voxel is defined as a three-dimensional pixel [157], for example, in our studies with the size of 1x1x1 mm^3. There are a number of gray matter segmentation tools available such as Statistical Parametric Mapping (SPM), FMRIB's Software Library (FSL) and FreeSurfer, each having strengths and weaknesses, but all showing high reliability [158].

The automated or semi-automated processes involved in these cortical parcellation softwares can be divided into the steps of preprocessing and parcellation. The preprocessing usually includes skull stripping, bias field correction and tissue classification (also known as segmentation) into gray matter, white matter, cerebrospinal fluid and other [159]. With parcellation the brain is divided into smaller anatomical regions and labeled based on atlases and algorithms of the software [159].

The VBM method involves spatially normalizing the images to a stereotactic space; extracting gray matter by characterizing the voxels as either gray matter, white matter, cerebrospinal fluid or background noise based on voxel intensities; smoothing; and finally statistical comparison of groups [160]. The output from the VBM method is a statistical parametric map showing regions where gray matter concentration differs significantly between groups [160]. With modulated VBM, a jacobian deformation field is used to be able to interpret the statistical parametric maps as the volume of gray matter instead of gray matter concentration/density [161].

The work on gray matter morphometry included in this thesis used FreeSurfer, a freely available suite of tools for surface-based analysis (http://surfer.nmr.mgh.harvard.edu/) with high level of automated processes. The FreeSurfer reliability have been shown to be excellent with intraclass

correlation coefficients, for cortical regions>0.87 and for subcortical regions>0.95 [162].

In surface-based analysis, morphometric measures are derived from geometric models of the cortical surface [161]. Surface-based method used by FreeSurfer includes intensity normalization, skull stripping, segmentation, tessellation, and inflation [163, 164]. The segmentation procedure is based on a combination of the voxel intensities and local geometric information identifying the gray–white mater border [163]. This boundary between gray and white matter is used as a reference point for morphological measures, and the pial-gray matter interface (the outer surface of the cortical gray matter) is determined [156] by 'growing' the gray-white matter boarder until the gray matter-cerebrospinal fluid limit is detected [159]. When the cortical sheet has been identified this way, the cortical surface is inflated to a spherical representation with minimization of metric distortion so that the spherical surface accurately represents the shape of the folding pattern of the cortex [165]. The alignment of the spherical representation (to an average or a template) is based on folding patterns, which yields more accurate registration of cortical areas across individuals than for instance the 3D Talairach coordinate system [165].

The spherical sheet of cortical gray matter can then be used to calculate cortical thickness, surface area, volumes etc. Different atlases can be used to label the different anatomical regions [156]. We used the Destrieux atlas [166] for cortical parcellation, although other atlases are also available such as the Desikan-Killiany atlas [159].

The automated segmentation of subcortical regions can be based on a combination of MRI image properties (such as different intensity histograms), and local spatial relationships between structures (such as the amygdala is situated anterior and superior to the hippocampus) [167].

Four morphological measures were computed and extracted for each cortical parcellation: volume (V), surface area (SA), cortical thickness (CT), and mean curvature (MC). The different measurements represent distinct features of the cortex. Cortical thickness and surface area are genetically unrelated with two distinct sources of genetic influences [168]. Volume is genetically and phenotypically more closely related to surface area than cortical thickness [169]. Mean curvature is a measurement of the folding of the cortex, with increased mean curvature meaning sharper cortical folds [170]. Increased mean curvature has been interpreted as white matter atrophy, primarily in studies of neurodegenerative disorders [171].

In manuscript III, we wanted to investigate the different aspects of the gray matter morphometry of the sensorimotor network and therefore used all four measures. For manuscript IV, we chose only volumes as there where many instances with significant correlations between the different measurements in the same region, which is not suitable for graph analysis. Volumes were chosen since it captures information of both surface area and cortical thickness (although indirect so if they goes in opposite directions it will be missed), and since volume is the most widely used measure in the literature, thereby facilitating comparisons between studies.

3.4 STATISTICAL AND DATA ANALYSES

The main results of paper I are based on mixed-models, an extension of general linear models (GLMs), where we are particularly interested in interaction effects. Paper II uses mediation analysis based on a combination of stepwise GLMs and bootstrapping. The main results of manuscript III are based on ANOVAs and ANCOVAs, which are also variants of GLMs. Lastly, the main results of manuscript IV is based on network connectivity and graph analysis. The next sections give some background information about the different methods used. In addition, all papers use basic statistical analyses for describing and comparing group characteristics.

3.4.1 GLMS, MIXED MODELS AND INTERACTION EFFECTS

The general equation of the GLM is: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_i Xo_i$, where Y is the dependent variable (also known as the predicted or response variable), all X are independent variables (predictor or explanatory variables), the β_0 is the intercept (the value Y takes if all X=0) and the β are the coefficients (or weights) assigned to the independent variables [172]. The independent variables are not allowed to correlate, if having more than one independent variable. If the independent variable(s) are numerical/quantitative/continuous, they are called covariate(s), and if they represent groups/categorical (e.g. males/females), they are called factors.

GLMs can be used in different statistical procedures, such as
-the analysis of variance/ANOVA, where all independent variables are factors;
-the analysis of covariance/ANCOVA, which is a GLM with at least one factor and one covariate;
-the linear regression, where all variables are covariates [172].

A β gives the predicted change in Y for one unit increase in X, and the unit varies with the independent variable. Therefore, one cannot compare the magnitude of the βs for different variables unless they are standardized. Standardized β are related to standard deviations; one standard deviation change in variable X_1 will result in a β_1 standard deviation change in Y [172].

The linear mixed models are extensions of the GLM [173]. You could rewrite the GLM equation as: Outcome = Intercept + Fixed effects + Error. The Fixed effects are the independent variables you are interested in, or want to control for. The equation for Mixed models would then be: Outcome = Intercept + Fixed effects + Random effects + Error, with Fixed effects being to variables you are interested in, or want to control for, and Random effects being variables that you are not interested in but need to be accounted for.

In a study with repeated measures, the repeated measures cannot be treated as independent variables as they come from the same individual and are expected to correlate. In paper I, we use several different distension steps to evaluate the progression of intensity ratings between groups. By using mixed methods, we can specify subject as a random effect (a variable we are not interested in but need to account for since we use the same subject multiple times), and thereby still being able to detect the fixed effect from the variables we are interested in such as sensitivity status, anxiety etc.

An interaction effect is an "it depends effect" [174]. The effect of variable X on Y depends on Z if there is an interaction effect between X and Z. In our paper I we have main effects, or independent effects, for example sensitivity status (being normosensitive or hypersensitive to rectal distension), distension level and depression on pain intensity ratings, but also interaction effects between for instance sensitivity status*distension level on intensity ratings. This is an example of a two-way interaction effect, investigating if the increase in pain intensity ratings with increased distension levels are dependent on whether you are normo- or hypersensitive to rectal distension. We also examined three-way interaction effects. A three-way interaction effect examines to what degree a third variable influence the two-way interaction effect. In paper I we used the three-way interaction effect to study if there are differences in the increase of intensity ratings with increased distension level based on the interaction between anxiety/depression/somatization and the sensitivity status (if the subject is normo- or hypersensitive).

3.4.2 MEDIATION AND BOOTSTRAPPING

Mediation analysis can be used when we think we know the mechanism through which a predictor variable affects the outcome through a mediator, and want to test this statistically. Mediation analysis is an attractive and well-used method as it can use cross-sectional data to indicate possible causal effects, given you know, or have good reason to postulate, the temporal precedence. However, if we are wrong in our presumptions, the model will be of little value, or worst case misleading [175]. The path going from the independent variable through the mediator to the dependent variable is called the indirect effect, and all other ways that the independent variable influences the dependent variable is called the direct effect [176].

The starting point of a mediation analysis is answering these questions [177]:

1) Does the independent variable (X) predict the dependent (outcome) variable (Y)? I.e. is there an effect that can be mediated?
2) Does the independent (X) variable predict the mediator (M)? I.e. can the mediator relate some information about the independent variable?
3) Does the mediator (M) affect the dependent variable (Y)? Is there a significant relationship between the mediator and the outcome after controlling for the independent variable?

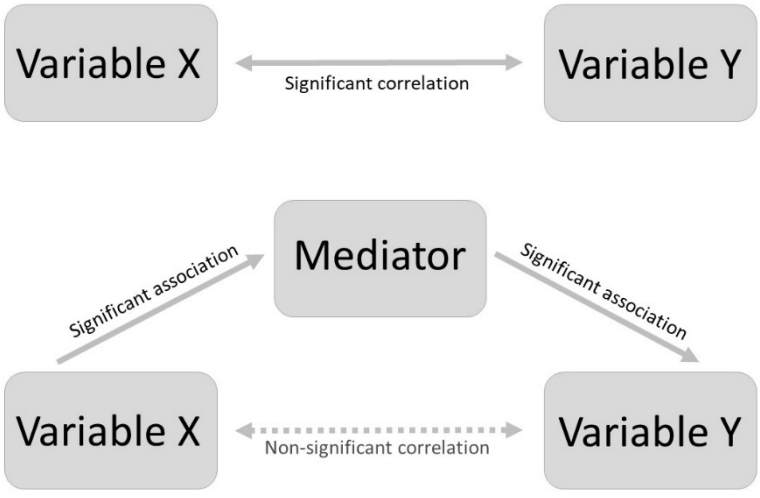

Figure 7. Illustration mediation effect.

In case of complete mediation, the effect of the independent variable on the outcome variable (direct effect) becomes non-significant when controlling for the mediator, if only reduced it is called partial mediation.

When mediation has been indicated by answering yes on the above-mentioned questions, we want to determine if the indirect effect (i.e. the path from Variable X → Mediator → Variable Y) is significant. This includes rejecting the null-hypothesis that the indirect effect = zero, and can be tested using a variety of methods such as the Sobel test, or as in our paper II, bootstrapping.

Bootstrapping is a nonparametric approach to estimate effect-size and test hypothesis that makes no assumptions about the shape of the distributions of the variables or the sampling distribution of the statistic [177]. In bootstrapping, the sample data is used to simulate new data sets. One bootstrap sample is created by randomly selecting the same number of observations as is in the sample data, from that pool of possible outcomes. The sampling is made with replacement so that the same value can appear many times in the simulated data, which by that is not identical as the sample data. This bootstrapping procedure is repeated many times, in paper II we used 1000 bootstrap samples, each with its own statistical properties such as mean, standard deviation and spread. The bootstrap procedure uses these sampling distributions as the foundation for creating confidence intervals and hypothesis testing of our sample data.

3.4.3 NETWORK CONNECTIVITY AND GRAPH ANALYSIS

"Graph theory provides a powerful method for quantifying the organization of brain connectivity" [178].

Analyzing the brain's structural connectivity is thought to capture neurobiologically important aspects of the brain organization [179]. Network analysis can help identify similarities and differences in the organization of neural networks [180], and abnormal connectivity has been found in neurological and psychological disorders by comparing structural or functional brain network properties [181].

The analysis of networks originated from the mathematical field of graph theory [180]. Networks in graph theory are based on nodes, also known as vertices, and links, also known as edges or connections [181]. Nodes are called neighbors if they have direct connections through an edge [180]. Links can be binary, i.e. connection defined as present or absent based on threshold(s), or weighted i.e. bear information about the connection strength between the nodes [180, 181]. In this thesis, we have used weighted graph analysis, since the results are presumably more straightforward, it is more robust, and it retains more information of the network properties, albeit at the cost of being more computationally demanding [182].

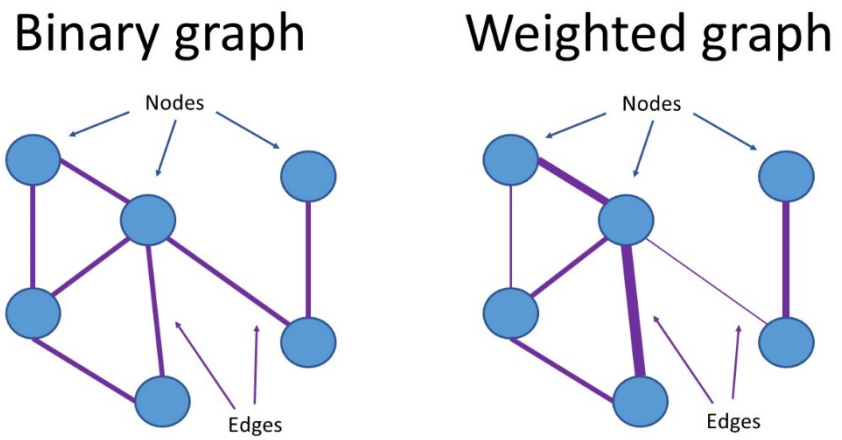

Figure 8. Binary vs weighted graphs.

There are several measures of brain connectivity graphs (=networks) that could be calculated, both at a global, and a local level. Since global measurements were not significantly different between the groups of interest in paper IV, these measurements are not specified in detail herein. Global measurements reflect overall estimates for the entire network, and "provide an indication of the entire network's capability for information integration" [183].

In this thesis, *nodes = regional gray matter volumes*, and *edges = Pearson correlations between regional brain volumes (corrected for total gray matter volume)*. There is an underlying assumption in this paradigm, that the correlations of brain regional volumes have a biological meaning. For example, brain regions where the volumes correlate may have similar functions, may be part of the same network, or may be influenced by the same factors.

In the following section, the local graph measures used in this thesis are described. Please note that we have used weighted graphs in this thesis, whereas the figures shows binary graph measures (except node strength where a weighted graph is shown) for simplification, the weighted equivalents are much more complicated.

3.4.4 GRAPH ANALYSIS MEASUREMENTS (LOCAL)

Node degree is the number of links connected to the node, and the weighted variant of node degree is called node strength. Node strength is defined as the sum of weights of all links connected to a specific node [181]. A change in a node with high node strength would strongly effect many other nodes [184].

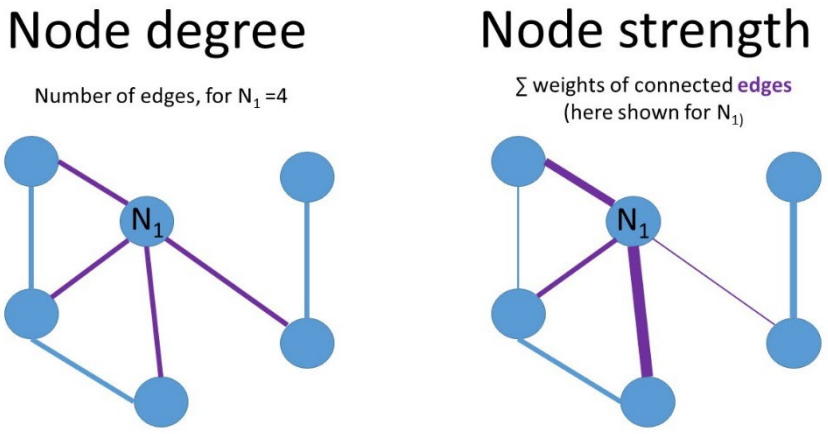

Figure 9. Node degree and node strength.

Paths are sequences of linked nodes. Path length in a weighted network is the total sum of individual link length, where link lengths are inversely related to link weights [181]. In anatomical networks paths represent potential routes of information flow, and shorter paths imply stronger potential integration [181]. Centrality regards the relative importance of a node or edge within the overall network architecture, one frequently used metric of centrality is betweenness centrality [185].

Betweenness centrality is defined as the fraction of shortest paths in the network that pass through a given node [181]. Nodes with high values of betweenness centrality participate in a large number of shortest paths [186]. Betweenness centrality represents how strongly a given node can influence

information flow in the network, an estimate of how a change in a given node would affect the rest of the network [184].

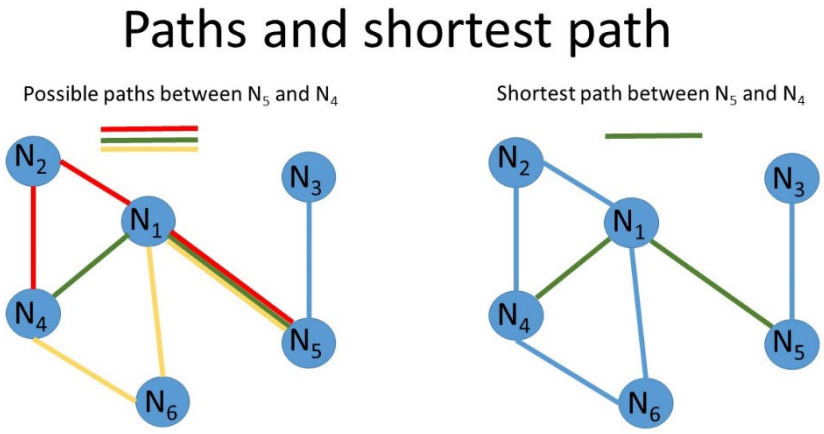

Figure 10. Paths and shortest path.

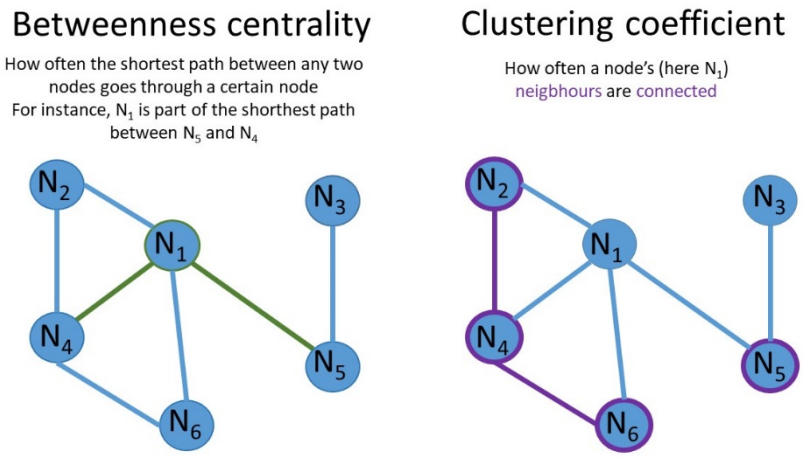

Figure 11. Betweenness centrality and clustering coefficient.

The local clustering coefficient is a measure of neighborhood connectivity [180]. It is defined as the number of connections between the neighbors of a node divided by the number of all potential connections between its neighbors [180]. High clustering is associated with robustness of a network, i.e. resilience against random network damage [187].

