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**THE EMOTIONAL MOTOR SYSTEM  
AND  
GASTROINTESTINAL SYMPTOMS**

**Pontus Karling**

*Department of Public Health and Clinical Medicine, Medicine, Umeå University  
SE-901 85 Umeå, Sweden  
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# The Emotional Motor System and Gastrointestinal Symptoms

## ABSTRACT

There is a significant comorbidity between anxiety/depression and functional gastrointestinal syndromes, such as irritable bowel syndrome (IBS) and functional dyspepsia. The pathophysiological link between emotions and the gut is not known. A model of an emotional motor system (EMS) which reacts to interoceptive and exteroceptive stress has been proposed. EMS consists of specific brain structures including anterior cingulate cortex (ACC), amygdala, hippocampus and hypothalamus and mediates their communication to the rest of the body (including the gastrointestinal tract) through the hypothalamus-pituitary-adrenal (HPA) axis, the autonomic nervous system (ANS) and by a pain modulation system. The aim of this thesis was to test the EMS model by studying the relationship between symptoms of anxiety and depression and IBS-like symptoms in patients with recurrent unipolar depression, in patients with IBS and in a sample of a normal Swedish population. The peripheral limb of EMS (ANS, HPA axis and the pain modulations system) was tested in patients with IBS and control subjects. Spectral heart rate variability was used to investigate ANS function in patients with refractory IBS and in healthy controls. The HPA axis function was tested by a weight adjusted low dose dexamethasone suppression test in control subjects. The influence of catecholamine degradation on pain modulation was tested by analyzing val158met catechol-o-methyl transferase (COMT) polymorphism in patients with IBS and in control subjects.

We found a significant relationship between symptoms of anxiety/depression and IBS-like symptoms in patients with recurrent unipolar depression, in patients with IBS and in a sample of the normal population. Interestingly, patients with recurrent unipolar depression in remission had no more IBS-like symptoms than controls, indicating that the gastrointestinal symptoms may resolve when depression is treated to remission.

Patients with IBS have an increased mid-frequency power in rest and in supine position (after tilt test) compared to healthy controls indicating an increased sympathetic ANS drive.

The symptoms of diarrhea and early satiety has in the literature been associated to the stimulation of corticotropin releasing hormone (CRH) receptors and was also in our study related to HPA axis function tested by a low dose dexamethasone test. Interestingly both hypo- and hyperfunction of the HPA axis was related to these symptoms in control subjects.

The val158met COMT polymorphism was associated to IBS-like symptoms. Control subjects with IBS-like symptoms (defined by the upper quartile in total GSRS-IBS score) had a higher frequency of the met/met and a significantly lower frequency of the val/met genotype. Also patients with IBS tended to have a lower frequency of the heterozygous val/met genotype so we conclude that this genotype may be protective against IBS/IBS-like symptoms. In addition, the val/val genotype in patients with IBS was associated to diarrhea symptoms.

**Conclusions:** Our results support the model of an emotional motor system in the genesis of functional gastrointestinal symptoms by the finding of the association of IBS-like symptoms and mood disturbances, and by finding alterations in the peripheral limbs of EMS (ANS, HPA axis and catecholamines) in subjects with IBS and IBS-like symptoms.

**Key words:** Anxiety, ANS, Autonomic nervous system, Cortisol, DST, Emotional motor system, Functional, Gastrointestinal symptoms, GSRS-IBS, Gut, HADS, Heart rate variability, HPA axis, IBS, Irritable bowel syndrome, Stress, Unipolar depression, Val158met COMT polymorphism

## ORIGINAL ARTICLES

- I Karling P, Danielsson Å, Adolfsson R, Norrback K-F. No difference in symptoms between healthy subjects and patients with unipolar depression in remission. *Neurogastroenterol Motil* 2007; 19: 896-904
- II Karling P, Nyhlin H, Wiklund U, Sjöberg M, Olofsson B-O, Bjerle P. Spectral analysis of heart rate variability in patients with irritable bowel syndrome. *Scand J Gastroenterol* 1998; 33: 572-76.
- III Karling P, Norrback K-F, Adolfsson R, Danielsson Å. Gastrointestinal symptoms are associated with hypothalamic-pituitary-adrenal axis suppression in healthy individuals. *Scand J Gastroenterol.* 2007; 14:1294-301
- IV Karling P, Danielsson Å, Pettersson M, Söderström I, Del-Favero J, Adolfsson R, Norrback K-F. The val158met Catechol-O-methyltransferase (COMT) polymorphism is associated with irritable bowel syndrome-like symptoms. Manuscript.

## ABBREVIATIONS

ACC	Anterior cingulate cortex
ACTH	Adrenocorticotrophic hormone
ANS	Autonomic nervous system
BMI	Body mass index
CBG	Cortisol binding protein
CNS	Central nervous system
COMT	Catechol-o-methyltransferase
CRH	Corticotropin releasing hormone
CRP	C-reactive protein
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-IV
DST	Dexamethasone Suppression Test
ECG	Electrocardiogram
EMS	Emotional motor system
ENS	Enteric nervous system
GABA	Gamma-aminobutyric acid
GCs	Glucocorticoids
GI	Gastrointestinal
GSRS	Gastrointestinal symptoms rating scale
GSRS-IBS	Gastrointestinal symptoms rating scale – Irritable bowel syndrome
GR	Glucocorticoid receptor
HADS	Hospital anxiety and depression scale
HLA	Human leukocyte antigen
HPA	Hypothalamic-pituitary-adrenal
HRV	Heart rate variability
IBS	Irritable bowel syndrome
IBS-C	Constipation dominated IBS
IBS-D	Diarrhoea dominated IBS
IgG	Immunoglobulin G
IL-10	Interleukin -10
IL-12	Interleukin - 12
LC	Locus ceruleus
MF/HF	Mid-frequency/High-frequency band
MR	Mineralcorticoid receptor
mRNA	Messenger Ribonucleic Acid
OR	Odds ratio
PI-IBS	Postinfectious IBS
PVN	Paraventricular nucleus
SNP	Single nucleotide polymorphism
SSRI	Serotonin reuptake inhibitors
5-HT	5-hydroxytryptamine (Serotonin)

## BACKGROUND

### Irritable bowel syndrome (IBS)

Unexplained gastrointestinal symptoms exist frequently in the general population and lead to many visits to physicians (1). The gastrointestinal tract has a limited repertoire of responses to disease or disturbance in function and one gastrointestinal symptom or symptom cluster can have different pathophysiological causes (2). At present, in most patients who seek medical care, no obvious structural or biochemical abnormality can be found and for these patients consensus has been to characterize their gastrointestinal complaints by symptom clusters into different functional gastrointestinal disorders (3). The most prevalent symptom clusters of functional gastrointestinal disorders are functional dyspepsi (nausea, early satiety and upper gastrointestinal pain/discomfort) and irritable bowel syndrome (bloating, defecation disturbances and abdominal pain/discomfort) (3) (Figure 1).

An early description of the irritable bowel syndrome (IBS) was presented by Cumming in 1849, “The bowels are at one time constipated, another lax, in the same person. How the disease has two such different symptoms - I do not profess to explain” (4). This mysterious gastrointestinal syndrome has had many names through the last century, such as mucous colitis described by Osler in 1892, and colonic spasm as it was called in the 1920s. Jordan and Kiefer in 1929 introduced the term irritable colon, which changed to colon irritable and then to the current used terminology: irritable bowel syndrome (5).

## IBS SYMPTOMS

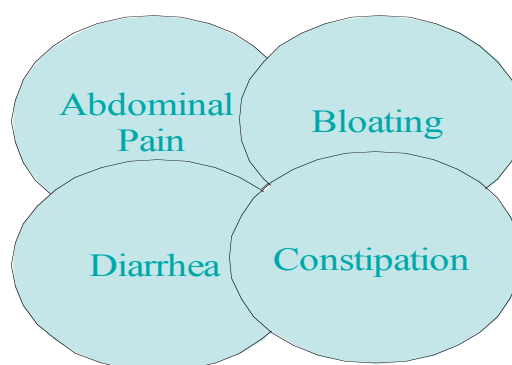


Figure 1: IBS symptoms

IBS is responsible for 30% of all gastrointestinal complaints in general practice (6) and functional GI disorders accounts for 35-48% of symptomatic outpatient diagnoses in a gastroenterology department (7-8). The Rome classification system characterizes the IBS in terms of multiple physiological determinants contributing to a common set of symptoms rather than a single disease entity. Subdivision of IBS by the predominant symptom (i.e diarrhea-dominated (IBS-D) and constipation-dominant (IBS-C)) is often used in research (3). Panel I lists the revised Rome III diagnostic criteria for IBS (3).

**Panel I: Rome III Diagnostic Criteria for Irritable Bowel Syndrome**

<p>Recurrent abdominal discomfort or pain at least three days per month the last three months and with the symptom onset at least six months ago. The abdominal discomfort or pain has 2 of 3 features:</p> <ol style="list-style-type: none"> <li>1. Relieved/Improved with defecation, and/or</li> <li>2. Onset associated with a change in frequency of stool, and/or</li> <li>3. Onset associated with a change in form (appearance) of stool.</li> </ol>
<p>Symptoms that cumulatively support the diagnosis of IBS:</p> <ol style="list-style-type: none"> <li>1. Abnormal stool frequency (for research purposes, “abnormal” may be defined as greater than 3 bowel movements per day and less than 3 bowel movements per week);</li> <li>2. Abnormal stool form (lumpy/hard or loose/watery stool);</li> <li>3. Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);</li> <li>4. Passage of mucus;</li> <li>5. Bloating or feeling of abdominal distention.</li> </ol>

The prevalence of IBS varies due to definitional criteria and to differences in the specific questions used to retrieve the information. Most studies show prevalences between 3-15% and with a female/male ratio approximately of 2:1 (9-10). The majority of patients with IBS reported at least one GI symptom in over half of the days, and pain was reported one third of the time (11). In a longer perspective (years) the point prevalence of IBS in a population remains almost unchanged but in 40% of subjects the symptoms had disappeared and were replaced by new cases (12-13). However, IBS is still common in elderly people (14). 30-90% of persons with IBS symptoms do not seek medical attention for their symptoms (15). Cultural factors, the presence and degree of pain, psychological disturbance and the possibility of access to health care may influence consulting rate (15). Consulters for IBS also have more non-GI complaints and consult physicians more for these symptoms compared with persons with IBS not seeking health care or persons without bowel symptoms (16-17). Persons with IBS miss three times as many work days as those without bowel symptoms (13.4 days vs 4.9 days) (18).



The cause of IBS as for the other functional gastrointestinal disorders is unknown. Below follows a brief summary of the areas which research has been focused on (Figure 2).