The efficiency of a network measures how well information propagates over the network [188, 189]. Local efficiency is the averaged efficiency of all first-order neighborhoods [190], the inverse of the average shortest path connecting all neighbors of that node [187]. Local efficiency measures how fault tolerant the system is at a local level, how efficient the communication between neighbors would be if one of the nodes were removed [188].

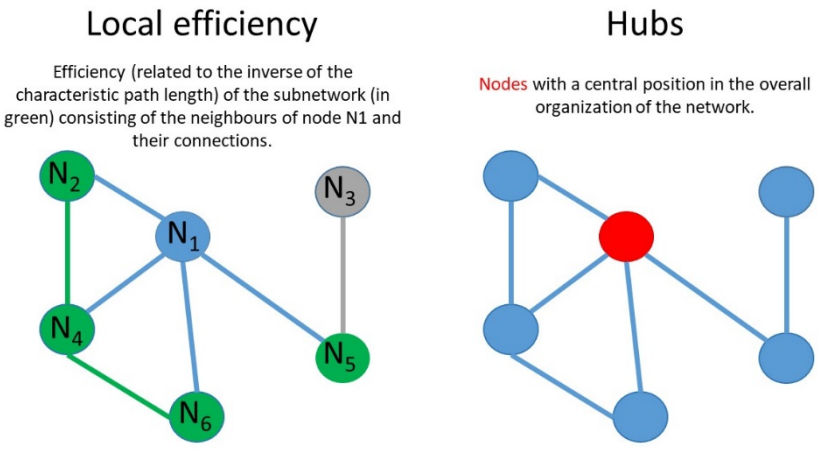

Figure 12. Local efficiency and hubs.

A hub is a node with a central position in the overall organization of the network [185]. There are no single measure for defining network hubs; instead, it is often preferable to detect hubs by aggregating rankings across different measures, most of which express aspects of node centrality [185]. In paper IV we have used four criteria for detecting hubs. Using dummy scores with one point for each of the four: 20% of nodes showing highest strength, highest betweenness centrality, lowest path length and lowest local clustering coefficient, hubs were defined as hub score ≥2.

Modules are subgroup of nodes within a network that have a larger number of connections within the module, and a smaller number of connections to nodes outside of their module [185]. Modules are also called clusters, communities [180], modular structure or community structure [181]. The nodes in a module should have maximal within-module connections and minimal between-

modules connections and represents functional segregation [181]. Nodes in the same modules tend to have similar functions [180].

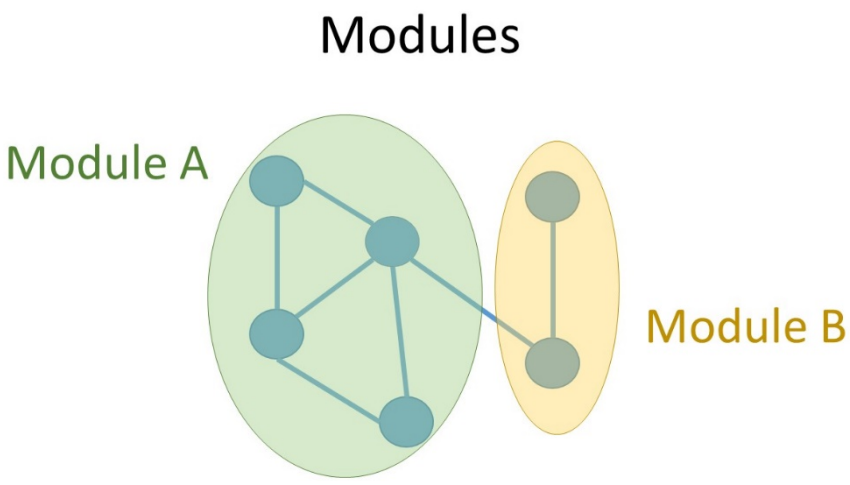

Figure 13. Modules and modular structure.

4 RESULTS & DISCUSSION

In this chapter, the main results from the four papers/manuscripts will be described separately. The results will be described without numbers, as these can be found in the respective paper/manuscript, to simplify reading. The results will be presented in three ways: a brief summary, bullet points, and graphical abstract, to try to convey the results as clearly as possible.

Thereafter, I will discuss results arching over more than one paper/manuscript, and address some aspects that I would like to discuss more in depth, moving beyond what have already been discussed in the respective paper/manuscript.

4.1 MAIN RESULTS PAPER I

Paper I showed that depression and somatization were associated with increased perception of painful rectal sensations in hypersensitive, but not in normosensitive IBS patients. Anxiety was associated with augmented ratings of painful rectal sensation in hypersensitive IBS, and non-painful rectal sensations were increased by anxiety in both hypersensitive and normosensitive IBS patients alike. The results indicated upregulation of WDR afferent pathways, either solely or in combination with high threshold afferent pathways, as the intensity ratings were increased in hypersensitive IBS patients for the full range of distension levels, including innocuous stimuli.

Main results in bullet format:

1) Anxiety and hypersensitivity independently increased the slope of the distension-perception curves for both pain and unpleasantness ratings.
2) When including the interaction between anxiety and sensitivity status, anxiety increased the pain ratings – distension level curve only in hypersensitive IBS patients.
3) Anxiety had the same effect on the slope of the distension-unpleasantness curve in hypersensitive and normosensitive IBS patients.
4) Depression had no independent effect on the slope of the distension-perception curve, for pain nor unpleasantness ratings.
5) When including the interaction between depression and sensitivity status, depression increased the slope of the

distension-pain curve in hypersensitive IBS patients only, but had no effect on unpleasantness ratings.

6) Somatization increased the slope of the distension-pain curve. When including interaction with sensitivity status, this increase was seen only in hypersensitive IBS.
7) Somatization had no effect on the slope of the distension-unpleasantness curve, neither alone nor when considering sensitivity status.

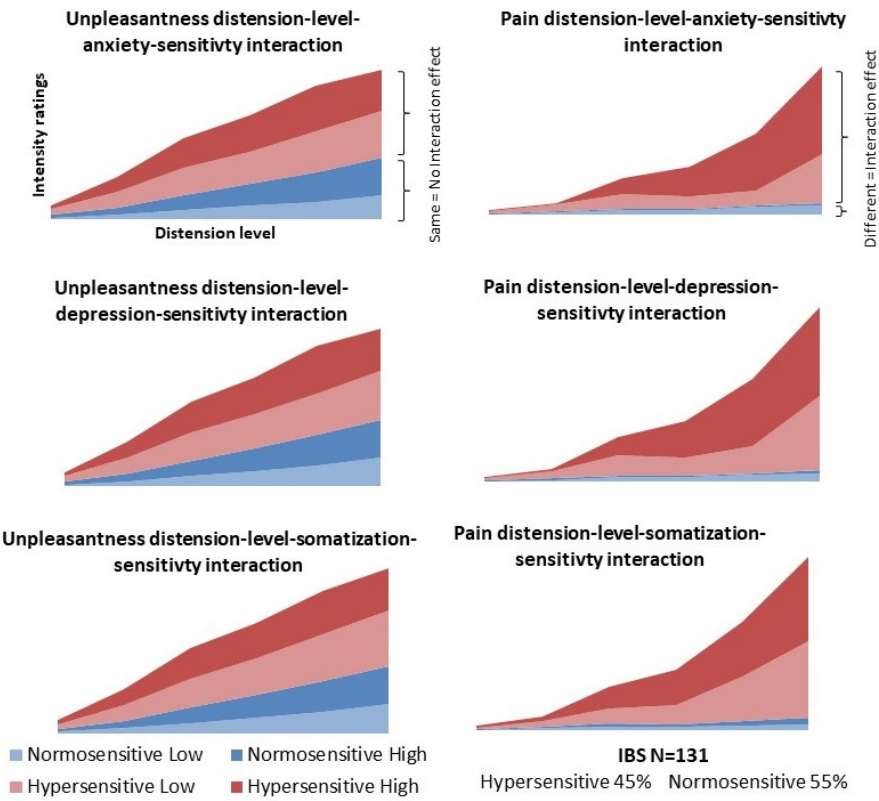

Figure 14. Paper I included 131 IBS patients, 45% of whom were hypersensitive to rectal distension, and 55% were normosensitivie. Shown are the 3-way-interaction-effects for the three psychological variables x two sensation ratings during increased level of rectal distension. The high and low category are only created for illustrative purposes, based on a median split for the respective measure: anxiety, depression and somatization. The mixed models used these variables as continuous. The progression of the intensity ratings curve with increased distension levels are shown in blue for normosensitive IBS patients and in red for hypersensitive IBS patients. The 3-way-interaction-effect can be interpreted as: if the change in the slope between the red and the blue fields are different there is an interaction-effect, if they are not different there is no interaction-effect. In the upper left corner we can see that the increase in the progression of the unpleasantness ratings with

increased distension level is the same for both hypersensitive and normosensitive IBS (there is no 3-way-ineraction-effect), i.e. the progression of the slope is not dependent on the combination of sensitivity status and anxiety. In the upper right corner we can see that the increase of the progression of the pain ratings with increased distension level is different between hypersensitive and normosensitive IBS (there is a 3-way-interaction-effect). Specifically, the progression of pain ratings depends on the combination of anxiety level and sensitivity status. Similar interpretations can be done in the second row for depression, and the third row for somatization level.

4.2 MAIN RESULTS PAPER II

Paper II showed that somatization was associated with various measurements of rectal pain sensitivity in IBS. Somatization mediated the effect GI-specific anxiety had on pain referral area, and the effect depression had on pain intensity ratings. Neither anxiety, depression nor GI-specific anxiety had any independent effects in the final models. Sexual abuse in adulthood had an independent effect on rectal pain thresholds in IBS.

Main results in bullet format:

1) Somatization was significantly associated with most measures of rectal pain sensitivity (pain threshold when distension went back to baseline between distensions, pain referral area, pain intensity ratings at 36 mmHg; but not pain threshold when distensions were successively increased without going back to baseline).
2) Somatization mediates the effect of GI-specific anxiety on pain thresholds (when the pressure goes back to baseline between distensions).
3) Somatization mediates the effect of depression on pain intensity ratings (at 36 mmHg rectal distension level).
4) Sexual abuse in adulthood was the only form of abuse with an (independent) effect on pain threshold (in both distension protocols). Experience of adult sexual abuse was associated with lower thresholds (i.e. higher sensitivity).

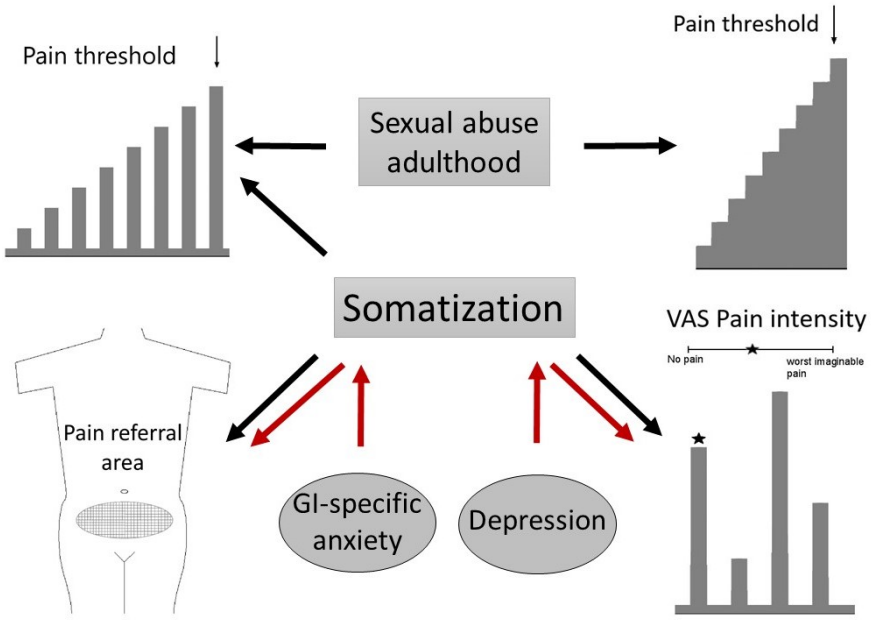

Figure 15. Paper II used two different IBS cohorts and two different rectal distension protocols to assess four measurements of visceral sensitivity: pain threshold from the two different protocols illustrated in the figure, pain referral area (using the rectal distension protocol to the left), and VAS pain intensity ratings at 36 mmHg rectal distension. Anxiety, depression, GI-specific anxiety and four types of abuse (sexual abuse in childhood and adulthood, physical abuse in childhood and adulthood) were assessed through questionnaires. Black arrows in the figure represent main effects/direct effects on the sensory measure as dependent variable, and the red arrows show mediation though somatization to influence the visceral sensory measures.

4.3 MAIN RESULTS MANUSCRIPT III

Manuscript III showed that, at the uncorrected significance level, there were differences in regional gray matter morphometry between IBS and healthy controls, especially in posterior insula and supplementary motor cortex, which in women were related to psychological distress. Evoked pain in IBS showed positive associations with gray matter metrics in primary sensory and motor regions, while spontaneous abdominal pain was associated with reduced thalamic volume and secondary somatosensory cortex area.

Main results in bullet format:

1) In the entire sample, IBS patients showed a trend towards thinner cortex in right posterior insula and bilateral supplementary motor area, related to psychological distress.
2) There were indications of many disease-by-sex interaction effects on gray matter morphometry in IBS.
3) In women, IBS-related gray matter differences in the sensorimotor network were related to psychological distress; this was not seen in men.
4) When controlling for sex and age, increased rectal pain sensitivity assessed with rectal barostat indicated associations with increased size of primary motor cortex and primary somatosensory cortex.
5) When controlling for sex and age, increased symptoms of abdominal pain indicated associations with decreased thalamus and secondary somatosensory cortex volumes.

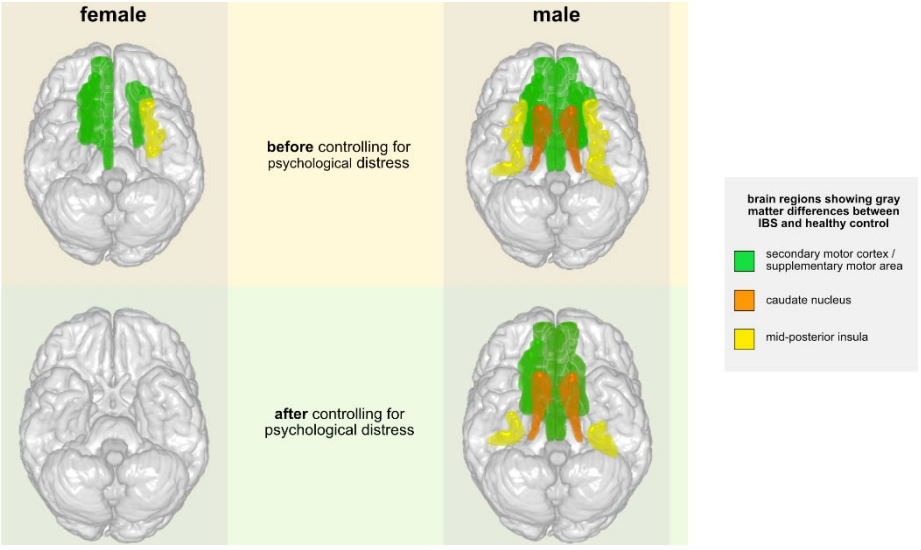

Figure 16. Highlights results in manuscript III. Group differences between IBS and healthy controls vanished in women after taking into account psychological distress, whereas the results in men were virtually unaltered. Courtesy of Hyo Ryu, who made this figure.

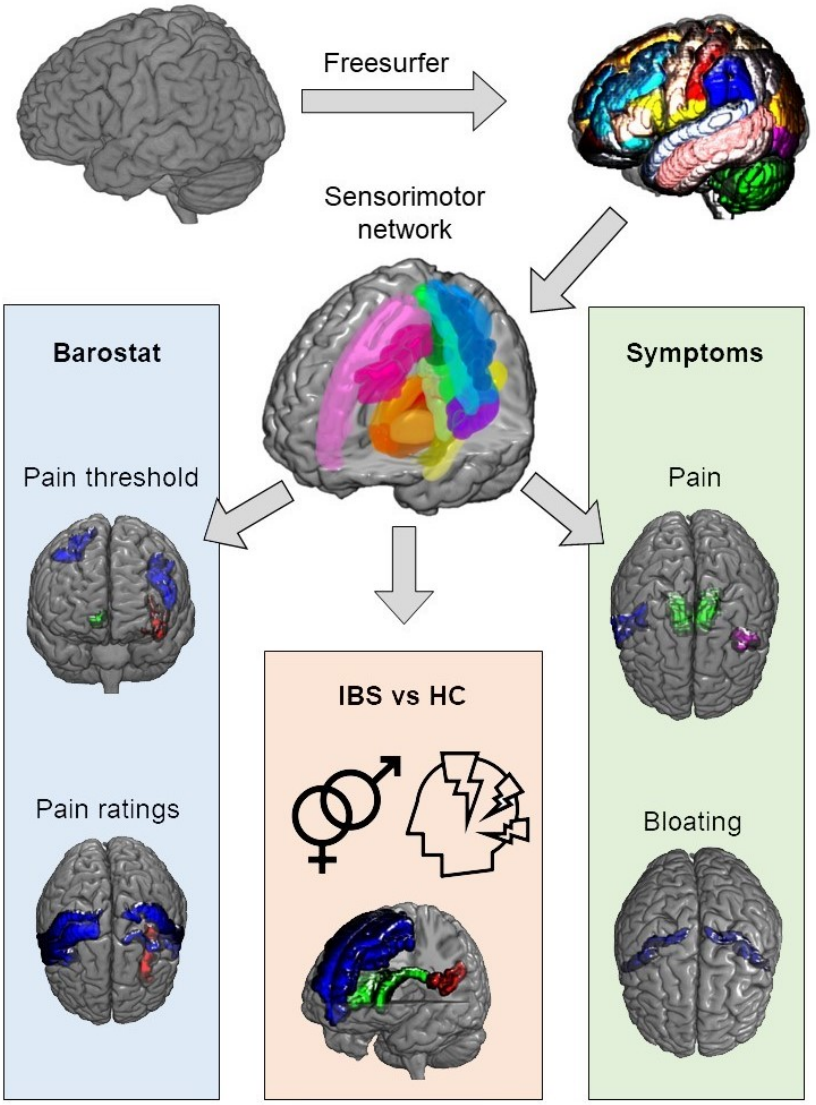

Figure 17. An overview of the study of manuscript III. Structural brain MRI scans of IBS patients (n=67) and healthy controls (n=31) were acquired and parcellated using FreeSurfer. Regions of the sensorimotor network were selected as regions of interest. IBS and healthy controls were compared with and without taking psychological distress and sex into account. In IBS patients only, pain threshold and pain intensity rating during rectal barostat distensions, and abdominal pain and bloating from the GSRS-IBS were correlated with subregions of the sensorimotor network.