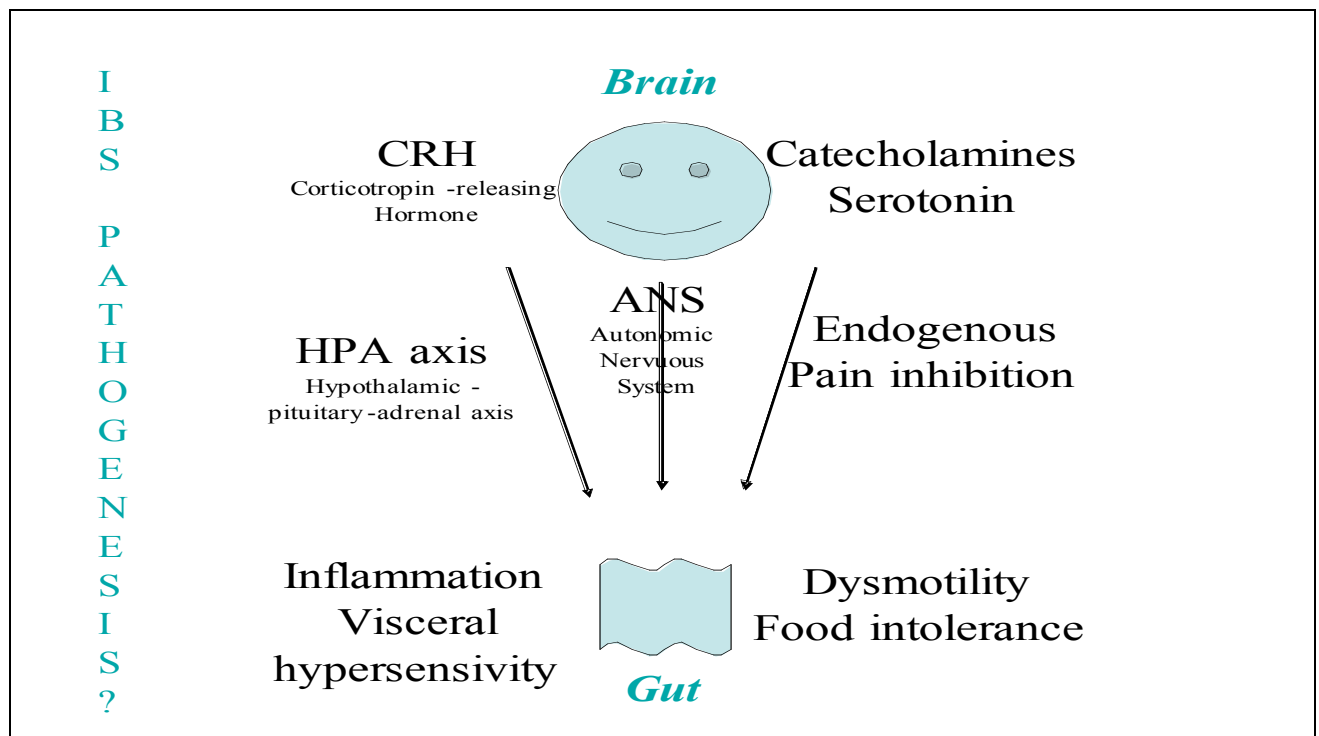


Figure 2: Possible pathogenic factors for IBS

### Abnormal Motility in Patients with IBS

There have been many studies of gut motility in patients with IBS (19). In the ileum, colon and rectum, IBS patients show an exaggerated response to a variety of provocative stimuli including meals (20), distention (21), stress (22-23), cholecystokinin (24), neostigmine (22) and corticotropin-releasing hormone (CRH) injection (25-26). In the proximal small intestine and in the stomach stress inhibits contractions (27-28), whereas meals increase contractions (19). Overall bowel transit is accelerated in diarrhea-dominated IBS and slowed or normal in constipated-dominated IBS (29-31). Among IBS patients with diarrhea and abdominal pain, there are more high-amplitude propagating contractions as well as abnormal contractions and these are more likely to be associated with a sensation of pain (32-33). A decreased inhibition of postprandial colonic motility upon rectal stimulation has been demonstrated in patients with IBS (34). Delayed transit of gas causes greater abdominal perception in IBS (35) and IBS patients are more likely than healthy controls to perceive the occurrence of normal migrating motor complexes (36). Since 25-75% of IBS patients exhibit no motility “abnormalities” motility parameters cannot yet be used as diagnostic markers (19).

## **Visceral Hypersensitivity in IBS**

Different studies have reported that 20-80% of IBS patients have lower threshold of pain when a balloon is inflated in the bowel (37) and enhanced perception of visceral events is also documented throughout the GI tract, including esophagus (38), stomach (39), small bowel (40) and colon (41). However, whether IBS patients show somatic hypersensitivity to pain is at present uncertain (40, 42). Consistent with visceral hypersensitivity IBS patients are more likely than controls to notice intestinal contractions and gas (35-36), and their pain thresholds are correlated, to some degree with the level of clinical pain they experience (43).

## **Inflammation and infection in IBS**

In unselected patients with IBS increased numbers of mast cells in the muscularis externa of the colon and the ileal and colonic mucosa (44-46) as well as an increased cellularity of the colon mucosa and lamina propria have been found (47-48). There is also evidence that IBS patients have an increased amount of inflammatory cells in close proximity to mucosal nerve endings (49-51). IBS patients show slightly increased levels of nitric oxide in bowel gas (52), calprotectin in faeces (53) and highly sensitive C reactive protein (CRP) in serum (54), all these are markers of inflammation. The proinflammatory cytokines TNF-alpha, IL-1beta, IL-6 and LPS-induced IL-6 levels in peripheral blood cells (in vitro) are increased in IBS patients compared with healthy controls (55).

4-26% of IBS patients displayed their onset of IBS symptoms after an episode of gastroenteritis (56) and the risk of IBS is 10 times higher the year after a culture-verified gastroenteritis (57). This entity of IBS is termed postinfectious-IBS (PI-IBS) and is one of the main fields in IBS research at present. Patients with PI-IBS show increased cellularity of lamina propria in rectum, an increase in CD3+ and CD25+ lymphocytes in lamina propria as well as an increase in colonic enterochromaffin cells and an increased expression of interleukin-1beta mRNA in the colon mucosa (58-60). PI-IBS exhibits more urgency and diarrhea symptoms than non PI-IBS. Risk factors for developing PI-IBS is the virulence of the pathogen (61), long duration of diarrhea, female gender, hypochondria, anxiety/depression and the presence of a significant life stressor the year before the gastroenteritis (60, 62)). Also, more than half of western travellers to foreign countries develop gastrointestinal symptoms (mostly diarrhea) which in a majority of cases were persistent (63). Yet, no specific bacteria have been linked to PI-IBS and there is no evidence of higher prevalence of IBS in countries with a higher incidence of gastrointestinal infections (64).

Studies of the gut flora in unselected IBS patients have shown an increase in *Blastocystis hominis* (65-66) and a decrease in coliforms, lactobacilla and bifidobacteria compared to healthy controls (67-69). Probiotics have in some studies decreased IBS symptoms (70-73) and one study also showed that

bifidobacteria increases the serum IL-10/IL-12 ratio, indicating a beneficial effect on humoral immunosystem (72). The use of antibiotics has been described as a risk factor for IBS (74). However, a subset of IBS patients with abdominal bloating and flatulens and/or with a positive lactulos breath test exhibited symptom improvement on antibiotics therapy indicating small bowel bacterial contamination (75-76).

26-60% of patients with inflammatory bowel disease in remission have IBS-like symptoms (77-79) supporting a link of a low grade inflammation to a cause of IBS. The influence of gut inflammation on visceral hypersensitivity is complex (80). Acute gut inflammation results in sensitization of peripheral, spinal, and central transmission (81) and is associated with lower threshold for pain in rectum (82-83) but in chronic gut inflammation it seems that an adaptive down-regulation of perceptual sensitivity occurs (84-85).

### **Food intolerance and IBS**

The prevalence of IgE mediated food allergy in adults is estimated to 0.3% (86) but as many as 20% of a normal population and 60% of IBS patients believe they are intolerant to food components (87-88). Patients with IBS often seek information about dietary changes (89). 60-70 % of patients with IBS have exaggerated symptoms after food intake (90-91). This could partly be explained by onset of postprandial motility and visceral hypersensitivity presented above (92). Many IBS patients describe intolerance to milk and lactose but the prevalence of diagnosed lactose intolerance is not higher in an IBS population (93). Also yeast and yeast products have been discussed as a trigger for IBS symptoms (94). Exclusion of food associated with raised specific IgG antibodies improved symptoms in IBS patients, and over 80% of these patients excluded milk and yeast (95).

Patients with celiac disease show IBS like symptoms and gluten enteropathy must be excluded in the investigations of IBS patients (96). Twin studies have shown an association of IBS to gluten enteropathy and also reumatoid arthritis (97). Diarrhea-dominated IBS patients with normal villi structure on duodenal biopsy and normal antibodies to endomysium had a higher presence of the genotype HLA-DQ2 (35%), an increased amount of intraepithelial lymphocytes in duodenal biopsies and an elevated amount of endomysium antibodies in duodenal aspirate. In IBS patients with these findings, gluten free diet decreased symptoms more than in those IBS patients without these findings (98).

### **IBS and comorbidity**

Many other chronic non-gastrointestinal somatic disorders, often of unknown origin are about twice as common in patients with IBS (panel II, III) and 78% of excess health care visits for IBS patients is due to non-gastrointestinal complaints (16-17). Also, IBS non-consulters demonstrate high rates of comorbidity (99). The higher incidence in IBS patients of irritable bladder (100),

Panel II. The prevalence of other somatic disorders in IBS (adopted from 16)

	<b>In patients with IBS</b>	<b>Controls</b>
<b>Fibromyalgia</b>	32%	2%
<b>Chronic fatigue syndrome</b>	14%	0.4%
<b>Chronic pelvic pain</b>	35%	14%
<b>Temporomandibular joint disorder</b>	16%	21%

bronchial hyperreactivity (101), fibromyalgia (102) and chronic pelvic pain (103), all diagnoses of unknown etiology, have brought on speculations of a common feature of enhanced visceral and somatic perception or a so called functional somatic syndrome (104). Anxiety, depression and chronic fatigue are also more common in fibromyalgia and chronic pelvic pain as in IBS and may be involved in the functional somatic syndrome (104-106). A dual hypothesis has been proposed for IBS. Whereas IBS patients with multiple symptoms from other parts of the body have a central cause for their bowel symptoms, IBS patients without extra-intestinal symptoms have a gut related cause for their bowel symptoms (16).

In addition, other functional gastrointestinal disorders such as gastroesophageal reflux disease and non-ulcer dyspepsia are more common in patients with IBS (107-108), and the IBS patients more often proceed to abdominopelvic and gallbladder surgery and, thus, an associated risk for experiencing morbidity and mortality associated with these surgical procedures (109).

However, a recent published large study by Whitehead et al (110) stated that comorbidity in IBS is due to a general amplification of symptom reporting and physician consultation rather than a few unique associations, which suggests biased symptom perception rather than shared pathophysiology. They also show that excess comorbidity is present only in a subset of IBS patients (16%).

Panel III. The prevalence of IBS in other somatic disorders (adopted from 16)

	<b>Prevalence of IBS</b>
<b>In a normal population</b>	5-15%
<b>Fibromyalgia</b>	48%
<b>Chronic fatigue syndrome</b>	51%
<b>Chronic pelvic pain</b>	50%
<b>Gynecological referrals (dyspareunia, dysmenorrhea, urinary symptoms and nonmenstrual bleeding)</b>	37%
<b>Interstitial cystitis</b>	30%
<b>Temporomandibular joint disorder</b>	64%
<b>Bronchial hyperresponsiveness</b>	23%

## **IBS and affective disorders**

There are many studies presented in the literature that have explored the impact of psychosocial and psychiatric disorders in patients with IBS (111, 112). The prevalence of anxiety and/or depression disorders in subjects with IBS attending gastroenterology clinics is as high as 33-59% (113-122). Anxiety and depression in IBS patients have also been associated with more IBS symptoms and worse prognosis (121-125) but interestingly also with increased proinflammatory cytokines (55). However, it has also been argued that psychiatric illness is not increased in the total IBS population and that anxiety and depression instead act as confounders, leading a person with IBS to seek medical care (126-127). Despite frequent studies of psychiatric disorders in IBS, there are relatively few studies on IBS in psychiatric patients. Two small studies reported a prevalence of 27-29% of IBS in patients with recent onset or recurrent episode of major depression (128-129). Increased prevalence of IBS have also been reported in dysthymia (59%) (130) and in anxiety disorders (17-46%) (128, 131-132). Physical symptoms are common in patients with anxiety and depression. In patients about to be hospitalized for major depression 92% complained of at least one painful symptom and 76% complained of multiple painful symptoms (133). The presence of painful symptoms in depressed patients also predicts a greater severity of the depressive disorder (134). Physical symptoms decrease with recovery and treatment of depression (135). Anti-depressant medication is often used in refractory IBS (136) and there is some evidence of parallel improvement in IBS symptoms and depression in patients with concurrent depression and IBS when treated with antidepressants (137).