4.4 MAIN RESULTS MANUSCRIPT IV

In manuscript IV, we demonstrate differences in structural gray matter connectivity, defined as correlations between (total gray matter volume corrected) gray matter volumes of regions previously identified to be involved in IBS. This was seen between IBS patients with high vs low levels of somatization (IBS high somatization and IBS low somatization, respectively) as well as between healthy controls and both IBS groups. These differences were found at the local level, but no group differences were found in the global network structure. The most robust findings were associated with altered network metrics in parts of the prefrontal cortex, insula and cerebellum.

Main results in bullet format:

1) At the more robust significance level, FDR-corrected $p<0.05$:
    a. IBS low somatization had increased hub scores in anterior insula and left cerebellum, compared to IBS high somatization.
    b. Healthy controls had increased hub scores in anterior and middle insula, compared to IBS low somatization.
    c. IBS high somatization had increased hub scores and clustering coefficients in subregions of prefrontal cortex, compared to healthy controls.
2) On the more exploratory significance level (uncorrected $p<0.005$) several differences were found, see Figures 18, 19 and 20 below for details.
3) The modular structure and the clustering of hubs were characterized, but these results are descriptive and hard to examine with statistical tests. All these results can be found in manuscript IV supplementary material, some will be discussed in the Results & Discussion section 4.10 ‘Hippocampus, Amygdala and cingulate cortex = memories, fear and stress in IBS high somatization?’.

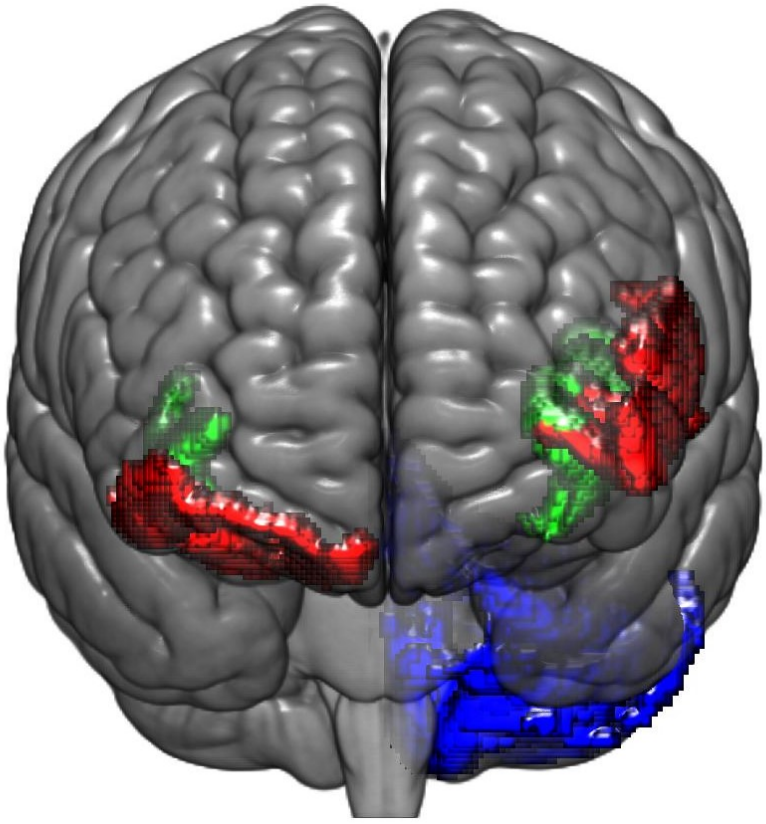

Figure 18. Frontal view of the brain, with the left of the figure being the right of the brain, showing all regions with differences in graph measures between groups at the FDR-corrected significance level. Prefrontal regions are shown in red (left triangular part of inferior frontal gyrus and right orbital gyrus had higher hub scores in IBS high somatization compared to healthy controls; left triangular part of inferior frontal gyrus and left horizontal ramus of the anterior segment of the lateral sulcus had increased clustering coefficients in IBS high somatization compared to healthy controls). Insular regions are shown in green (left short insular gyri had higher hub score in IBS low somatization than in IBS high somatization, right short insular gyri and left superior segment of the central sulcus of the insula had increased hub scores in healthy controls than IBS low somatization). Cerebellum is shown in blue (left cerebellum had higher hub score in IBS low somatization than IBS high somatization).

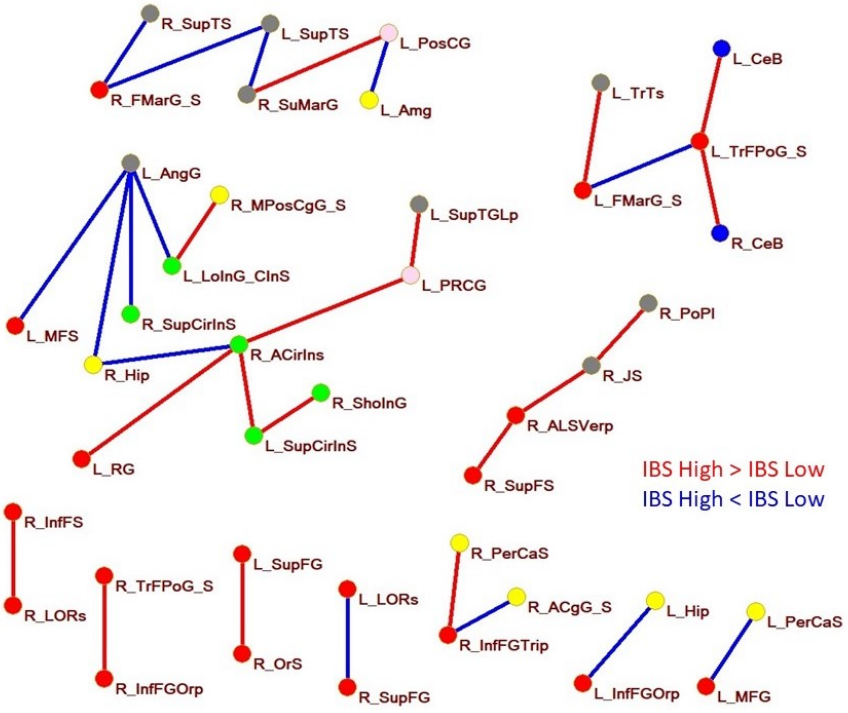

Figure 19. Differences in connectivity between groups significant at p<0.005. Regions belonging to the prefrontal cortex are shown as red nodes; insular regions are shown in green; subcortical and cingulate regions in yellow; S1/M1 in pink; superior temporal and inferior parietal lobe in gray; and cerebellum in blue. R = right, L = left, for full list of the anatomical abbreviations please see supplementary material of manuscript IV.
A. Shows all correlations that differs between IBS high somatization and IBS low somatization. IBS high somatization > IBS low somatization are shown with red lines, IBS high somatization < IBS low somatization are shown with blue lines.
B (Next page). Shows all correlations that differ between IBS high somatization and healthy controls. IBS high somatization > healthy controls are shown with red lines, IBS high somatization < healthy controls in green lines.
C (Next page). Shows all correlations that differ between IBS low somatization and healthy controls. IBS low somatization > healthy controls are shown with blue lines, IBS low somatization < healthy controls in green lines.

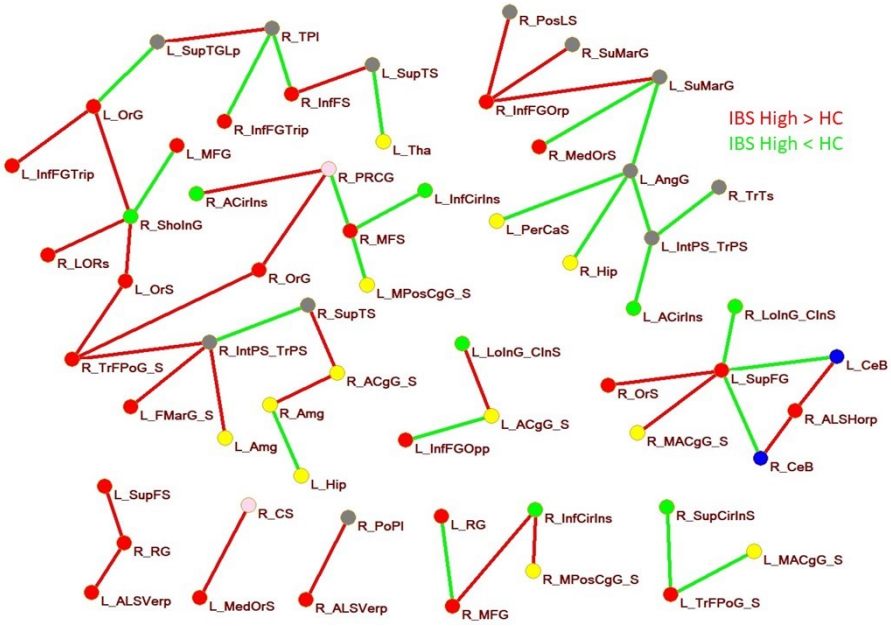

Figure 18 B. IBS high somatization vs healthy controls

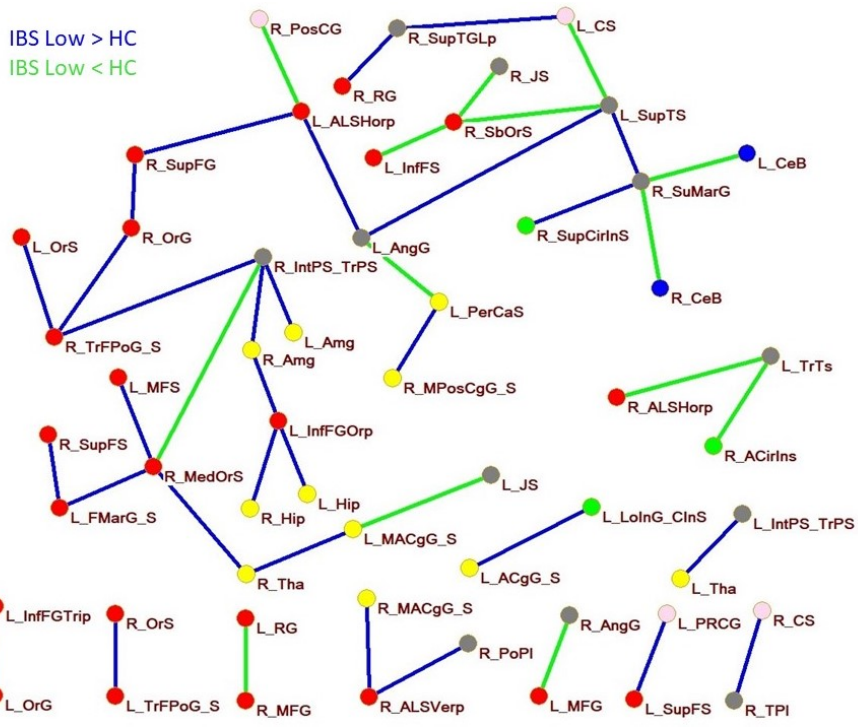

Figure 18 C. IBS low somatization vs healthy controls

4.5 SOMATIZATION AS A RED THREAD

In this thesis, a clear pattern illustrating the importance of somatization in IBS has emerged. In paper I, somatization was associated with increased pain ratings during rectal distension, particularly in IBS patients hypersensitive to rectal distension. In paper II, somatization was associated with all but one measure of rectal pain sensitivity, and mediated the effect some of the psychological distress variables had on rectal pain sensitivity. In paper IV, there were differences in the brain structural network depending on somatization level in IBS. This leads to a number of questions. What is somatization, what is central sensitization, and how are they related? What are we measuring with somatization questionnaires? How should we understand this phenomenon in relation to IBS?

4.5.1 SOMATIZATION OR CENTRAL SENSITIZATION?

Somatization is defined in a seminal paper by Lipowski [191] as "a tendency to experience and communicate somatic distress and symptoms unaccounted for by pathological findings, to attribute them to physical illness, and to seek medical help for them".

Four components of the Lipowski definition have been identified: (1) the presence of somatic symptoms or complaints, (2) somatic symptoms unaccounted for by pathological findings, (3) the participant attribute the somatic symptoms to physical illness, and (4) the participants seek medical help because of the somatic complaints assessed [62]. There is no golden standard for measuring somatization, which is a complicated concept to measure. In studies, somatization is commonly defined only by quantifying physical symptoms and their severity as a proxy [149].

In our studies, somatization were defined by (1) questionnaire measuring multiple somatic symptoms severity. (2) The GI symptoms were unaccounted for by pathological findings, and other major illnesses constituted exclusion criteria for participating in the study, although the possible pathology of different somatic symptoms were not systematically evaluated. The rather extensive workup protocol in the studies should have identified a number of other possible pathologies unknown to the participant explaining additional somatic symptoms. (3) We did not assess if the participants attributed the symptoms to physical illness. (4) The patients had sought medical help for their GI symptoms and thus constitute a 'patient-population', however, we did not systematically evaluate if the participants had sought medical care for their

other somatic symptoms. The definition used for somatization could thus be criticized [62], but adhere to, or could be considered somewhat better than the commonly used construct of somatization in research settings [62, 149].

The definition of sensitization by the International Association for the Study of Pain (IASP) is "Increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally subthreshold inputs" [5]. According to IASP, sensitization is a neurophysiological term that in clinical setting can be indirectly indicated by phenomena such as hyperalgesia or allodynia. Wider definitions have also been used, including increased behavioral, physiological, cognitive, and emotional responses to repeated stimuli. An alternative, broader definition of sensitization is "a non-associative learning process occurring when repeated administrations of a stimulus result in a progressive amplification of a response" [192].

On the cellular level, sensitization is an increased efficiency in a neural circuit, due to a change in the synapses from repeated use [192]. Sensitization of nociceptive neurons has been demonstrated at every location along the pain pathway, from peripheral nociceptors to cortical neurons [193]. In the spinal cord, increased excitability after repeated afferent stimulation of dorsal horn neurons have been well documented [193]. Cognitive bias have been proposed as a higher form of sensitization, called cognitive-emotional sensitization, or cognitive sensitization [194].

Central sensitization has also been attributed a broader definition: an amplified response of the central nervous system to peripheral input [61]. This view has been questioned, and some advocate that sensitization should only be used for the cellular process of enhanced excitability observed experimentally after repetitive stimulation of nociceptive afferents [193]. Similarly, central sensitization has been argued to specifically denote the C-fiber dependent plasticity in the spinal cord dorsal horn resulting in increased excitability in the CNS [195]. Others argue that the existence and clinical relevance of central sensitization, defined operationally as an amplification of neural signaling within the CNS that elicits pain hypersensitivity, is well documented [192, 196].

The term central sensitization in its wider definition has been shown to be clinically meaningful [197], even though there yet is not a clear path from cellular sensitization of nociceptive neurons to a behavioral process of enhanced pain sensitivity in chronic pain [193]. There is consistent evidence that having one type of chronic pain is associated with a higher likelihood than expected of having another chronic pain condition, and the risk increases with

the number of pain areas at baseline [198]. Central sensitization in clinical settings are characterized by excessive sensitivity to a variety of peripheral noxious stimuli, unpleasant after-stimuli sensations, hypersensitivity that extends beyond the territory of the simulated or injured nerve and secondary hyperalgesia [61], and may extend to non-noxious stimuli such as odors, noise etc. [192]. Central sensitization could both precede and be an effect of chronic pain in a circular fashion (central sensitization predispose an individual to develop chronic pain, and chronic painful sensations might enhance central sensitization) [61]. The presence of multiple somatic symptoms have repeatedly been argued as an indicator of central sensitization [61, 192, 196, 199], as well as an indicator of somatization [149]. These two phenomena have additional components making these concepts overlapping, but not the same. The total somatic symptom score has been shown to correlate with outcome (health status and healthcare use) independent of anxiety, depression and general medical illnesses, and should therefore not be regarded as only a reflection of underlying psychological distress [200].

According to Lipowski, somatization implies a discrepancy between subjective and objective health, and the somatizing patients has a pattern of predominantly somatic rather than cognitive response to stress and related emotional arousal [191]. Somatizers probably have central sensitization, and inaccurate perception [192].

What we call "somatization" in this thesis is probably an important component of central sensitization (if accepting its wider definition) maybe more so than actual somatization in its original definition. Somatization have behavioural components, and is usually assumed to be associated with psychological/ psychiatric illnesses, not necessarily present (but quite often is) for the definition of central sensitization.

4.5.2 CENTRAL SENSITIZATION IN IBS

Visceral hypersensitivity has consistently been shown in a large proportion of IBS patients [14], and somatic hypersensitivity has been demonstrated [39, 201]. In adolescents with IBS symptoms, increased sensitivity to cutaneous heat pain was found in a dose-response fashion: the more severe abdominal pain, the lower pain threshold [202]. Repetitive sigmoid distensions in IBS patients have been associated with induction of rectal hyperalgesia and viscerosomatic referral [203]. The presence of increased multiple somatic symptoms or 'somatization' has repeatedly been shown in IBS [204, 205]. IBS patients have diminished conditioned pain modulation indicative of both dysregulated descending pain modulation and central sensitization [40]. All

these factors indicate central sensitization in IBS. Further, many of the neuroanatomical findings consistently replicated in the IBS population are not specific to the IBS patient population [125], which also strengthens the assumption of a general central sensitization phenomenon in IBS.

In a recently published population based study from northern Sweden, not only high comorbidity in the IBS sample with fibromyalgia, posttraumatic stress disorder, generalized anxiety syndrome, panic syndrome, depression and migraine were found, but also intolerance to chemicals and sounds [205]. Further, compared to the reference group (individuals not reporting having a physician made diagnosis of IBS), IBS subjects had higher somatization levels, higher levels of stress and burnout, and worse perceived health assessed with validated questionnaires [205].

The work included in this thesis support central sensitization in IBS, and that IBS can be viewed as a central sensitization syndrome. We have shown that hypersensitive IBS patients are sensitive to both noxious and non-noxious rectal distensions, and the presence of multiple somatic symptoms (indicative of both central sensitization and somatization) were highly relevant for rectal pain perception. We also found evidence for a neurobiological substrate at the brain level for multiple somatic symptoms, possibly reflecting central sensitization in IBS, in the form of altered structural connectivity patterns.

Central sensitization is most likely present only in a subgroup of IBS patients, with other pathophysiological factors playing a larger role for IBS patients without this trait. In paper I, 45% were hypersensitive to rectal distension, and in manuscript IV, 53% of the IBS patients were in the high somatization group (based on a mean split). Since both hypersensitivity and multiple somatic symptoms are indications of central sensitization, my estimate would be that central sensitization is an important pathophysiological trait in up to half of the IBS patients at a specialized FGID clinic.

4.6 WHICH PAIN PATHWAYS ARE INVOLVED IN IBS?

In paper I, both pain and non-painful intensity ratings with increasing distension pressures were increased in hypersensitive compared to normosensitive patients. This indicates that IBS patients have upregulated wide dynamic range afferent pathways as a mechanism of rectal hypersensitivity. However, we cannot determine if this upregulation is specific, or occur in combination with upregulation of high-threshold afferent

pathways. When adding several psychological factors, the difference in intensity ratings between hypersensitive and normosensitive patients remained significant, showing that these psychological factors do not explain the group difference between hypersensitive and normosensitive patients, but may rather have an additive effect.

How or why upregulation of WDR afferents occurs in hypersensitive IBS patients cannot be determined based on our study. Central mechanisms are probably of importance, as reflected by the effect of anxiety, but does not fully explain this upregulation. Other central mechanisms, not psychological by nature, such as spinal cord sensitization, are also likely to be involved [44]. Peripheral sensitization of WDR afferents, nociceptors and activation of silent receptors [206], potentially mediated through inflammation, and changes in expression of TRVP1 receptors are also potential underlying mechanisms [207]. In mice, sensitized muscular-mucosal afferents and awakened silent afferents were shown to contribute significantly to the afferent input that sustains hypersensitivity to colorectal distension [208]. WDR neurons in the lumbal spinal cord of sheep with cutaneous innervation show sensitization to both noxious and non-noxious repeated stimuli [209].

Paper I thus indicates that both central and peripheral mechanisms play a role in the increased sensitivity to visceral stimuli in IBS patients. Psychological factors strongly influenced pain amplification in hypersensitive IBS patients, whereas amplification of non-painful visceral sensation seemed to be under a higher influence of peripheral mechanisms. Paper II and manuscript III further supports the importance of central factors in aberrant sensory information processing in IBS, but the methodology does not allow speculation which afferent pathways are involved.

4.7 PAIN THRESHOLDS VS PAIN INTENSITY RATINGS?

In paper II, somatization had no mediation effect on pain threshold, whereas it mediated the effect of GI-specific anxiety and depression on visceral referral area and pain intensity ratings, respectively. It thus seems, as it is the experience and/or evaluation of pain that is influenced by somatization, not the discriminatory aspect of pain measured by the pain threshold. In manuscript III the brain regions correlating with pain threshold and pain intensity ratings showed overlap in M1, but pain intensity ratings had correlations also with S1 and S2. This might indicate that pain ratings at the cortical level is a more complex phenomenon than detection of the pain threshold.

Sensory thresholds and sensory ratings seems to correlate, but not correspond to the same underlying mechanisms. In healthy women, placebo was associated with decreased pain and unpleasantness ratings during rectal distension and increased pain threshold, whereas nocebo was associated with increased pain and unpleasantness rating, but no change in the pain threshold [210]. Ergo, the expectation of pain was associated with increased pain ratings, but no change in the pain threshold. This suggests that intensity ratings are more susceptible for psychological and/or cognitive modulation than the pain threshold. Another study in healthy participants, when expecting a stimulus with lower intensity, the pain intensity were rated as less intense, despite that the same stimuli being delivered [112]. Also in IBS patients, substantial amounts of evidence support altered evaluation and/or pain reporting, irrespective of rectal pain thresholds [45].

There are, however, data supporting influence of psychological factors on pain thresholds [211]. In an elegant and highly cited paper, IBS patients had compared to healthy controls lower pain and urgency thresholds, similar ability to discriminate between two stimuli, and increased tendency to report all distensions as more intense, irrespective of the actual stimuli [211]. Further, somatization was inversely correlated with pain thresholds and directly with this 'response criterion' [211].

It thus seems as cognitive, psychological factors and somatization (and/or central sensitization) have the ability to influence both pain thresholds and pain intensity ratings, but to a greater extent in pain intensity ratings. Depending on the research question, one might therefore choose either thresholds or ratings or, as we have done, use both, arguing that it provides complementary information about sensory processing.

4.8 IBS SUBGROUPS NOT RELEVANT?

IBS is classified into subgroups based on predominate bowel habit (on the days with disturbed bowel habits), into IBS with predominant constipation (IBS-C), IBS with predominant diarrhea (IBS-D), IBS with mixed bowel habits (IBS-M) and IBS unclassified (IBS-U) [21].

In none of the studies of this theses, the predominant bowel habit has been taken into account in the analyses. From a clinical point of view, what type of disturbed bowel habit the IBS patient has is of great interest, and a possible treatment target [21, 212]. However, the bowel habit subtypes do not differ in colorectal pain thresholds or in overall IBS symptom severity [14]. In a meta-analysis showing diminished conditioned pain modulation in IBS, there were

no significant differences between IBS subgroups [40]. Over time, the patients might also move between the bowel habits subtypes [213]. In the studies of gray matter morphometry investigating possible differences based on IBS subtype, the differences between subtypes have been limited [129, 130].

On the other hand, somatization level based on PHQ-12 have been shown to be associated with IBS subtype, with IBS-M having higher levels of non GI-symptoms than IBS-C and IBS-D [204].

There are other potential ways of subgrouping IBS patients than based on bowel habits alone that might be both clinically and pathophysiological relevant [214]. Our group has recently used mixture model analysis to identify latent subgroups based on the combination of bowel habits with and without multiple extraintestinal somatic and psychological symptoms [29]. Subgroups with elevated non-GI symptoms showed more frequent healthcare utilization and medication usage [215]. In line with the discussion in 4.5.2 'Central sensitization in IBS', one might conclude that bowel habits are of clinical importance, but the combination of bowel habits and central sensitization (in the cited studies as non-GI symptoms measured with PHQ-12) is even more relevant clinically and pathophysiologically.

4.9 WHAT ABOUT ABUSE?

In paper II, only sexual abuse in adulthood had an independent effect on rectal pain sensitivity in IBS, and in manuscript III, there was unexpectedly no difference between the experience of abuse in IBS and healthy controls.

Overall, high levels of experienced abuse were seen in both groups; 65% of healthy controls and 54% IBS patients reported in this cohort some kind of experience of abuse, which was numerically but not statistically higher in healthy controls. A validity study in women on the questionnaire used, at a GI clinic in the USA, showed acceptable test-retest reliability and criterion validity, higher for sexual than physical abuse [151]. The questionnaire had more false negatives than false positives when compared to an interview, indicating a greater risk of underestimating than overestimating the true prevalence [151].

We used broad definitions of abuse. For sexual abuse including: being exposed to anyone else's sexual organs when one did not want it, and being threatened to have sex when one did not want to. For physical abuse including: being hit, kicked or beaten seldom, occasionally or often. Since the overall experience of abuse did not differ between groups, I further examined the available

information from manuscript III, using different abuse classifications. None of these classifications showed any statistical difference between IBS patients and healthy controls. Repeated physical abuse in childhood and adulthood were the closest to significance, with IBS showing a trend of being subjected to more repeated physical abuse (both p=0.09).

Table 4. Prevalence of experienced abuse. Comparisons between IBS and healthy controls based on cross-tabulation and Chi-square test or Fisher's exact test.

Abuse domain		**Healthy controls N (%)**	**IBS N (%)**	**p-value**
Overall abuse	Yes	20 (64.5%)	35 (53.8%)	0.32
	No	11 (35.5%)	30 (46.2%)	
Physical childhood	Yes	5 (16.1%)	17 (26.2%)	0.28
	No	26 (83.9%)	48 (73.8%)	
Sexual childhood	Yes	5 (16.1%)	10 (15.4%)	0.93
	No	26 (83.9%)	55 (84.6%)	
Physical adulthood	Yes	11 (35.5%)	18 (27.7%)	0.44
	No	20 (64.5%)	47 (72.3%)	
Sexual adulthood	Yes	8 (25.8%)	19 (29.2%)	0.73
	No	23 (74.2%)	46 (70.8%)	
Abuse quantification domains	0	11 (35.5%)	30 (46.2%)	0.47
	1	13 (41.9%)	16 (24.6%)	
	2	5 (16.1%)	11 (16.9%)	
	3	2 (6.5%)	7 (10.8%)	
	4	0 (0%)	1 (1.5%)	
Physical childhood more than seldom (i.e. occasionally or often)	Yes	0 (0%)	7 (10.8%)	0.09
	No	31 (100%)	58 (89.2%)	
Physical adulthood more than seldom (i.e. occasionally or often)	Yes	0 (0%)	7 (10.8%)	0.09
	No	31 (100%)	58 (89.2%)	
Any penetrative sexual abuse	Yes	1 (3.2%)	6 (9.2%)	0.42
	No	30 (96.8%)	59 (90.8%)	
Penetrative sexual abuse childhood	Yes	0 (0%)	1 (1.5%)	1.0
	No	31 (100%)	64 (98.5%)	
Penetrative sexual abuse adulthood	Yes	1 (3.2%)	6 (9.2%)	0.42
	No	30 (96.8%)	59 (90.8%)	

In a meta-analysis on the prevalence of childhood sexual abuse in 22 countries based on non-clinical cohorts, the mean prevalence of childhood sexual abuse in women were 20% and 8% in men [216]. A review on the prevalence of sexual abuse in Nordic countries showed a prevalence of childhood sexual abuse (broadly defined) between 3-23% for boys and 11-36% for girls [217]. Thus, our findings of childhood sexual abuse in 16% and 15% in healthy controls and IBS, respectively, are within the expected range based on international and Nordic population based studies.

A large national Swedish survey were conducted in 2012, examining the experience of violence/abuse and its associations with health [218]. The prevalence of adult sexual abuse was 28% in women, and 6% in men [218]. Our results with 26% and 29% having experienced sexual abuse in adulthood in healthy controls and IBS respectively, seems to be consistent with these national findings.

The data we have for the individual categories of physical abuse also seems to be coherent with the literature [218], even though the overall experience of abuse might be slightly higher than expected.

4.9.1 ABUSE AND SYMPTOMATOLOGY

The large Swedish survey showed that severe abuse was associated with a 2- to 5-fold increase in posttraumatic stress disorder (PTSD) symptomatology, depression, and somatic symptoms measured with PHQ-15, compared to those who had not experienced severe abuse [218].

In an American specialized GI clinic, the prevalence of abuse (sexual or physical) was approximately 50%, regardless of functional or organic diagnosis, but the functional group had experienced more severe abuse [219]. Those with abuse history had poorer health status and were more likely to report other symptoms [219]. In a more recent study [220], IBS patients with experience of abuse had, compared to non-abused IBS patients, more severe abdominal pain and illness-related disability, an effect partially mediated by comorbid anxiety and depression (and to a lesser extent multiple somatic symptoms). Further, multiple abuse experiences exerted negative effects on IBS symptoms and health-related quality of life in an additive fashion [220].

Sexual abuse is the most studied form of childhood abuse in relationship to IBS [221]. It has rather consistently showed increased prevalence of experience of sexual childhood abuse in IBS compared to IBD patients and other reference groups [221]. The relationship between physical abuse in childhood and IBS is less clear, as several studies have not shown increased prevalence of childhood physical abuse in adult IBS [221].

It seems important to note that other kinds of abuse can also have severe impact on physical and mental health. An association between psychological abuse and several health-related issues was documented in the large Swedish survey [218]. In a study on abuse, parental styles and symptomatology in IBS patients by Lackner et al [222], both maternal and paternal rejection was associated with abuse. However, current abdominal pain level was not associated with either abuse history or parental style. Only paternal (not maternal) hostility and

rejection was correlated with somatization, whereas abuse did not show an association with somatization [222].

In one study, no effect of sexual abuse on pain thresholds during rectal barostat testing were found, in IBS nor in healthy controls [223]. When comparing IBS patients with and without experience of severe physical and/or sexual abuse, the abused patients even had higher thresholds in another study [224]. These studies differed from our paper II, in their definition of the pain threshold, abuse definition, and the abuse was not specified as childhood or adult abuse, which in our study showed different associations with rectal pain threshold. A third study using clustering analysis in severe IBS found two clusters with low rectal discomfort threshold; one with higher prevalence of childhood sexual abuse, psychiatric comorbidity and high rates of doctors' consultation, and one with lower prevalence of childhood sexual abuse, moderate psychiatric comorbidity and low rates of doctors' consultation [225]. This indicates that there might be an interaction effect between childhood abuse and psychiatric comorbidity in the relationship with rectal sensitivity in IBS.

To conclude this paragraph, sexual and physical abuse are associated with reduced health overall, but not equivocally associated specifically with IBS. Other kinds of maltreatment, not studied herein, might be important for the development of IBS symptoms. The association between abuse and visceral sensitivity seems to depend on the type of abuse, when it occurred, and potentially also on the severity of abuse, as well as on interactions with other factors such as psychiatric comorbidity. The results so far have been inconclusive, possibly due to different methods of determining sensory thresholds as well as the classification of abuse.

4.10 HIPPOCAMPUS, AMYGDALA AND CINGULATE CORTEX = MEMORIES, FEAR AND STRESS IN IBS HIGH SOMATIZATION?

In manuscript IV, the discussion focused on the prefrontal cortex, insula and cerebellum, since these were the most robust findings. There were, however, other interesting findings at the uncorrected significance level $p<0.005$, and in the modular structure.

In healthy controls and IBS low somatization, but not in IBS high somatization, hippocampus and amygdala clustered, as would be expected due to their

connected role in fear, memory and learning [99]. In IBS high somatization, amygdala clustered with anterior cingulate cortex instead, which was not seen in the other groups. The anterior cingulate cortex-amygdala complex reacts to stress and has two important descending projections: the activation of locus coeruleus that causes a hypervigilant state, and increased activation in the sympathetic nervous system through the hypothalamus-pituatary-adrenal axis [95]. This fits well with the clustering of regional gray matter of anterior cingulate cortex and amygdala seen in our IBS high somatization group, which may be involved in the hypervigilant state and/or disturbed hypothalamus-pituatary-adrenal axis function observed in IBS and thought to be associated with increased somatic symptom generation [15].

IBS patients with high somatization levels showed less local efficiency of amygdala and hippocampus compared to healthy controls, indicating the networks' vulnerability to changes in these brain regions. The hippocampus is a key structure for spatial and declarative memory formation, and important for plasticity and adaptive brain functions [97]. The amygdala-hippocampal interconnections are important for memory and learning, and critical for fear conditioning and extinction [99]. It is possible that already learnt schemes of the reality can shape the hippocampal representation of new events [98]. For example, if we have vast experience of pain, and this pain triggers a certain maladaptive reaction, it is more likely that any new pain will make our brain react in the same way, compared to someone who has not had these painful experiences. We may speculate that an imbalance between amygdala and hippocampal function, and their integration within a larger network, is involved in the generation of multiple somatic symptoms in high somatization IBS, possibly by reducing extinction abilities after fear conditioning to interceptive threats.

We also found that right anterior middle cingulate cortex had a more central role in IBS high somatization compared to IBS low somatization. The aMCC is involved in fear, and generates avoidance responses to fear, by generating implicit premotor signals rather than conscious emotional feelings [92]. The anterior middle cingulate cortex is also frequently activated in human pain studies, and atrophy in the vaMCC has been correlated with catastrophizing in migraine headache patients [92]. In a fMRI study using rectal distension at the individual discomfort level in IBS patients, anxiety scores correlated significantly with pain-induced activation of the right anterior middle cingulate cortex [226]. This might be interpreted as the aMCC mediating increased attention to visceral stimuli and pain amplification by emotions of fear and anxiety in IBS [226]. The increased centrality of aMCC in IBS high somatization could thus reflect a stronger role of fear and avoidance responses

in this group of high somatization IBS patients, distinguishing them from IBS patients with low somatization levels.

In addition to the differences described in manuscript IV, the above-mentioned altered clustering and network properties in IBS high somatization, implicates that regions known for their importance in memory, fear and stress seem to be important in IBS high somatization.

4.11 INCIDENTAL FINDINGS AND NEUROETHICS

For any investigation there is the possibility of incidental findings, and this is especially true for imaging modalities with detailed anatomical representation. The study participants had verbal and written informed consent before taking part in the study, in accordance with established research praxis. The autonomy ethical principal should thereby be addressed. Many subjects expressed thoughts or comments about the possibility of findings on the MRI as: “If there is something wrong in my brain I would sure like to know about it”. Nevertheless, how well do the study participants understand the meaning of an anomaly or possible pathology on a brain MRI scan? In the possible case of an asymptomatic malignant tumor, it would of course be of great value for the person to know, and possible increase the chances of survival. However, the majority of incidental findings are without clinical consequences [227]. How will an accidental finding with limited clinical relevance affect a person? The IBS cohort overall has increased levels of anxiety, catastrophizing etc., so it is reasonable to think that these participants might suffer more than other study subjects from accidental findings with uncertain clinical relevance.

In our studies, all brain MRI scans were evaluated by an experienced neuroradiologist. If needed, we discussed or referred to a neurologist, with vast experience both from clinical work and research, for clinical evaluation and follow-up.