## **Brain-Gut Interactions in IBS**

There is complex bidirectional interplay between the gut and the brain. The central nervous system (CNS) modulates motility, afferent visceral signals, secretion, immune function and blood flow in the gastrointestinal tract (138-141). Afferent signals from the gut influence reflex regulation, mood states and pain perceptions in the CNS (19, 81, 142). The CNS modulation of gut sensations occur at different levels both centrally and in the periphery (81, 143), and the CNS act as a “filter” where most visceral afferent signals reach the brainstem and thalamus but only a very few are consciously perceived in the cortex (19, 144).

Emotional and cognitive states influence the perception of signals from the gut. In patients with functional bowel disorders, stress, anxiety, the confrontation with fearful faces or recall of aversive memories all can enhance perception (145-148) and conversely distraction, hypnosis and relaxation decrease perceptual sensitivity (143, 149).

A majority of people experience stress-induced GI symptoms. For example a study by Drossman et al showed that up to 67% of healthy controls and 84% of patients with IBS reported that stress led to abdominal pain and/or change in stool pattern (150). Acute stressors induce different motor effects in the upper and lower GI tract. In healthy human subjects, anger, fear, painful stimuli and stress delay gastric emptying and enhance colonic motor activity (22-23, 27-28). In rats, colonic motility is more responsive to stress than the upper gut (151-152). Life events and daily stress is more common before the onset of IBS but also before a flare of inflammatory bowel disease (153-155) and as mentioned earlier there is a high comorbidity between psychiatric disorders (most commonly anxiety and depression) and IBS (111, 156).

### **The Emotional Motor System**

Stress, defined as acute threats of the homeostasis of an organism can be caused either by *interoceptive* stressors, such as infections, haemorrhage and inflammation or by *exteroceptive* stressors such as psychological strains or threats. Both types of stressors evoke similar adaptive responses (allostasis) which serve to defend the stability of the internal milieu and to assure the survival of the organism (138, 140). Normally, the elaborate neurobiological response system is well-adjusted to the specific stressor in a given situation for a specific individual. It is rapidly turned on and off, synchronising the physiological stress response to the stress episode and limiting the exposure time of the potentially harmful effects of the stress response. A pathological stressor is an acute or a chronic stressor that causes a maladaptive response, resulting in a chronic overactivity or underactivity in the physiological stress response systems which can lead to or exacerbate disease. The length, severity and type of the stressor in combination with the impact of genetic factors, early life events, cognitive factors and environmental support influences the outcome from pathological stressors upon an individual (138, 140).

The organism's response to stress is generated by a network comprised of integrated brain structures, in particular subregions of the hypothalamus (paraventricular nucleus, PVN), amygdala and periaqueductal grey. These regions receive input from visceral and somatic afferents and from cortical regions including medial prefrontal cortex and subregions of anterior cingulate and insular cortices. In turn, output from this integrated network to the pituitary and pontomedullary nuclei mediates the neuroendocrine and autonomic responses to the body (140-141). Ascending serotonergic projections from raphe nuclei, and noradrenergic projections from locus coeruleus, and circulating glucocorticoids exert feedback control of the central stress circuitry. The parallel output of this central stress circuitry is referred to the "emotional motor system (EMS)" (140, 157). The EMS is activated in response to different types of stressors and includes responses of the autonomic nervous system, the HPA axis, the endogenous pain modulation system, and ascending monoaminergic

pathways. The central coordinators of the EMS is the parvocellular corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) neurons of the PVN of the hypothalamus, the CRH neurons of the paragigantocellular and other nuclei of the medulla, as well as the catecholaminergic neurons of locus ceruleus (LC) and other cell groups of the medulla and the pons (138). The peripheral limb of the EMS is the HPA axis, the efferent sympathetic/adrenomedullary system and the endogenous pain modulation system (140) (Figure 3).