In this study with a total of 113 brain scans, there were five with findings that could clearly be considered as normal variance. Five additional anomalies were discussed with the neurologist, and four of these subjects were referred for clinical neurological evaluation. Only one had a finding of possible clinical relevance, an infratentorial meningioma. This person is now being followed with regular MRI scans, but has so far not been subject for surgical treatment. One study participant had an increased intensity signal on T2-weighted images near the insula of initially unknown clinical relevance. After thorough

neurological exam and follow-up MRI scan, it was discarded as clinically irrelevant. However, the study participant got highly distressed by the finding. In combination with other stressors, this MRI finding culminated in sick leave, despite repeated reassurance of the benign nature of the lesion.

Routine inspection of brain MRI scans acquired for research purposes have been advocated [228, 229]. Even the presence of such a debate indicates that it is not self-evident in all research communities. Another consideration is if all participants should get a full MRI review report, or only in the cases where the findings are thought to have clinical relevance. According to a study on subject that had previously taken part in a study including a brain MRI scan (n=196), almost 80% of the study participants wanted to receive information about the MRI result regardless of what it showed [228]. Having a brain MRI scan in a study serves as a good reference if the subjects later on needs a brain MRI scan based on a clinical question [228]. Retrospectively reported anxiety created by the MRI report were in this study low, but significantly correlated with overall health related anxiety [228]. This study also had focus groups interviews, disclosing a few themes such as difficulty understanding the radiology report because it was written in medical terminology [230]. They also showed that 10% of the investigated participants sought follow up care without this being recommended, and 67% did not seek medical care when recommended to do so [230].

Another setting where incidental findings are a potential issue is for example research using whole body MRI scans. The psychosocial consequences of receiving information of incidental findings in a general population study using whole body MRI were studied retrospectively [231]. This study showed strong distress while awaiting a potential notification of incidental findings in 10%, and 29% reported moderate to severe psychological distress after getting this notification. The distress increased with the participant's subjective evaluation of the severity of the findings, which differed substantially from that of the radiologists. Despite this, 96% of the participants were content with their participation in the whole body MRI study, and 36.5% stated they would have refused taken part if findings of relevance had potentially not been disclosed to them [231].

With this brief discussion, I wish to illustrate that in research we may think of a MRI scan as a non-invasive examination, harmless for the participant. It is however important to reflect on the possible unintended psychosocial effects our investigations might have for the study participant.

4.12 CLINCAL RELEVANCE

Based on our studies, we know that roughly half of the IBS patients have visceral hypersensitivity, and rectal pain perception is enhanced by somatization, psychological distress and experience of sexual abuse in adulthood. Further, we showed that somatization, measured as multiple somatic symptoms, can be visualized with structural brain imaging analyzed with graph theory. In this paragraph, I highlight some of the potential clinical relevance of our findings.

First, multiple somatic symptoms are an indication of central sensitization, and the more symptoms from different bodily regions a patient has, the more likely that the patient has a central sensitization syndrome or functional somatic syndrome. In a meta-analysis of longitudinal studies investigating how many of the patients initially diagnosed with a functional somatic symptom that turned out to have an underlying somatic disease, the prevalence of missed somatic diagnosis was 0.5% (95% CI 0.01-1.5) [232]. After an initial, proper, diagnostic evaluation in accordance with the recommendations from the Rome foundation [21] resulting in an IBS diagnosis, the more widespread the symptoms, the more confident you can be of the diagnosis, and the less need for additional advanced medical investigations. This patient group has increased levels of psychological distress, and increased stress might deteriorate IBS symptoms. As a clinician, you must contain your distress about possibly missing an underlying somatic diagnosis, and be careful not to transfer it to the patient.

Second, so called functional or psychosomatic symptoms is not ‘all in their minds’, but it might be ‘partially in their brains’, seen as altered structural or functional circuits. The symptoms patient experience are real, and quite often devastating, but the cause or reason for these symptoms might not always be what the patient believe. As medical professionals, we need to educate our patients about how complex the sensation of pain is, and help them find tools to cope with their situation. Pain can be learnt to be autonomous by repeated experience under unfortunate conditions, but in many cases, it can also be unlearnt if given the right knowledge and support. The brain plasticity goes both ways.

Third, there might come a day, hopefully not too far away, when a medication targeting visceral hypersensitivity becomes available. It is probable that this drug will have a peripheral target. In that case, it is important to recognize the possible synergistic effect of that drug and centrally acting treatments, targeting for instance anxiety (such as SSRI and CBT [233]). Investigating the

individual patient pathophysiological mechanisms in research settings will help to develop more effective treatment options, and in clinical settings, it will help tailoring the treatment options to the individual patient.

5 FUTURE PERSPECTIVES

In addition to the joy of better understanding the pathophysiology of IBS, a main goal of my research is to contribute to improved care and therapeutic options for suffering people. There are many ways forward in improving our knowledge on brain-gut interactions in IBS in order to meet this goal.

Brain imaging studies have several problems. These include lack of replicability, source of variance based on neuroimaging techniques, different results based on different software used for analyzing neuroimaging data, generally low statistical power, publication bias etc. [234]. To combat this, larger studies would be helpful, as well as pure replications of existing studies, and increased appreciation of studies with sound methodological approaches that 'fails' to detect differences between groups. To increase the robustness of the results, the studies should have standardized protocols and pre-inclusion power calculations for the selected regions of interest [162]. As discussed in manuscript III, sex, psychological factors and other co-morbidities need to be taken into account when performing gray matter morphometric studies. Since there are so many possible research questions, and analytical considerations, the sprawling results of brain imaging in IBS can be expected for some additional time to come.

Gray matter changes are commonly seen in cross-sectional studies. Longitudinal studies of gray matter morphometry is greatly needed, to further evaluate the relevance of findings from these cross-sectional studies. So far, I have not came across any longitudinal gray matter morphometry study in IBS. Further, the cellular substrate for the gray matter changes in disease and health is another question that would make these results more understandable, and potentially more clinically relevant.

Below are a few suggestions and hypotheses, developed during the work of this thesis.

Hypothesis 1:

At present it is common to divide IBS patients into hypersensitive and normosensitive based on pain thresholds. An alternative grouping of IBS patients could be based on the slope or shape of their sensory ratings during rectal distension, as an attempt to better reflect the putative physiologic mechanisms. By adding a measurement of central sensitization, additional subgrouping will be possible, and lead to added information about the possible underlying pathophysiological mechanisms.

The central sensitization inventory (CSI) was developed to assess key somatic and emotional complaints often associated with Central Sensitivity Syndromes [235]. The term Central Sensitivity Syndrome was proposed by Yunus for nonorganic disorders that have evidence to share central sensitization as etiology [236]. The CSI part A consists of 25 questions measured on a 5-point temporal Likert scale from never to always, yielding a score ranging from 0 to 100 [235]. The measurement properties of the CSI have been evaluated in a recent review, and it has been shown to be reliable, consistent, and valid [237]. Further, it has been shown to correlate with serum levels of brain-derived neurotrophic factor (BDNF), and those subjects who respond with inhibition to a conditioned pain modulation (normal reaction) had lower scores on CSI than those who showed deficiency in the descending modulatory systems [238]. It thus seems as if the CSI is a good option for evaluating possible central sensitization in this postulated study.

a) Low VAS pain ratings and low-medium unpleasantness ratings up to near pain threshold implies 'normal' sensitivity. These patients are expected to have low levels of central sensitization, and their symptom profile might be dominated by primarily disturbed bowel habits.
b) High VAS pain ratings and high unpleasantness ratings only at higher distension levels might imply up-regulated high threshold/ nociceptive pathways.
    a. In combination with low level of central sensitization, these patients can be expected to have primarily peripheral sensitization with the possible effect of treatments affecting the local gut environment.
    b. In combination with high level of central sensitization, these patients should have predominantly painful symptoms and gain from treatments targeting pain caused by central sensitization.
c) High VAS pain ratings and high unpleasantness ratings, through the range of distensions, might imply up-regulated WDR afferent pathways. These patients might have symptoms like urgency, feeling of incomplete bowel movement, bloating etc.
    a. In combination with low levels of central sensitization, they would be expected to have symptoms restricted to the GI tract, and psychological distress mainly focusing on, or secondarily to GI symptoms.
    b. In combination with high level of central sensitization, they would be expected to have a wide range of symptoms from different bodily organs. They might be having both painful and non-painful sensitization symptoms (such as tinnitus,

chemical hypersensitivity etc.), and probably have high levels of psychological distress.

Hypothesis 2:

I paper III, we found an indication of psychological distress causing disease related alterations in women but not in men. This possible disease-by-psychological distress-by-sex interaction warrants further investigation in a replication study. Among the regions within the sensorimotor network, posterior insula and supplementary motor cortex seems to be the most relevant to IBS.

Specific hypothesis: There are IBS status-by-psychological distress-by sex interactions effects, with psychological distress causing the disease related alterations in women, but not in men, with reduced posterior insula and supplementary motor cortex gray matter in disease.

Basically what I suggest is a replication study of one of the most interesting results in manuscript III. By a priory focusing on the differences seen in our study, the likelihood increases for the results remaining statistically significant after correction for multiple comparisons.

Hypothesis 3:

Learning is associated with an initial increase in gray matter, followed by selection and normalization of gray matter [81]. Chronic pain has by definition been present for a long time, and the initial increase has probably passed by the time of investigation with MRI brain scan. Hence, an overall decrease of gray matter is seen in chronic pain states.

Hypothetically, the brain might try to decrease the impact of chronic pain by diminishing the brain regions where the pain is processed and perceived. Speculatively, this decrease of gray matter in pain processing regions, which also has cognitive functions, may as a side effect reduce the cognitive flexibility by reducing the possible initial increase in gray matter associated with new learning.

One way to test this could be to follow patients with chronic pain, such as IBS patients with central sensitization, longitudinally, with multiple brain scans and at the same time assess painful symptoms and symptom severity as well as cognitive functions and/or cognitive flexibility. The symptom severity should, if the hypothesis above is correct, correlate with the changes of gray matter

volume/cortical thickness over time, and changes in cognitive functions would be associated with opposite changes with delay in gray matter changes associated with pain. Given the importance of sex and the possible disease-by-sex interaction effect displayed in manuscript III, I would suggest to study only females initially, as they outnumber males in many chronic pain conditions and central sensitivity syndromes.

6 CONCLUSION

Anxiety, depression and somatization exert different influences on intensity ratings with increasing rectal distension levels for painful and non-painful sensations (unpleasantness). All three factors increased the pain intensity ratings in hypersensitive IBS patients for the full range of distension levels. Non-painful intensity ratings were influenced only by anxiety and did not show an interaction-effect with sensitivity status. Upregulation of wide dynamic range afferent pathways, either solely or in combination with nociceptive pathways, seems to be relevant to hypersensitivity in IBS patients.

Somatization, measured as multiple somatic symptoms, is associated with several measurements of rectal pain sensitivity, and mediates the effects of depression and GI-specific anxiety on rectal pain perception. Sex and age also influenced rectal pain sensitivity. Sexual abuse in adulthood was the only abuse domain investigated associated with increased rectal pain sensitivity.

Group differences in gray matter morphometry of the sensorimotor network between IBS and healthy controls, as well as correlations with brain and rectal distension evoked pain and reported symptoms were only indicated, not confirmed, since the results were not significant after correction for multiple comparisons. The results implied that gray matter morphometry differences between IBS and healthy controls might depend on a sex-by-psychological distress-interaction effect. Further, pain evoked by rectal distension indicated positive association with the size of primary sensorimotor regions, whereas symptoms of abdominal pain indicated negative association with thalamus and secondary somatosensory cortex. A possible interpretation is that these clinical measures from a neurobiological perspective reflects different aspects of visceral pain sensory mechanisms.

The connectivity between brain regions, measured as correlations between regional gray matter volumes corrected for total gray matter volume, differs between IBS with high and low levels of somatization and between both IBS groups and healthy controls. The most robust findings showed increased importance of prefrontal regions in a graduated fashion: IBS high > IBS low > healthy controls, and insula showed the opposite pattern of importance in healthy controls> IBS low> IBS high. Further, cerebellum was specifically central in IBS with low levels of somatization. On a more exploratory significance level, anterior middle cingulate cortex, hippocampus and amygdala showed altered connectivity in IBS high somatization. Altogether, this study indicated that for the neurobiological process associated with

multiple somatic symptoms, cognitive factors are more important than primary visceral perception in IBS, and depends on the level of multiple somatic symptoms. Further, regions known for their involvement in stress, memory and fear are implicated in IBS high somatization.

This thesis argues for multiple somatic symptoms and visceral hypersensitivity both being indicators of central sensitization. Central sensitization seems to be an important pathophysiological mechanism in a subgroup of IBS patients, possibly the subgroup with the worst health status. The findings in this thesis also support that central sensitization is an important component of somatization.

The overall conclusion of this thesis in one sentence is; somatization, measured as multiple somatic symptoms, are important for visceral (hyper-) sensitivity in IBS, and can be seen as altered structural connectivity within the brain, mainly in regions important for cognition and visceral sensory processing.

In order to help a patient with IBS as a medical professional, one cannot only focus on the GI symptoms. One have to look at the entire person, including, but not limited to, information about other symptoms, general background, social situation, fears, cognitive functions and coping strategies. Not until both the medical professional and the patient has acknowledged the importance of all of these factors and their interactions, one can expect to *really* improve the health for a person living with IBS. Remember that painful experiences and maladaptive functioning can alter the brain structure, and so can behavior! So, "g*o out and shape the brain you want to have!"*

ACKNOWLEDGEMENT

There are so many people of importance for the work of this thesis; it would be too many to thank everyone individually. A collective 'thank you' to all of you! Some, I would like to thank in particular:

Magnus Simrén –My supervisor. Thank you for showing me the world of research and excellent guidance through it. Thank you for your knowledge, support, enthusiasm and the trust given in me to do this work on your behalf. You have been a true mentor.

Lukas Van Oudenhove –My co-supervisor. Thank you for making statistics interesting and accessible. Thank you for always quick replies with impressive skills in identifying the problem and reasoning on approaches to deal with anything that comes up.

Mikael Elam –My former co-supervisor. Thank you for interesting discussions and helping me develop critical thinking in the world of brain imaging.

The staff at Mag- och tarmlab. Thank you for the hard work you do every day making it possible for many people to do high-end research. Thank you for the warmth, nice company and fun times. But most of all, thank you for giving me a 'home' to come back to, again and again and again...

Hans Törnblom –The gastroenterologist who evaluated many of the included participants, and a co-author. Thank you for your hard work, and sharing you vast clinical knowledge.

The co-authors and co-workers at UCLA: Emeran Mayer, Jennifer Labus, Hyo Ryu, Arpana Gupta, Kirsten Tillisch, Cody Ashe-McNalley (previously) and Florian Kurth (previously). Thank you for sharing your knowledge and all the time spent on our projects. Thank you for being excellent hosts during my stays in Los Angeles.

The co-authors and co-workers at KU Leuven: Lukas, Jan Tack, Patrick Dupont and Egbert Clevers. It has been an efficient pleasure working with you! Jan, thank you for sharing your vast knowledge. Patrick, in a short time you have taught me so much about science and advanced analytical techniques, in such a humble and collegial way. Egbert, thank you for being a friendly and helpful mastermind –we could to do great things together ☺.

Fellow PhD-students and other researchers –Thank you for rewarding discussions, nice company and fun times.

The staff at the MRI unit. Thank you for making the brain imaging studies possible and for all the nice evenings spent together during scanning.

Maria Ljungberg –The medical physicist who implemented the MRI protocol. Thank you for the meticulousness of your work.

All the participants in the studies –Thank you! Without you, there would be no thesis, no research, and no progress.

My colleagues at the Queen Silvia Children´s Hospital –Thank you for all support. Both clinically, but also the support I have received in the sometimes difficult task of combining research and clinical work.

Katarina Wilpart –My friend and colleague. Thank you for your invaluable support and friendship. Thank you for reading my work to stop me from writing stupid things like 'breast test' instead of 'breath test'. But most of all, thank you for all the fun times you have made me forget about work.

Marit Stockfelt –My friend and colleague. Thank you for all interesting discussions in science, life, family and everything else in between. In addition, thank you for reviewing my work, making it a bit more pleasant to read.

Gunvor Linde Grinsvall –My mom. Thank you for making me believe I can do anything, and for helping with the kids when I've needed to work. Thank you for all the love you given me over the years.

Bo Grinsvall –My dad. Thank you for giving me my work ethics and for showing me what you can achieve with hard work.

Inglis and Jan Karlsson –My parents-in-law. Thank you for being here for us during the intense phase of writing the thesis, taking care of the children. And thank you for being just awesome in general.

Linus Grinsvall –My husband. Thank you for always being there for me, for being my foundation in life. Thank you for helping me with everything from making decisions, excel programing, to taking care of our kids when I work.

Casper and Melvin Grinsvall –My children. You make me want to do my best, all the time. Thanks to you, I will never give up.

REFERENCES

1. Kuner, R. and Flor, H., *Structural plasticity and reorganisation in chronic pain.* Nat Rev Neurosci, **2017**. 18(2): p. 113.
2. Feng, B. and Gebhart, G.F., *Characterization of silent afferents in the pelvic and splanchnic innervations of the mouse colorectum.* Am J Physiol Gastrointest Liver Physiol, **2011**. 300(1): p. G170-180.
3. Camilleri, M., Coulie, B., and Tack, J.F., *Visceral hypersensitivity: facts, speculations, and challenges.* Gut, **2001**. 48(1): p. 125-131.
4. *https://en.wikipedia.org/wiki/Morphometrics*. 2018 [cited 2018 November 13].
5. *The International Association for the Study of Pain*. December 14, 2017.
6. Robinson, D.R. and Gebhart, G.F., *Inside information: the unique features of visceral sensation.* Mol. Interv., **2008**. 8(5): p. 242-253.
7. *https://en.wikipedia.org/wiki/Perception*. 2018 [cited 2018 November 13].
8. Molina, D.K. and DiMaio, V.J., *Normal Organ Weights in Women: Part II-The Brain, Lungs, Liver, Spleen, and Kidneys.* Am J Forensic Med Pathol, **2015**. 36(3): p. 182-187.
9. Molina, D.K. and DiMaio, V.J., *Normal organ weights in men: part II-the brain, lungs, liver, spleen, and kidneys.* Am J Forensic Med Pathol, **2012**. 33(4): p. 368-372.
10. Goldstein, A.M., Hofstra, R.M., and Burns, A.J., *Building a brain in the gut: development of the enteric nervous system.* Clin Genet, **2013**. 83(4): p. 307-316.
11. Mayer, E.A., *Gut feelings: the emerging biology of gut-brain communication.* Nat Rev Neurosci, **2011**. 12(8): p. 453-466.
12. Enck, P., Aziz, Q., Barbara, G., Farmer, A.D., Fukudo, S., Mayer, E.A., . . . Spiller, R.C., *Irritable bowel syndrome.* Nat Rev Dis Primers, **2016**. 2: p. 16014.
13. Drossman, D.A., *Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features and Rome IV.* Gastroenterology, **2016**.
14. Simren, M., Tornblom, H., Palsson, O.S., van Tilburg, M.A.L., Van Oudenhove, L., Tack, J., and Whitehead, W.E., *Visceral hypersensitivity is associated with GI symptom severity in functional GI disorders: consistent findings from five different patient cohorts.* Gut, **2018**. 67(2): p. 255-262.
15. Van Oudenhove, L., Crowell, M.D., Drossman, D.A., Halpert, A.D., Keefer, L., Lackner, J.M., . . . Levy, R.L., *Biopsychosocial Aspects of Functional Gastrointestinal Disorders.* Gastroenterology, **2016**.