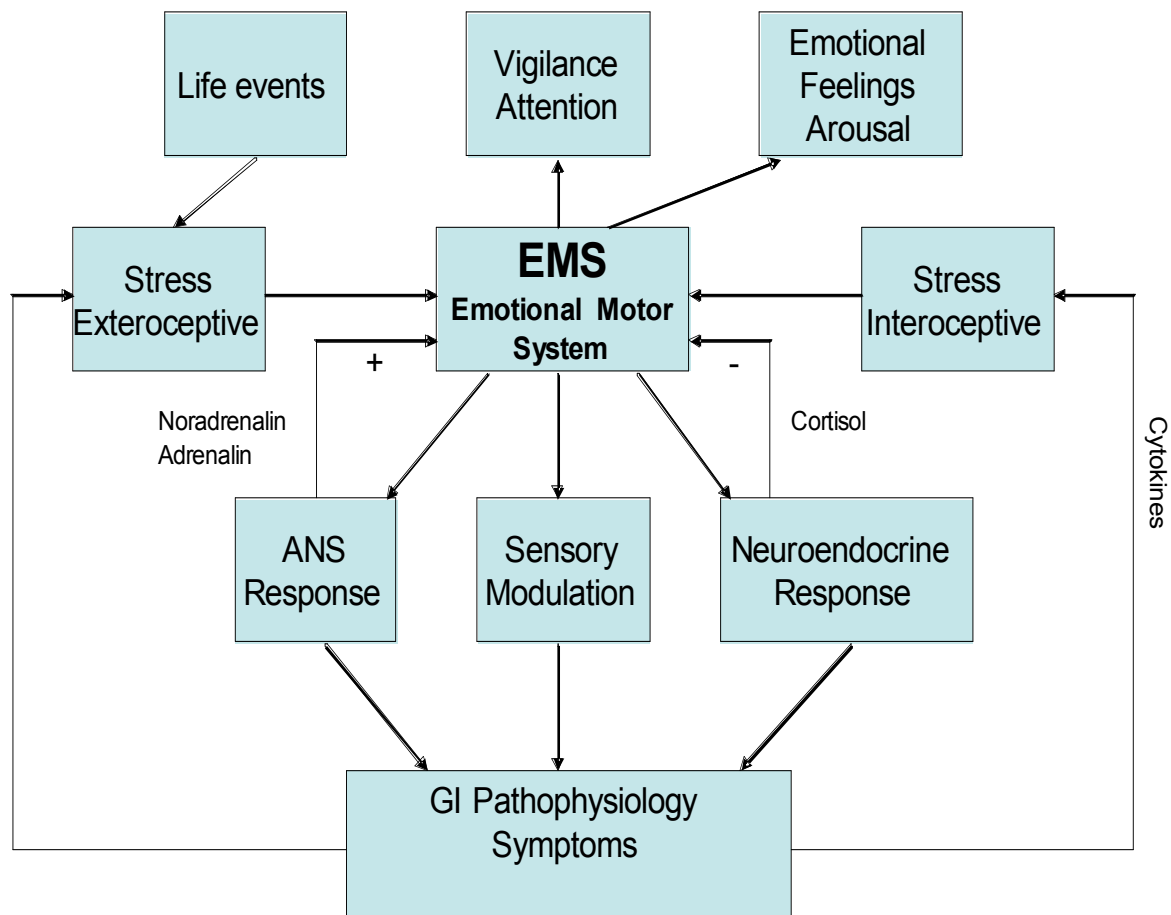


Figure 3: The emotional motor system (adopted from 140)

The developmental setting of the EMS depends partially on genetic factors, and show considerable plasticity in response to early life events and to certain types of pathological stress (140, 158). For example, pathological stress can alter the responsiveness of the feedback systems by downregulation of glucocorticoid receptors and of pre- and postsynaptic adrenergic and serotonergic receptors. The results can be up- or downregulation of the HPA axis, of the pain perception, and decreased or increased activity of the ANS. Typically observed

central adaptations to pathological stress are an increase in CRH synthesis and secretion, an increase in the activity and sensitivity of central noradrenergic systems, and either downregulation or sensitization of glucocorticoid receptors and ACTH release. As a consequence, changes in spinal or peripheral target cells can occur. Long-lasting or severe pathological stress can also cause structural changes in certain brain regions, such as in hippocampus with subsequent changes in mood and memory function (140, 159).

### Corticotropin releasing hormone (CRH)

Corticotropin releasing hormone (CRH) is a central hormone in EMS. During acute stress it activates the sympathetic nervous system followed by activation of the HPA axis (138, 140). CRH, also plays a central role in the pathophysiology of symptoms of anxiety and depression (140, 160) and CRH<sub>1</sub> receptors are abundant in amygdala mediating anxiety symptoms (161). Interestingly, the same receptors are located in colon and when injected intravenously CRH augments colonic motility and increases rectal sensitivity. All these responses are elevated in patients with IBS (25-26) (Figure 4). CRH blockage reduces colonic motility, visceral hypersensitivity and IBS symptoms (25-26) but also normalizes abnormal EEG pattern seen in IBS patients indicating that CRH might have a central role in patients with IBS (162). In addition, CRH<sub>2</sub> receptors are abundant in the stomach, and when activated decrease upper GI motility (151).

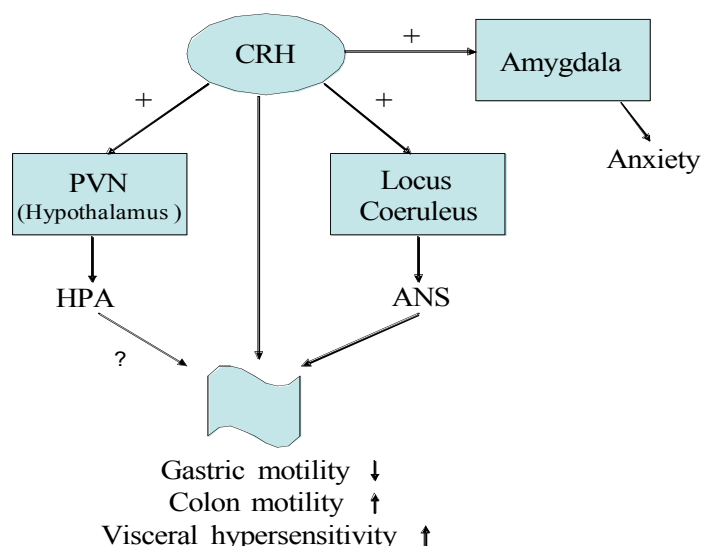


Figure 4: Corticotropin releasing hormone (CRH) effects

### The Hypothalamic-Autonomic Nervous System (ANS) axis

The parasympathetic part of the ANS consists of the vagal nerve which innervates the entire gut except the distal third of the colon and the parasympathetic sacral innervation which innervate the distal colon, rectum and



internal anal sphincters. The vagal nerve consists of 70-90% of unmyelinated afferent neurons with cell bodies located in the nodosa ganglia lying just below the jugular foramen. The vagal afferents acting via the brain stem are believed to mediate both nonnoxious physiological sensations such as satiety and nausea but also inhibitory influences on spinal nociceptive transmission. The vagal motor pathways from the nucleus ambiguus have efferents to the striated musculature of the upper GI tract and the dorsal motor nucleus is the source of efferents to the smooth muscle region of the gut. The sacral parasympathetic innervation projects from preganglionic neurons located in the intermediate grey matter of spinal cord segments S1-S5 to pelvic ganglia. Postganglionic pelvic nerve fibers from pelvic ganglia then innervate the enteric ganglia. The sacral parasympathetic innervation is involved in the spinal reflexes that regulate colonic motility and defecation (139).

The sympathetic part of the ANS, which is only efferent, has their preganglionic (cholinergic) neurons which originate from the intermediate gray region of the thoracolumbar spinal cord and terminates in the spinal ganglia. The postganglionic (noradrenergic) neurons to the stomach, small intestine and proximal colon are located in the superior celiac mesenteric ganglion. The distal colon is innervated by the inferior mesenteric ganglion and the rectum is innervated by the pelvic ganglion (139).

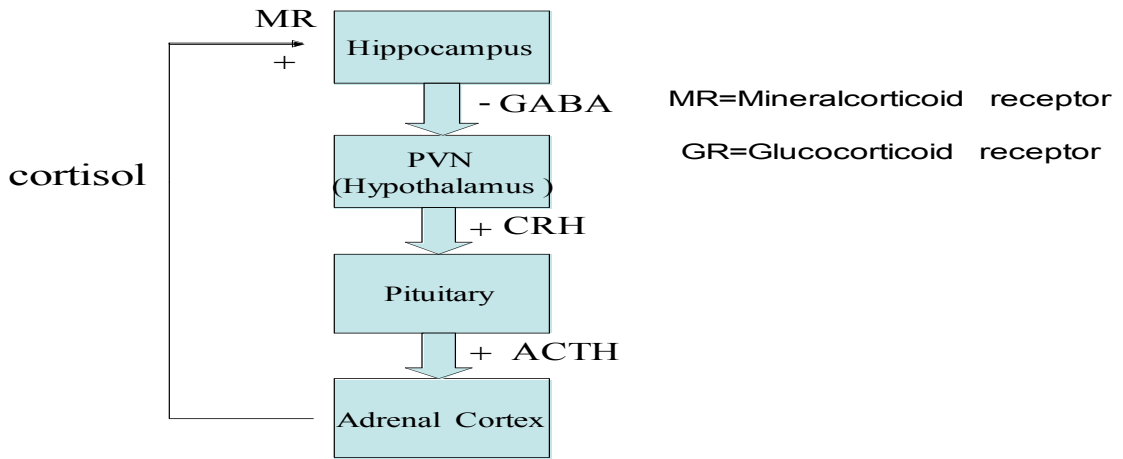
The ANS is a link between the CNS and the enteric nervous system (ENS) and influences gastrointestinal motility, secretion and gut immune system (163). Impairments of the autonomic regulation are likely to play an important role in gastrointestinal symptoms and diseases. For example, diabetes mellitus, Parkinsonism and amyloidosis are examples of diseases where autonomic neuropathy causes pronounced gastrointestinal symptoms (163-165). Also, both adrenergic and cholinergic dysfunction is suggested to be involved in the pathophysiology of IBS (22, 166-169). During acute stress, the ANS provides a rapid response to the body including the gut (138). The central activation of the sympathetic branch of ANS mainly originates from CRH neurons and catecholaminergic neurons of the locus ceruleus. For example, CRH enhances the firing rate of noradrenergic neurons in the locus ceruleus in a dose-dependent manner (170). In addition to activation of sympathetic pathways various acute stressors produce a characteristic biphasic pattern of parasympathetic activation, consisting of gastrovagal inhibition and activation of sacral parasympathetic output (138, 140), which all together contribute to the characteristic stress-induced slowing of gastric emptying, the increase in distal colonic motility and the acceleration of intestinal transit. There are also bidirectional projections between the PVN and locus ceruleus and there is an integrated activation of both HPA axis and ANS during stress. However, the systems are separated from another, suggesting variability in the recruitment of the systems in response to stress (138, 140).

## **Hippocampal-hypothalamic-pituitary-adrenal axis**

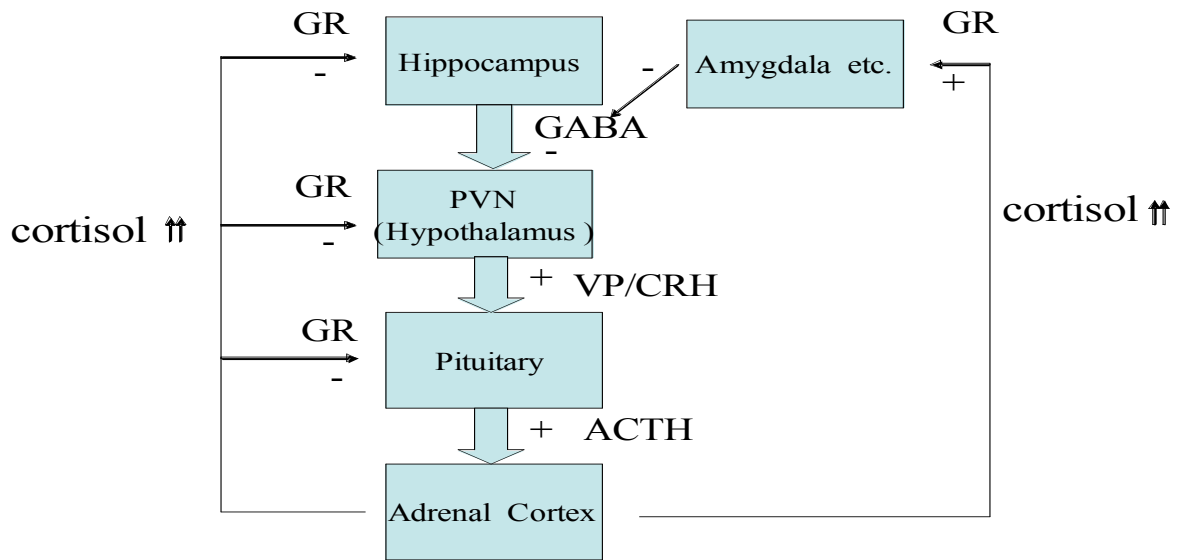
The hypothalamic-pituitary-adrenal axis (HPA axis) and its hormones; CRH, ACTH and the final effectors, the glucocorticoids (GC) are essential for the stress response and control of the body homeostasis. Neurons in the paraventricular nucleus (PVN) of the hypothalamus, express CRH with its cosecretagogue vasopressin, and other neuropeptides that drive the activity of the sympatho-adrenomedullary systems and the HPA system (138, 159, 171). As mentioned above, these two systems are not separate entities but exert control over each other's activity (138, 159). The HPA system is slower but more persistent in its actions than the sympatho-adrenomedullary system (159, 172). Like other components of the stress system, glucocorticoids display two modes of operation, a "proactive" and a "reactive" mode. In the "proactive" mode, the glucocorticoids maintain basal activity of the HPA system and control the sensitivity or threshold of the system's response to stress. It promotes coordination of circadian events, such as the sleep/wake cycle and food intake and is involved in processes underlying selective attention, integration of sensory information and response selection (159). In the "reactive" mode, the peripheral GC response to stress, in parallel with the sympathetic response plays a prominent role in suppression of inflammation (140), but also glucocorticoid feedback after a transient time delay helps to terminate the stress-induced HPA activation and thereby helps an individual to cope with, adapt to, and recover from stress. Thereby it restrains defense reactions to stress, which would themselves become damaging if left uncontrolled and redirects metabolism to meet the energy demand during stress (159).