16. Lovell, R.M. and Ford, A.C., *Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis.* Clin Gastroenterol Hepatol, **2012**. 10(7): p. 712-721 e714.
17. Whitehead, W.E., Burnett, C.K., Cook, E.W., 3rd, and Taub, E., *Impact of irritable bowel syndrome on quality of life.* Dig Dis Sci, **1996**. 41(11): p. 2248-2253.
18. Drossman, D.A., Morris, C.B., Schneck, S., Hu, Y., Norton, N.J., Norton, W.F., . . . Bangdiwala, S.I., *International Survey of Patients With IBS: Symptom Features and Their Severity, Health Status, Treatments, and Risk Taking to Achieve Clinical Benefit.* Journal of Clinical Gastroenterology, **2009**. 43(6): p. 541-550.
19. Chang, J.Y., Locke, G.R., III, McNally, M.A., Halder, S.L., Schleck, C.D., Zinsmeister, A.R., and Talley, N.J., *Impact of Functional Gastrointestinal Disorders on Survival in the Community.* Am J Gastroenterol, **2010**. 105(4): p. 822-832.
20. Canavan, C., West, J., and Card, T., *Review article: the economic impact of the irritable bowel syndrome.* Aliment Pharmacol Ther, **2014**. 40(9): p. 1023-1034.
21. Lacy, B.E., Mearin, F., Chang, L., Chey, W.D., Lembo, A.J., Simren, M., and Spiller, R., *Bowel Disorders.* Gastroenterology, **2016**. 150(6): p. 1393-1407.e1395.
22. Thompson, W.G., Longstreth, G.F., Drossman, D.A., Heaton, K.W., Irvine, E.J., and Muller-Lissner, S.A., *Functional bowel disorders and functional abdominal pain.* Gut, **1999**. 45 Suppl 2: p. II43-47.
23. Longstreth, G.F., Thompson, W.G., Chey, W.D., Houghton, L.A., Mearin, F., and Spiller, R.C., *Functional bowel disorders.* Gastroenterology, **2006**. 130(5): p. 1480-1491.
24. Wiklund, I.K., Fullerton, S., Hawkey, C.J., Jones, R.H., Longstreth, G.F., Mayer, E.A., . . . Naesdal, J., *An irritable bowel syndrome-specific symptom questionnaire: development and validation.* Scand. J. Gastroenterol., **2003**. 38(9): p. 947-954.
25. Clevers, E., Vaes, B., Henrard, S., Goderis, G., Tack, J., Tornblom, H., . . . Van Oudenhove, L., *Health problems associated with irritable bowel syndrome: analysis of a primary care registry.* Aliment Pharmacol Ther, **2018**. 47(10): p. 1349-1357.
26. Aziz, I., Palsson, O.S., Tornblom, H., Sperber, A.D., Whitehead, W.E., and Simren, M., *The Prevalence and Impact of Overlapping Rome IV-Diagnosed Functional Gastrointestinal Disorders on Somatization, Quality of Life, and Healthcare Utilization: A Cross-Sectional General Population Study in Three Countries.* Am J Gastroenterol, **2017**. 113(1): p. 86-96.
27. Halder, S.L., Locke, G.R., 3rd, Schleck, C.D., Zinsmeister, A.R., Melton, L.J., 3rd, and Talley, N.J., *Natural history of functional gastrointestinal disorders: a 12-year longitudinal population-based study.* Gastroenterology, **2007**. 133(3): p. 799-807.

28. Clevers, E., Tack, J., Tornblom, H., Ringstrom, G., Luyckx, K., Simren, M., and Van Oudenhove, L., *Development of Irritable Bowel Syndrome Features Over a 5-year Period.* Clin Gastroenterol Hepatol, **2018**. 16(8): p. 1244-1251 e1241.
29. Polster, A., Van Oudenhove, L., Jones, M., Ohman, L., Tornblom, H., and Simren, M., *Mixture model analysis identifies irritable bowel syndrome subgroups characterised by specific profiles of gastrointestinal, extraintestinal somatic and psychological symptoms.* Aliment Pharmacol Ther, **2017**. 46(5): p. 529-539.
30. Mulak, A. and Bonaz, B., *Irritable bowel syndrome: a model of the brain-gut interactions.* Med Sci Monit, **2004**. 10(4): p. RA55-62.
31. Aziz, Q. and Thompson, D.G., *Brain-gut axis in health and disease.* Gastroenterology, **1998**. 114(3): p. 559-578.
32. Bouin, M., Plourde, V., Boivin, M., Riberdy, M., Lupien, F., Laganière, M., . . . Poitras, P., *Rectal distention testing in patients with irritable bowel syndrome: Sensitivity, specificity, and predictive values of pain sensory thresholds.* Gastroenterology, **2002**. 122(7): p. 1771-1777.
33. Posserud, I., Syrous, A., Lindström, L., Tack, J., Abrahamsson, H., and Simrén, M., *Altered Rectal Perception in Irritable Bowel Syndrome Is Associated With Symptom Severity.* Gastroenterology, **2007**. 133(4): p. 1113-1123.
34. Naliboff, B., Derbyshire, S.W., Munakata, J., Berman, S., Mandelkern, M., Chang, L., and Mayer, E.A., *Cerebral Activation in Patients With Irritable Bowel Syndrome and Control Subjects During Rectosigmoid Stimulation.* Psychosomatic Medicine May/June, **2001**. 63(3): p. 365-375.
35. Yuan YZ, T.R., Xu B, Sun J, Chen KM, Miao F, Zhang ZW, Xu JY, *Functional brain imaging in irritable bowel syndrome with rectal balloon-distention by using fMRI.* World Journal of Gastroenterology, **2003**. 9(6): p. 1356-1360.
36. Tillisch, K., Mayer, E.A., and Labus, J.S., *Quantitative Meta-analysis Identifies Brain Regions Activated During Rectal Distension in Irritable Bowel Syndrome.* Gastroenterology, **2011**. 140(1): p. 91-100.
37. Mayer, E.A., Aziz, Q., Coen, S., Kern, M., Labus, J.S., Lane, R., . . . Tracey, I., *Brain imaging approaches to the study of functional GI disorders: a Rome working team report.* Neurogastroenterol Motil, **2009**. 21(6): p. 579-596.
38. Wilder-Smith, C.H., Schindler, D., Lovblad, K., Redmond, S.M., and Nirkko, A., *Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls.* Gut, **2004**. 53(11): p. 1595-1601.
39. Wilder-Smith CH, R.-Y.J., *Abnormal endogenous pain modulation and somatic and visceral hypersensitivity in female patients with*

irritable bowel syndrome. World Journal of Gastroenterology, **2007**. 13(27): p. 3699-3704.
40. Albusoda, A., Ruffle, J.K., Friis, K.A., Gysan, M.R., Drewes, A.M., Aziz, Q., and Farmer, A.D., *Systematic review with meta-analysis: conditioned pain modulation in patients with the irritable bowel syndrome.* Aliment Pharmacol Ther, **2018**. 48(8): p. 797-806.
41. Knowles, C.H. and Aziz, Q., *Basic and clinical aspects of gastrointestinal pain.* Pain, **2009**. 141(3): p. 191-209.
42. Keszthelyi, D., Troost, F.J., and Masclee, A.A., *Irritable bowel syndrome: methods, mechanisms, and pathophysiology. Methods to assess visceral hypersensitivity in irritable bowel syndrome.* Am. J. Physiol.-Gastroint. Liver Physiol., **2012**. 303(2): p. G141-154.
43. Keszthelyi, D., Troost, F.J., Simrén, M., Ludidi, S., Kruimel, J.W., Conchillo, J.M., and Masclee, A.A., *Revisiting concepts of visceral nociception in irritable bowel syndrome.* European Journal of Pain, **2012**. 16(10): p. 1444-1454.
44. Anand, P., Aziz, Q., Willert, R., and van Oudenhove, L., *Peripheral and central mechanisms of visceral sensitization in man.* Neurogastroenterol. Motil., **2007**. 19(1 Suppl): p. 29-46.
45. Elsenbruch, S., *Abdominal pain in Irritable Bowel Syndrome: a review of putative psychological, neural and neuro-immune mechanisms.* Brain Behav. Immun., **2011**. 25(3): p. 386-394.
46. De Winter, B.Y., Deiteren, A., and De Man, J.G., *Novel nervous system mechanisms in visceral pain.* Neurogastroenterol Motil, **2016**. 28(3): p. 309-315.
47. Wu, J.C., *Psychological Co-morbidity in Functional Gastrointestinal Disorders: Epidemiology, Mechanisms and Management.* J Neurogastroenterol Motil, **2012**. 18(1): p. 13-18.
48. Pae, C.U., Masand, P.S., Ajwani, N., Lee, C., and Patkar, A.A., *Irritable bowel syndrome in psychiatric perspectives: a comprehensive review.* Int. J. Clin. Pract., **2007**. 61(10): p. 1708-1718.
49. Mykletun A, J.F., Williams L, Pasco J, Henry M, Nicholson GC, Kotowicz MA, Berk M, *Prevalence of mood and anxiety disorder in self reported irritable bowel syndrome (IBS). An epidemiological population based study of women.* BMC Gastroenterol., **2010**. 5(10): p. 88.
50. Vandvik, P.O., Lydersen, S., and Farup, P.G., *Prevalence, comorbidity and impact of irritable bowel syndrome in Norway.* Scand. J. Gastroenterol., **2006**. 41(6): p. 650-656.
51. Hillilä, M.T., Siivola, M.T., and Färkkilä, M.A., *Comorbidity and use of health-care services among irritable bowel syndrome sufferers.* Scand. J. Gastroenterol., **2007**. 42(7): p. 799-806.
52. Surdea-Blaga, T., Baban, A., and Dumitrascu, D.L., *Psychosocial determinants of irritable bowel syndrome.* World J. Gastroenterol., **2012**. 18(7): p. 616-626.

53. Lee, C., Doo, E., Choi, J.M., Jang, S.H., Ryu, H.S., Lee, J.Y., . . . Motility, *The Increased Level of Depression and Anxiety in Irritable Bowel Syndrome Patients Compared with Healthy Controls: Systematic Review and Meta-analysis.* J Neurogastroenterol Motil, **2017**. 23(3): p. 349-362.
54. Geng, Q., Zhang, Q.E., Wang, F., Zheng, W., Ng, C.H., Ungvari, G.S., . . . Xiang, Y.T., *Comparison of comorbid depression between irritable bowel syndrome and inflammatory bowel disease: A meta-analysis of comparative studies.* J Affect Disord, **2018**. 237: p. 37-46.
55. Wouters, M.M. and Boeckxstaens, G.E., *Is there a causal link between psychological disorders and functional gastrointestinal disorders?* Expert Review of Gastroenterology & Hepatology, **2016**. 10(1): p. 5-8.
56. Bridges, K., Goldberg, D., Evans, B., and Sharpe, T., *Determinants of somatization in primary care.* Psychol Med, **1991**. 21(2): p. 473-483.
57. De Gucht, V. and Fischler, B., *Somatization: a critical review of conceptual and methodological issues.* Psychosomatics, **2002**. 43(1): p. 1-9.
58. Wessely, S., Nimnuan, C., and Sharpe, M., *Functional somatic syndromes: one or many?* Lancet, **1999**. 354(9182): p. 936-939.
59. Boeckxstaens, G.E., Drug, V., Dumitrascu, D., Farmer, A.D., Hammer, J., Hausken, T., . . . members, C.A.B.G., *Phenotyping of subjects for large scale studies on patients with IBS.* Neurogastroenterol Motil, **2016**. 28(8): p. 1134-1147.
60. Levy, R.L., Von Korff, M., Whitehead, W.E., Stang, P., Saunders, K., Jhingran, P., . . . Feld, A.D., *Costs of care for irritable bowel syndrome patients in a health maintenance organization.* Am J Gastroenterol, **2001**. 96(11): p. 3122-3129.
61. Yunus, M.B., *Editorial review: an update on central sensitivity syndromes and the issues of nosology and psychobiology.* Curr Rheumatol Rev, **2015**. 11(2): p. 70-85.
62. Crombez, G., Beirens, K., Van Damme, S., Eccleston, C., and Fontaine, J., *The unbearable lightness of somatisation: a systematic review of the concept of somatisation in empirical studies of pain.* Pain, **2009**. 145(1-2): p. 31-35.
63. Boeckxstaens, G., Camilleri, M., Sifrim, D., Houghton, L.A., Elsenbruch, S., Lindberg, G., . . . Parkman, H.P., *Fundamentals of Neurogastroenterology: Physiology/Motility - Sensation.* Gastroenterology, **2016**.
64. Khlevner, J., Park, Y., and Margolis, K.G., *Brain-Gut Axis: Clinical Implications.* Gastroenterol Clin North Am, **2018**. 47(4): p. 727-739.
65. Vermeulen, W., De Man, J.G., Pelckmans, P.A., and De Winter, B.Y., *Neuroanatomy of lower gastrointestinal pain disorders.* World J Gastroenterol, **2014**. 20(4): p. 1005-1020.

66. Weltens, N., Iven, J., Van Oudenhove, L., and Kano, M., *The gut-brain axis in health neuroscience: implications for functional gastrointestinal disorders and appetite regulation.* Ann N Y Acad Sci, **2018**. 1428(1): p. 129-150.
67. Brookes, S.J., Spencer, N.J., Costa, M., and Zagorodnyuk, V.P., *Extrinsic primary afferent signalling in the gut.* Nat Rev Gastroenterol Hepatol, **2013**. 10(5): p. 286-296.
68. Whitehead, W.E. and Delvaux, M., *Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. The Working Team of Glaxo-Wellcome Research, UK.* Dig Dis Sci, **1997**. 42(2): p. 223-241.
69. Ludidi, S., Conchillo, J.M., Keszthelyi, D., Van Avesaat, M., Kruimel, J.W., Jonkers, D.M., and Masclee, A.A., *Rectal hypersensitivity as hallmark for irritable bowel syndrome: defining the optimal cutoff.* Neurogastroenterol Motil, **2012**. 24(8): p. 729-733, e345-726.
70. Fu, M. and Zuo, Y., *Experience-dependent structural plasticity in the cortex.* Trends Neurosci, **2011**. 34(4): p. 177-187.
71. May, A., *Experience-dependent structural plasticity in the adult human brain.* Trends Cogn Sci, **2011**. 15(10): p. 475-482.
72. Valkanova, V., Eguia Rodriguez, R., and Ebmeier, K.P., *Mind over matter--what do we know about neuroplasticity in adults?* Int Psychogeriatr, **2014**. 26(6): p. 891-909.
73. Smallwood, R.F., Laird, A.R., Ramage, A.E., Parkinson, A.L., Lewis, J., Clauw, D.J., . . . Robin, D.A., *Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume.* J Pain, **2013**. 14(7): p. 663-675.
74. Rodriguez-Raecke, R., Niemeier, A., Ihle, K., Ruether, W., and May, A., *Brain gray matter decrease in chronic pain is the consequence and not the cause of pain.* J Neurosci, **2009**. 29(44): p. 13746-13750.
75. Teutsch, S., Herken, W., Bingel, U., Schoell, E., and May, A., *Changes in brain gray matter due to repetitive painful stimulation.* NeuroImage, **2008**. 42(2): p. 845-849.
76. Gwilym, S.E., Filippini, N., Douaud, G., Carr, A.J., and Tracey, I., *Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study.* Arthritis Rheum, **2010**. 62(10): p. 2930-2940.
77. Obermann, M., Nebel, K., Schumann, C., Holle, D., Gizewski, E.R., Maschke, M., . . . Katsarava, Z., *Gray matter changes related to chronic posttraumatic headache.* Neurology, **2009**. 73(12): p. 978-983.
78. Seminowicz, D.A., Wideman, T.H., Naso, L., Hatami-Khoroushahi, Z., Fallatah, S., Ware, M.A., . . . Stone, L.S., *Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function.* J Neurosci, **2011**. 31(20): p. 7540-7550.

79. Chen, B., He, Y., Xia, L., Guo, L.L., and Zheng, J.L., *Cortical plasticity between the pain and pain-free phases in patients with episodic tension-type headache.* J Headache Pain, **2016**. 17(1): p. 105.
80. Stankewitz, A., Valet, M., Schulz, E., Woller, A., Sprenger, T., Vogel, D., . . . Tolle, T.R., *Pain sensitisers exhibit grey matter changes after repetitive pain exposure: a longitudinal voxel-based morphometry study.* Pain, **2013**. 154(9): p. 1732-1737.
81. Wenger, E., Brozzoli, C., Lindenberger, U., and Lovden, M., *Expansion and Renormalization of Human Brain Structure During Skill Acquisition.* Trends Cogn Sci, **2017**. 21(12): p. 930-939.
82. Tardif, C.L., Gauthier, C.J., Steele, C.J., Bazin, P.L., Schafer, A., Schaefer, A., . . . Villringer, A., *Advanced MRI techniques to improve our understanding of experience-induced neuroplasticity.* Neuroimage, **2016**. 131: p. 55-72.
83. Kanai, R. and Rees, G., *The structural basis of inter-individual differences in human behaviour and cognition.* Nat Rev Neurosci, **2011**. 12(4): p. 231-242.
84. Janak, P.H. and Tye, K.M., *From circuits to behaviour in the amygdala.* Nature, **2015**. 517(7534): p. 284.
85. Sweatt, J.D., *Neural plasticity and behavior - sixty years of conceptual advances.* J Neurochem, **2016**. 139 Suppl 2: p. 179-199.
86. Herrero, M.T., Barcia, C., and Navarro, J.M., *Functional anatomy of thalamus and basal ganglia.* Childs Nerv Syst, **2002**. 18(8): p. 386-404.
87. Haber, S.N., *Corticostriatal circuitry.* Dialogues Clin Neurosci, **2016**. 18(1): p. 7-21.
88. Schwabe, L., *Memory under stress: from single systems to network changes.* Eur J Neurosci, **2016**. 45(4): p. 478-489.
89. Roostaei, T., Nazeri, A., Sahraian, M.A., and Minagar, A., *The human cerebellum: a review of physiologic neuroanatomy.* Neurol Clin, **2014**. 32(4): p. 859-869.
90. Koziol, L.F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., . . . Yamazaki, T., *Consensus paper: the cerebellum's role in movement and cognition.* Cerebellum, **2014**. 13(1): p. 151-177.
91. Adamaszek, M., D'Agata, F., Ferrucci, R., Habas, C., Keulen, S., Kirkby, K.C., . . . Verhoeven, J., *Consensus Paper: Cerebellum and Emotion.* Cerebellum, **2017**. 16(2): p. 552-576.
92. Vogt, B.A., *Midcingulate cortex: Structure, connections, homologies, functions and diseases.* Journal of Chemical Neuroanatomy, **2016**. 74: p. 28-46.
93. Stanislav, K., Alexander, V., Maria, P., Evgenia, N., and Boris, V., *Anatomical Characteristics of Cingulate Cortex and Neuropsychological Memory Tests Performance.* Procedia - Social and Behavioral Sciences, **2013**. 86: p. 128-133.

94. Al Omran, Y. and Aziz, Q., *Functional brain imaging in gastroenterology: to new beginnings.* Nat Rev Gastroenterol Hepatol, **2014**. 11(9): p. 565-576.
95. Peters, A., McEwen, B.S., and Friston, K., *Uncertainty and stress: Why it causes diseases and how it is mastered by the brain.* Prog Neurobiol, **2017**. 156: p. 164-188.
96. Genon, S., Reid, A., Langner, R., Amunts, K., and Eickhoff, S.B., *How to Characterize the Function of a Brain Region.* Trends in Cognitive Sciences, **2018**. 22(4): p. 350-364.
97. von Bohlen und Halbach, O., Draguhn, A., and Storm-Mathisen, J., *Recent advances in hippocampal structure and function.* Cell and Tissue Research, **2018**. 373(3): p. 521-523.
98. Ranganath, C., *Time, memory, and the legacy of Howard Eichenbaum.* Hippocampus, **2018**.
99. McDonald, A.J. and Mott, D.D., *Functional neuroanatomy of amygdalohippocampal interconnections and their role in learning and memory.* J Neurosci Res, **2017**. 95(3): p. 797-820.
100. Toda, T. and Gage, F.H., *Review: adult neurogenesis contributes to hippocampal plasticity.* Cell Tissue Res, **2018**. 373(3): p. 693-709.
101. Pugnaghi, M., Meletti, S., Castana, L., Francione, S., Nobili, L., Mai, R., and Tassi, L., *Features of somatosensory manifestations induced by intracranial electrical stimulations of the human insula.* Clin Neurophysiol, **2011**. 122(10): p. 2049-2058.
102. Obaid, S., Zerouali, Y., and Nguyen, D.K., *Insular Epilepsy: Semiology and Noninvasive Investigations.* J Clin Neurophysiol, **2017**. 34(4): p. 315-323.
103. Namkung, H., Kim, S.H., and Sawa, A., *The Insula: An Underestimated Brain Area in Clinical Neuroscience, Psychiatry, and Neurology.* Trends Neurosci, **2017**. 40(4): p. 200-207.
104. Stephani, C., Fernandez-Baca Vaca, G., Maciunas, R., Koubeissi, M., and Luders, H.O., *Functional neuroanatomy of the insular lobe.* Brain Struct Funct, **2011**. 216(2): p. 137-149.
105. Singh-Curry, V. and Husain, M., *The functional role of the inferior parietal lobe in the dorsal and ventral stream dichotomy.* Neuropsychologia, **2009**. 47(6): p. 1434-1448.
106. Caspers, S., Eickhoff, S.B., Rick, T., von Kapri, A., Kuhlen, T., Huang, R., . . . Zilles, K., *Probabilistic fibre tract analysis of cytoarchitectonically defined human inferior parietal lobule areas reveals similarities to macaques.* NeuroImage, **2011**. 58(2): p. 362-380.
107. Caspers, S., Geyer, S., Schleicher, A., Mohlberg, H., Amunts, K., and Zilles, K., *The human inferior parietal cortex: cytoarchitectonic parcellation and interindividual variability.* NeuroImage, **2006**. 33(2): p. 430-448.

108. Decety, J. and Chaminade, T., *When the self represents the other: a new cognitive neuroscience view on psychological identification.* Conscious Cogn, **2003**. 12(4): p. 577-596.
109. Shamay-Tsoory, S.G., *The neural bases for empathy.* Neuroscientist, **2011**. 17(1): p. 18-24.
110. Funahashi, S. and Andreau, J.M., *Prefrontal cortex and neural mechanisms of executive function.* J Physiol Paris, **2013**. 107(6): p. 471-482.
111. Fuster, J.M. and Bressler, S.L., *Past makes future: role of pFC in prediction.* J Cogn Neurosci, **2015**. 27(4): p. 639-654.
112. Wiech, K., Ploner, M., and Tracey, I., *Neurocognitive aspects of pain perception.* Trends Cogn Sci, **2008**. 12(8): p. 306-313.
113. Loggia, M.L., Berna, C., Kim, J., Cahalan, C.M., Martel, M.O., Gollub, R.L., . . . Edwards, R.R., *The lateral prefrontal cortex mediates the hyperalgesic effects of negative cognitions in chronic pain patients.* J Pain, **2015**. 16(8): p. 692-699.
114. Georgopoulos, A.P., *Neural aspects of cognitive motor control.* Curr Opin Neurobiol, **2000**. 10(2): p. 238-241.
115. Bonnard, M., de Graaf, J., and Pailhous, J., *Interactions between cognitive and sensorimotor functions in the motor cortex: evidence from the preparatory motor sets anticipating a perturbation.* Rev Neurosci, **2004**. 15(5): p. 371-382.
116. Frot, M., Magnin, M., Mauguiere, F., and Garcia-Larrea, L., *Cortical representation of pain in primary sensory-motor areas (S1/M1)--a study using intracortical recordings in humans.* Hum Brain Mapp, **2013**. 34(10): p. 2655-2668.
117. Raij, T.T., Forss, N., Stancak, A., and Hari, R., *Modulation of motor-cortex oscillatory activity by painful Adelta- and C-fiber stimuli.* Neuroimage, **2004**. 23(2): p. 569-573.
118. Ogino, Y., Nemoto, H., and Goto, F., *Somatotopy in human primary somatosensory cortex in pain system.* Anesthesiology, **2005**. 103(4): p. 821-827.
119. Sewards, T.V. and Sewards, M., *Separate, parallel sensory and hedonic pathways in the mammalian somatosensory system.* Brain Res Bull, **2002**. 58(3): p. 243-260.
120. Worthen, S.F., Hobson, A.R., Hall, S.D., Aziz, Q., and Furlong, P.L., *Primary and secondary somatosensory cortex responses to anticipation and pain: a magnetoencephalography study.* Eur J Neurosci, **2011**. 33(5): p. 946-959.
121. Mazzola, L., Isnard, J., and Mauguiere, F., *Somatosensory and pain responses to stimulation of the second somatosensory area (SII) in humans. A comparison with SI and insular responses.* Cereb Cortex, **2006**. 16(7): p. 960-968.
122. Liang, M., Mouraux, A., and Iannetti, G.D., *Parallel processing of nociceptive and non-nociceptive somatosensory information in the*

human primary and secondary somatosensory cortices: evidence from dynamic causal modeling of functional magnetic resonance imaging data. J Neurosci, **2011**. 31(24): p. 8976-8985.
123. Cona, G. and Semenza, C., *Supplementary motor area as key structure for domain-general sequence processing: A unified account.* Neurosci Biobehav Rev, **2016**. 72: p. 28-42.
124. Hwang, K., Bertolero, M.A., Liu, W.B., and D'Esposito, M., *The Human Thalamus Is an Integrative Hub for Functional Brain Networks.* J Neurosci, **2017**. 37(23): p. 5594-5607.
125. Weaver, K.R., Sherwin, L.B., Walitt, B., Melkus, G.D., and Henderson, W.A., *Neuroimaging the brain-gut axis in patients with irritable bowel syndrome.* World J Gastrointest Pharmacol Ther, **2016**. 7(2): p. 320-333.
126. Lovden, M., Wenger, E., Martensson, J., Lindenberger, U., and Backman, L., *Structural brain plasticity in adult learning and development.* Neurosci Biobehav Rev, **2013**. 37(9 Pt B): p. 2296-2310.
127. Davis, K.D., Pope, G., Chen, J., Kwan, C.L., Crawley, A.P., and Diamant, N.E., *Cortical thinning in IBS: implications for homeostatic, attention, and pain processing.* Neurology, **2008**. 70(2): p. 153-154.
128. Blankstein, U., Chen, J., Diamant, N.E., and Davis, K.D., *Altered Brain Structure in Irritable Bowel Syndrome: Potential Contributions of Pre-Existing and Disease-Driven Factors.* Gastroenterology, **2010**. 138(5): p. 1783-1789.
129. Seminowicz, D.A., Labus, J.S., Bueller, J.A., Tillisch, K., Naliboff, B.D., Bushnell, M.C., and Mayer, E.A., *Regional Gray Matter Density Changes in Brains of Patients With Irritable Bowel Syndrome.* Gastroenterology, **2010**. 139(1): p. 48-57.e42.
130. Jiang, Z., Dinov, I.D., Labus, J., Shi, Y., Zamanyan, A., Gupta, A., . . . Mayer, E.A., *Sex-related differences of cortical thickness in patients with chronic abdominal pain.* PLoS One, **2013**. 8(9): p. e73932.
131. Piche, M., Chen, J.I., Roy, M., Poitras, P., Bouin, M., and Rainville, P., *Thicker posterior insula is associated with disease duration in women with irritable bowel syndrome (IBS) whereas thicker orbitofrontal cortex predicts reduced pain inhibition in both IBS patients and controls.* J Pain, **2013**. 14(10): p. 1217-1226.
132. Labus, J.S., Dinov, I.D., Jiang, Z., Ashe-McNalley, C., Zamanyan, A., Shi, Y., . . . Mayer, E.A., *Irritable bowel syndrome in female patients is associated with alterations in structural brain networks.* Pain, **2014**. 155(1): p. 137-149.
133. Hong, J.Y., Labus, J.S., Jiang, Z., Ashe-Mcnalley, C., Dinov, I., Gupta, A., . . . Mayer, E.A., *Regional neuroplastic brain changes in patients with chronic inflammatory and non-inflammatory visceral pain.* PLoS One, **2014**. 9(1): p. e84564.
134. Orand, A., Gupta, A., Shih, W., Presson, A.P., Hammer, C., Niesler, B., . . . Chang, L., *Catecholaminergic Gene Polymorphisms Are*

Associated with GI Symptoms and Morphological Brain Changes in Irritable Bowel Syndrome. PLoS One, **2015**. 10(8): p. e0135910.

135. Labus, J.S., Van Horn, J.D., Gupta, A., Alaverdyan, M., Torgerson, C., Ashe-McNalley, C., . . . Mayer, E.A., *Multivariate morphological brain signatures predict patients with chronic abdominal pain from healthy control subjects.* Pain, **2015**. 156(8): p. 1545-1554.
136. Hubbard, C.S., Becerra, L., Heinz, N., Ludwick, A., Rasooly, T., Wu, R., . . . Nurko, S., *Abdominal Pain, the Adolescent and Altered Brain Structure and Function.* PLoS One, **2016**. 11(5): p. e0156545.
137. Chua, C.S., Bai, C.H., Shiao, C.Y., Hsu, C.Y., Cheng, C.W., Yang, K.C., . . . Hsu, J.L., *Negative correlation of cortical thickness with the severity and duration of abdominal pain in Asian women with irritable bowel syndrome.* PLoS One, **2017**. 12(8): p. e0183960.
138. Labus, J.S., Hollister, E.B., Jacobs, J., Kirbach, K., Oezguen, N., Gupta, A., . . . Mayer, E.A., *Differences in gut microbial composition correlate with regional brain volumes in irritable bowel syndrome.* Microbiome, **2017**. 5(1): p. 49.
139. Pitiot, A., Smith, J.K., Humes, D.J., Garratt, J., Francis, S.T., Gowland, P.A., . . . Marciani, L., *Cortical differences in diverticular disease and correlation with symptom reports.* Neurogastroenterol Motil, **2018**. 30(7): p. e13303.
140. Vandenberghe, J., Vos, R., Persoons, P., Demyttenaere, K., Janssens, J., and Tack, J., *Dyspeptic patients with visceral hypersensitivity: sensitisation of pain specific or multimodal pathways?* Gut, **2005**. 54(7): p. 914-919.
141. Francis, C.Y., Morris, J., and Whorwell, P.J., *The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress.* Aliment Pharmacol Ther, **1997**. 11(2): p. 395-402.
142. Lisspers, J., Nygren, A., and Soderman, E., *Hospital Anxiety and Depression Scale (HAD): some psychometric data for a Swedish sample.* Acta Psychiatr Scand, **1997**. 96(4): p. 281-286.
143. Zigmond, A.S. and Snaith, R.P., *The hospital anxiety and depression scale.* Acta Psychiatr Scand, **1983**. 67(6): p. 361-370.
144. Cosco, T.D., Doyle, F., Ward, M., and McGee, H., *Latent structure of the Hospital Anxiety And Depression Scale: a 10-year systematic review.* J Psychosom Res, **2012**. 72(3): p. 180-184.
145. Labus, J.S., Bolus, R., Chang, L., Wiklund, I., Naesdal, J., Mayer, E.A., and Naliboff, B.D., *The Visceral Sensitivity Index: development and validation of a gastrointestinal symptom-specific anxiety scale.* Aliment Pharmacol Ther, **2004**. 20(1): p. 89-97.
146. Labus, J.S., Mayer, E.A., Chang, L., Bolus, R., and Naliboff, B.D., *The central role of gastrointestinal-specific anxiety in irritable bowel syndrome: further validation of the visceral sensitivity index.* Psychosom Med, **2007**. 69(1): p. 89-98.

147. Derogatis, L., *SCL-90R administration, scoring and procedures manual-II*. Clinical Psychometric Research. **1992**, Baltimore.
148. Kroenke, K., Spitzer, R.L., and Williams, J.B., *The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms.* Psychosom Med, **2002**. 64(2): p. 258-266.
149. Sitnikova, K., Dijkstra-Kersten, S.M.A., Mokkink, L.B., Terluin, B., van Marwijk, H.W.J., Leone, S.S., . . . van der Wouden, J.C., *Systematic review of measurement properties of questionnaires measuring somatization in primary care patients.* J Psychosom Res, **2017**. 103: p. 42-62.
150. Zijlema, W.L., Stolk, R.P., Lowe, B., Rief, W., BioShaRe, White, P.D., and Rosmalen, J.G., *How to assess common somatic symptoms in large-scale studies: a systematic review of questionnaires.* J Psychosom Res, **2013**. 74(6): p. 459-468.
151. Leserman, J., Drossman, D.A., and Li, Z., *The reliability and validity of a sexual and physical abuse history questionnaire in female patients with gastrointestinal disorders.* Behav Med, **1995**. 21(3): p. 141-150.
152. Mertz, H., Naliboff, B., Munakata, J., Niazi, N., and Mayer, E.A., *Altered rectal perception is a biological marker of patients with irritable bowel syndrome.* Gastroenterology, **1995**. 109(1): p. 40-52.
153. Grinsvall, C., Tornblom, H., Tack, J., Van Oudenhove, L., and Simren, M., *Relationships between psychological state, abuse, somatization and visceral pain sensitivity in irritable bowel syndrome.* United European Gastroenterol J, **2018**. 6(2): p. 300-309.
154. Cremonini, F., Houghton, L.A., Camilleri, M., Ferber, I., Fell, C., Cox, V., . . . Whorwell, P.J., *Barostat testing of rectal sensation and compliance in humans: comparison of results across two centres and overall reproducibility.* Neurogastroenterol Motil, **2005**. 17(6): p. 810-820.
155. May, A., Ashburner, J., Büchel, C., McGonigle, D.J., Friston, K.J., Frackowiak, R.S.J., and Goadsby, P.J., *Correlation between structural and functional changes in brain in an idiopathic headache syndrome.* Nature Medicine, **1999**. 5(7): p. 836.
156. Helms, G., *Segmentation of human brain using structural MRI.* MAGMA, **2016**. 29(2): p. 111-124.
157. Lerch, J.P., van der Kouwe, A.J., Raznahan, A., Paus, T., Johansen-Berg, H., Miller, K.L., . . . Sotiropoulos, S.N., *Studying neuroanatomy using MRI.* Nat Neurosci, **2017**. 20(3): p. 314-326.
158. Johnson, E.B., Gregory, S., Johnson, H.J., Durr, A., Leavitt, B.R., Roos, R.A., . . . Scahill, R.I., *Recommendations for the Use of Automated Gray Matter Segmentation Tools: Evidence from Huntington's Disease.* Front Neurol, **2017**. 8: p. 519.
159. Mikhael, S., Hoogendoorn, C., Valdes-Hernandez, M., and Pernet, C., *A critical analysis of neuroanatomical software protocols reveals*

clinically relevant differences in parcellation schemes. Neuroimage, **2018**. 170: p. 348-364.
160. Ashburner, J. and Friston, K.J., *Voxel-based morphometry--the methods.* Neuroimage, **2000**. 11(6 Pt 1): p. 805-821.
161. Greve, D.N., *An Absolute Beginner's Guide to Surface- and Voxel-based Morphometric Analysis.* Proc. Intl. Soc. Mag. Reson. Med., **2011**. 19.
162. Liem, F., Merillat, S., Bezzola, L., Hirsiger, S., Philipp, M., Madhyastha, T., and Jancke, L., *Reliability and statistical power analysis of cortical and subcortical FreeSurfer metrics in a large sample of healthy elderly.* Neuroimage, **2015**. 108: p. 95-109.
163. Dale, A.M., Fischl, B., and Sereno, M.I., *Cortical surface-based analysis. I. Segmentation and surface reconstruction.* Neuroimage, **1999**. 9(2): p. 179-194.
164. Fischl, B., Sereno, M.I., and Dale, A.M., *Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system.* Neuroimage, **1999**. 9(2): p. 195-207.
165. Fischl, B., Sereno, M.I., Tootell, R.B., and Dale, A.M., *High-resolution intersubject averaging and a coordinate system for the cortical surface.* Hum Brain Mapp, **1999**. 8(4): p. 272-284.
166. Destrieux, C., Fischl, B., Dale, A., and Halgren, E., *Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature.* NeuroImage, **2010**. 53(1): p. 1-15.
167. Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., . . . Dale, A.M., *Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain.* Neuron, **2002**. 33(3): p. 341-355.
168. Panizzon, M.S., Fennema-Notestine, C., Eyler, L.T., Jernigan, T.L., Prom-Wormley, E., Neale, M., . . . Kremen, W.S., *Distinct genetic influences on cortical surface area and cortical thickness.* Cereb Cortex, **2009**. 19(11): p. 2728-2735.
169. Winkler, A.M., Kochunov, P., Blangero, J., Almasy, L., Zilles, K., Fox, P.T., . . . Glahn, D.C., *Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies.* Neuroimage, **2010**. 53(3): p. 1135-1146.
170. King, J.B., Lopez-Larson, M.P., and Yurgelun-Todd, D.A., *Mean cortical curvature reflects cytoarchitecture restructuring in mild traumatic brain injury.* Neuroimage Clin, **2016**. 11: p. 81-89.
171. Deppe, M., Marinell, J., Kramer, J., Duning, T., Ruck, T., Simon, O.J., . . . Meuth, S.G., *Increased cortical curvature reflects white matter atrophy in individual patients with early multiple sclerosis.* Neuroimage Clin, **2014**. 6: p. 475-487.
172. Carey, G., *Chapter 9 The General Linear Model (GLM): A gentle introduction*, in *Quantitative Methods in Neuroscience* **2013**:

http://psych.colorado.edu/~carey/qmin/qminchapters/qmin09-glmintro.pdf.
173. *Chapter 30, The GLM procedure*. Available from: http://www.math.wpi.edu/saspdf/stat/chap30.pdf.
174. Frost, J., *Understanding Interaction Effects in Statistics*. http://statisticsbyjim.com/regression/interaction-effects/.
175. Kenny, D.A., *Reflections on Mediation.* Organizational Research Methods, **2008**. 11(2): p. 353-358.
176. Lange, T., Hansen, K.W., Sorensen, R., and Galatius, S., *Applied mediation analyses: a review and tutorial.* Epidemiol Health, **2017**. 39: p. e2017035.
177. Preacher, K.J. and Hayes, A.F., *SPSS and SAS procedures for estimating indirect effects in simple mediation models.* Behav Res Methods Instrum Comput, **2004**. 36(4): p. 717-731.
178. Singh, M.K., Kesler, S.R., Hadi Hosseini, S.M., Kelley, R.G., Amatya, D., Hamilton, J.P., . . . Gotlib, I.H., *Anomalous gray matter structural networks in major depressive disorder.* Biol Psychiatry, **2013**. 74(10): p. 777-785.
179. Bassett, D.S. and Bullmore, E.T., *Human brain networks in health and disease.* Curr Opin Neurol, **2009**. 22(4): p. 340-347.
180. Kaiser, M., *A tutorial in connectome analysis: topological and spatial features of brain networks.* Neuroimage, **2011**. 57(3): p. 892-907.
181. Rubinov, M. and Sporns, O., *Complex network measures of brain connectivity: uses and interpretations.* Neuroimage, **2010**. 52(3): p. 1059-1069.
182. Wang, Y., *Graph Analysis of the Associative-Semantic Network*, in *Biomedical Sciences Group, Faculty of Medicine, Department of Neurosciences, Laboratory for Cognitive Neurology*. **2015**, KU Leuven.
183. Fleischer, V., Radetz, A., Ciolac, D., Muthuraman, M., Gonzalez-Escamilla, G., Zipp, F., and Groppa, S., *Graph Theoretical Framework of Brain Networks in Multiple Sclerosis: A Review of Concepts.* Neuroscience, **2017**.
184. Dalege, J., Borsboom, D., van Harreveld, F., and van der Maas, H.L.J., *Network Analysis on Attitudes: A Brief Tutorial.* Soc Psychol Personal Sci, **2017**. 8(5): p. 528-537.
185. van den Heuvel, M.P. and Sporns, O., *Network hubs in the human brain.* Trends Cogn Sci, **2013**. 17(12): p. 683-696.
186. *https://sites.google.com/site/bctnet/measures/list*. Brain Connectivity Toolbox.
187. *http://home.kpn.nl/stam7883/graph_introduction.html*. Department of Clinical Neurophysiology VU University Medical Center, **Accessed November 1st 2018**.

188. Latora, V. and Marchiori, M., *Economic small-world behavior in weighted networks.* The European Physical Journal B - Condensed Matter and Complex Systems, **2003**. 32(2): p. 249-263.
189. Latora, V. and Marchiori, M., *Efficient behavior of small-world networks.* Phys Rev Lett, **2001**. 87(19): p. 198701.
190. Ginestet, C.E. and Simmons, A., *Statistical parametric network analysis of functional connectivity dynamics during a working memory task.* Neuroimage, **2011**. 55(2): p. 688-704.
191. Lipowski, Z.J., *Somatization: the concept and its clinical application.* Am J Psychiatry, **1988**. 145(11): p. 1358-1368.
192. Ursin, H., *Brain sensitization to external and internal stimuli.* Psychoneuroendocrinology, **2014**. 42: p. 134-145.
193. Cervero, F., *Spinal cord hyperexcitability and its role in pain and hyperalgesia.* Experimental Brain Research, **2009**. 196(1): p. 129-137.
194. Brosschot, J.F., *Cognitive-emotional sensitization and somatic health complaints.* Scand J Psychol, **2002**. 43(2): p. 113-121.
195. Hansson, P., *Translational aspects of central sensitization induced by primary afferent activity: what it is and what it is not.* Pain, **2014**. 155(10): p. 1932-1934.
196. Woolf, C.J., *Central sensitization: implications for the diagnosis and treatment of pain.* Pain, **2011**. 152(3 Suppl): p. S2-15.
197. Nijs, J., Paul van Wilgen, C., Van Oosterwijck, J., van Ittersum, M., and Meeus, M., *How to explain central sensitization to patients with 'unexplained' chronic musculoskeletal pain: practice guidelines.* Man Ther, **2011**. 16(5): p. 413-418.
198. Croft, P., Dunn, K.M., and Von Korff, M., *Chronic pain syndromes: you can't have one without another.* Pain, **2007**. 131(3): p. 237-238.
199. Moshiree, B., Zhou, Q., Price, D.D., and Verne, G.N., *Central sensitisation in visceral pain disorders.* Gut, **2006**. 55(7): p. 905-908.
200. Tomenson, B., Essau, C., Jacobi, F., Ladwig, K.H., Leiknes, K.A., Lieb, R., . . . Sumathipala, A., *Total somatic symptom score as a predictor of health outcome in somatic symptom disorders.* British Journal of Psychiatry, **2013**. 203(5): p. 373-380.
201. Piche, M., Arsenault, M., Poitras, P., Rainville, P., and Bouin, M., *Widespread hypersensitivity is related to altered pain inhibition processes in irritable bowel syndrome.* Pain, **2010**. 148(1): p. 49-58.
202. Stabell, N., Stubhaug, A., Flaegstad, T., Mayer, E., Naliboff, B.D., and Nielsen, C.S., *Widespread hyperalgesia in adolescents with symptoms of irritable bowel syndrome: results from a large population-based study.* J Pain, **2014**. 15(9): p. 898-906.
203. Munakata, J., Naliboff, B., Harraf, F., Kodner, A., Lembo, T., Chang, L., . . . Mayer, E.A., *Repetitive sigmoid stimulation induces rectal hyperalgesia in patients with irritable bowel syndrome.* Gastroenterology, **1997**. 112(1): p. 55-63.

204. Patel, P., Bercik, P., Morgan, D.G., Bolino, C., Pintos-Sanchez, M.I., Moayyedi, P., and Ford, A.C., *Irritable bowel syndrome is significantly associated with somatisation in 840 patients, which may drive bloating.* Aliment Pharmacol Ther, **2015**. 41(5): p. 449-458.
205. Stahlberg, L., Palmquist, E., and Nordin, S., *Intolerance to environmental chemicals and sounds in irritable bowel syndrome: Explained by central sensitization?* J Health Psychol, **2018**. 23(10): p. 1367-1377.
206. Gebhart, G.F., *Pathobiology of visceral pain: molecular mechanisms and therapeutic implications IV. Visceral afferent contributions to the pathobiology of visceral pain.* Am. J. Physiol.-Gastroint. Liver Physiol., **2000**. 278(6): p. G834-838.
207. Akbar, A., Walters, J.R., and Ghosh, S., *Review article: visceral hypersensitivity in irritable bowel syndrome: molecular mechanisms and therapeutic agents.* Aliment Pharmacol Ther, **2009**. 30(5): p. 423-435.
208. Feng, B., La, J.H., Schwartz, E.S., Tanaka, T., McMurray, T.P., and Gebhart, G.F., *Long-term sensitization of mechanosensitive and -insensitive afferents in mice with persistent colorectal hypersensitivity.* Am J Physiol Gastrointest Liver Physiol, **2012**. 302(7): p. G676-683.
209. Herrero, J.F. and Headley, P.M., *Sensitization of spinal neurons by non-noxious stimuli in the awake but not anesthetized state.* Anesthesiology, **1995**. 82(1): p. 267-275.
210. Elsenbruch, S., Schmid, J., Bäsler, M., Cesko, E., Schedlowski, M., and Benson, S., *How positive and negative expectations shape the experience of visceral pain: an experimental pilot study in healthy women.* Neurogastroenterology & Motility, **2012**. 24(10): p. 914-e460.
211. Dorn, S.D., Palsson, O.S., Thiwan, S.I., Kanazawa, M., Clark, W.C., van Tilburg, M.A., . . . Whitehead, W.E., *Increased colonic pain sensitivity in irritable bowel syndrome is the result of an increased tendency to report pain rather than increased neurosensory sensitivity.* Gut, **2007**. 56(9): p. 1202-1209.
212. Almquist, E., Tornblom, H., and Simren, M., *Practical management of irritable bowel syndrome: a clinical review.* Minerva Gastroenterol Dietol, **2016**. 62(1): p. 30-48.
213. Simren, M. and Tack, J., *New treatments and therapeutic targets for IBS and other functional bowel disorders.* Nat Rev Gastroenterol Hepatol, **2018**. 15(10): p. 589-605.
214. Polster, A., Van Oudenhove, L., Jones, M., Öhman, L., Törnblom, H., and Simrén, M., *Editorial: subgroups in irritable bowel syndrome—more than just diarrhoea and constipation? Authors' reply.* Alimentary Pharmacology & Therapeutics, **2017**. 46(7): p. 698-699.
215. Polster, A.V., Palsson, O.S., Törnblom, H., Öhman, L., Sperber, A.D., Whitehead, W.E., and Simrén, M., *Subgroups of IBS patients are characterized by specific, reproducible profiles of GI and non-GI*

symptoms and report differences in healthcare utilization: A population based study. Neurogastroenterol Motil, **2018**. In press.
216. Pereda, N., Guilera, G., Forns, M., and Gomez-Benito, J., *The prevalence of child sexual abuse in community and student samples: a meta-analysis.* Clin Psychol Rev, **2009**. 29(4): p. 328-338.
217. Kloppen, K., Haugland, S., Svedin, C.G., Maehle, M., and Breivik, K., *Prevalence of Child Sexual Abuse in the Nordic Countries: A Literature Review.* J Child Sex Abus, **2016**. 25(1): p. 37-55.
218. kvinnofrid, N.c.f., *VÅLD OCH HÄLSA En befolkningsundersökning om kvinnors och mäns våldsutsatthet samt kopplingen till hälsa.* NCK-rapport 2014:1, **2014**.
219. Drossman, D.A., *Abuse, trauma, and GI illness: is there a link?* Am J Gastroenterol, **2011**. 106(1): p. 14-25.
220. Kanuri, N., Cassell, B., Bruce, S.E., White, K.S., Gott, B.M., Gyawali, C.P., and Sayuk, G.S., *The impact of abuse and mood on bowel symptoms and health-related quality of life in irritable bowel syndrome (IBS).* Neurogastroenterol Motil, **2016**. 28(10): p. 1508-1517.
221. Sansone, R.A. and Sansone, L.A., *IRRITABLE BOWEL SYNDROME: Relationships with Abuse in Childhood.* Innovations in clinical neuroscience, **2015**. 12(5-6): p. 34-37.
222. Lackner, J.M., Gudleski, G.D., and Blanchard, E.B., *Beyond abuse: the association among parenting style, abdominal pain, and somatization in IBS patients.* Behav Res Ther, **2004**. 42(1): p. 41-56.
223. Whitehead, W.E., Crowell, M.D., Davidoff, A.L., Palsson, O.S., and Schuster, M.M., *Pain from rectal distension in women with irritable bowel syndrome: relationship to sexual abuse.* Dig Dis Sci, **1997**. 42(4): p. 796-804.
224. Ringel, Y., Whitehead, W.E., Toner, B.B., Diamant, N.E., Hu, Y., Jia, H., . . . Drossman, D.A., *Sexual and physical abuse are not associated with rectal hypersensitivity in patients with irritable bowel syndrome.* Gut, **2004**. 53(6): p. 838-842.
225. Guthrie, E., Creed, F., Fernandes, L., Ratcliffe, J., Van Der Jagt, J., Martin, J., . . . Tomenson, B., *Cluster analysis of symptoms and health seeking behaviour differentiates subgroups of patients with severe irritable bowel syndrome.* Gut, **2003**. 52(11): p. 1616-1622.
226. Elsenbruch, S., Rosenberger, C., Enck, P., Forsting, M., Schedlowski, M., and Gizewski, E.R., *Affective disturbances modulate the neural processing of visceral pain stimuli in irritable bowel syndrome: an fMRI study.* Gut, **2010**. 59(4): p. 489-495.
227. Bos, D., Poels, M.M., Adams, H.H., Akoudad, S., Cremers, L.G., Zonneveld, H.I., . . . Vernooij, M.W., *Prevalence, Clinical Management, and Natural Course of Incidental Findings on Brain MR Images: The Population-based Rotterdam Scan Study.* Radiology, **2016**. 281(2): p. 507-515.

228. Phillips, J.P., Cole, C., Gluck, J.P., Shoemaker, J.M., Petree, L., Helitzer, D., . . . Holdsworth, M., *Stakeholder Opinions And Ethical Perspectives Support Complete Disclosure Of Incidental Findings In MRI Research.* Ethics Behav, **2015**. 25(4): p. 332-350.
229. Bunnik, E.M., van Bodegom, L., Pinxten, W., de Beaufort, I.D., and Vernooij, M.W., *Ethical framework for the detection, management and communication of incidental findings in imaging studies, building on an interview study of researchers' practices and perspectives.* BMC Med Ethics, **2017**. 18(1): p. 10.
230. Rancher, C.E., Shoemaker, J.M., Petree, L.E., Holdsworth, M., Phillips, J.P., and Helitzer, D.L., *Disclosing neuroimaging incidental findings: a qualitative thematic analysis of health literacy challenges.* BMC Med Ethics, **2016**. 17(1): p. 58.
231. Schmidt, C.O., Hegenscheid, K., Erdmann, P., Kohlmann, T., Langanke, M., Volzke, H., . . . Grabe, H.J., *Psychosocial consequences and severity of disclosed incidental findings from whole-body MRI in a general population study.* Eur Radiol, **2013**. 23(5): p. 1343-1351.
232. Eikelboom, E.M., Tak, L.M., Roest, A.M., and Rosmalen, J.G.M., *A systematic review and meta-analysis of the percentage of revised diagnoses in functional somatic symptoms.* J Psychosom Res, **2016**. 88: p. 60-67.
233. Drossman, D.A., Tack, J., Ford, A.C., Szigethy, E., Tornblom, H., and Van Oudenhove, L., *Neuromodulators for Functional Gastrointestinal Disorders (Disorders of Gut-Brain Interaction): A Rome Foundation Working Team Report.* Gastroenterology, **2018**. 154(4): p. 1140-1171 e1141.
234. Kellmeyer, P., *Ethical and Legal Implications of the Methodological Crisis in Neuroimaging.* Camb Q Healthc Ethics, **2017**. 26(4): p. 530-554.
235. Mayer, T.G., Neblett, R., Cohen, H., Howard, K.J., Choi, Y.H., Williams, M.J., . . . Gatchel, R.J., *The development and psychometric validation of the central sensitization inventory.* Pain Pract, **2012**. 12(4): p. 276-285.
236. Yunus, M.B., *Fibromyalgia and Overlapping Disorders: The Unifying Concept of Central Sensitivity Syndromes.* Seminars in Arthritis and Rheumatism, **2007**. 36(6): p. 339-356.
237. Scerbo, T., Colasurdo, J., Dunn, S., Unger, J., Nijs, J., and Cook, C., *Measurement Properties of the Central Sensitization Inventory: A Systematic Review.* Pain Pract, **2018**. 18(4): p. 544-554.
238. Caumo, W., Antunes, L.C., Elkfury, J.L., Herbstrith, E.G., Busanello Sipmann, R., Souza, A., . . . Neblett, R., *The Central Sensitization Inventory validated and adapted for a Brazilian population: psychometric properties and its relationship with brain-derived neurotrophic factor.* J Pain Res, **2017**. 10: p. 2109-2122.