Cortisol action in the brain is mediated by two different types of intracellular receptors, glucocorticoid receptors (GRs) and mineralcorticoid receptors (MRs). MRs which are most abundant in hippocampus bind cortisol with high affinity and have major influence during basal conditions when glucocorticoid levels are low, whereas hippocampal GRs as well as GRs in other parts of the brain (i.e. amygdala) have low affinity to cortisol and are activated during stress and the circadian peak (159, 172-173) (Figure 5).

In general, during basal conditions the MR activation results in an inhibitory tone (GABAergic) of the HPA axis, while GR activation during acute stress depresses hippocampal output and activates HPA axis. Therefore the stress-induced activation of the HPA axis results in two contrary steroid actions on the PVN. First, negative feedback at the PVN level depressing HPA axis, and second, via decreased inhibitory hippocampal output a stimulatory effect on the



**Basal levels of cortisol**



**Cortisol rise during stress/ circadian peak**

Figure 5 Hippocampus-HPA axis during basal condition and stress

HPA axis (159). When a normal adaption to stress occurs, the negative feedback action via GRs in the PVN and neural inhibitory circuits overrides the excitatory extrahypothalamic influences on the HPA axis, whereas when a maladaptive response to stress occurs excitatory ascending aminergic and amygdaloid CRH systems may override the inhibitory influences (159, 171). A maladaptive stress response can be seen in various chronic disease states such as psychiatric, endocrine/metabolic, and/or autoimmune diseases or vulnerability to such diseases (138, 140). Depression is the disease with the most characteristics of a hyperactive HPA axis with about 50% of the depressed patients showing a

hyperactive HPA axis. In these patients CRH and vasopressin expression in the PVN is enhanced, the adrenals show hypertrophy, the basal cortisol levels are elevated and in the response to stress the HPA activation is both sluggish and persistent (159, 171, 173). Depressed patients show feedback resistance at the level of PVN and pituitary (173). Also, 50% of patients with Cushing syndrome suffer from depression and they improve their psychopathology after correction of the hypercortisolism (159, 173). There is evidence that healthy individuals at genetic risk for depression also have a mild hypercortisolism and feedback resistance as well (174).

The reverse situation with an enhanced feedback inhibition, resulting in a hypoactive HPA axis with resulting low cortisol levels have also been described. The mechanism for this phenomenon is not known (159). Long standing or chronic repetitive stress has in rats been associated with a gradual decrease in basal corticosterone levels implicating a time course in the development in HPA axis dysfunction with initially hyperactivity and later hypoactivity (175). Moreover, it has been suggested that hypercortisolism is obligatory for developing hippocampal atrophy, and that diseases with hypocortisolism and a small hippocampus, such as post traumatic stress syndrome have initially been caused by hypercortisolism (159, 176).

Why some individuals suffer from stress related brain pathology and others do not, is not known. One interesting observation is the pronounced individual differences in HPA activity seen (also in humans) (159, 171). Genetic factors and early life events appear to play a major role in exerting an influence upon individuals towards divergent emotional and adrenocortical reactivity in later life (159). Animal studies have shown that duration, frequency of stress exposure (separation from mother) and age when exposed determine the effects upon setting of the HPA-axis activity and the expressed receptor profile in adult life (159, 177).

The data on HPA axis function and IBS is sparse and conflicting (127, 178). Higher basal unstimulated cortisol levels have been shown in patients with IBS (179-182) but during CRH challenge both blunted (178) and exaggerated (25, 127) ACTH and serum cortisol responses have been reported.

### **Changes in pain modulation**

Some patients with IBS show an abnormal endogenous pain modulation (183). Pain perception depends on sensory, emotional and cognitive functions (141). The anterior cingulate cortex (ACC) is an important area in the brain which integrates visceral, attentional and emotional information. The ACC also regulates affects, arousal and by sending inhibitory efferent signals, directly and/or indirectly, via the amygdala and via pontomedullary networks that modulates pain signals (141). The endogenous pain inhibitory system, including ACC networks acts via descending opioidergic, serotonergic and noradrenergic systems which results in presynaptic inhibition of afferent pain signals in the

dorsal horn of the spinal cord (141). Interestingly, IBS patients show increased activation of the dorsal subdivision of ACC after rectal distention, which indicates a greater attentional attribution to visceral stimuli but decreased antral ACC activation in response to anticipated pain (141, 184). The latter suggests possible deficiencies in the cortical activation of the endogenous pain inhibitory system (95, 184-186). Also, cognitive therapy and low dose antidepressants may improve IBS symptoms by diminishing the inhibitory effects of stress on ACC function (141, 187). Whereas acute stress induces analgesia to somatic pain by descending pain inhibitory pathways (glutamatergic, serotonergic and opioidergic), the influence of chronic stress on modulation of visceral pain may instead result in visceral hyperalgesia (140). In analogy to this, patients with IBS show visceral hyperalgesia but also some evidence of cutaneous hypoalgesia (40, 42, 140, 188-189). In chronic pain associated with depression, a deficiency in noradrenergic and serotonergic pain inhibitory signals has been proposed (190). Also, antidepressants with dual action on noradrenergic and serotonergic reuptake displayed effectiveness on chronic pain (190, 191-192). In rats, early life events are associated with heightened colonic visceral hypersensitivity in adult life (193).

The endogenous opioid neurotransmitters (for example enkephalin peptides) and  $\mu$ -opioid receptors are centrally implicated in responses to stress, in the suppression of pain, and in the action of opiate analgesic drugs (194). In animals chronic activation of the dopaminergic-2 receptor, reduces the activity of the  $\mu$ -opioid system (185). Also in humans, polymorphism in the gene coding for catechol-o-methyltransferase (COMT), an enzyme that metabolizes catecholamines, thereby acting as a key modulator of dopaminergic and adrenergic/noradrenergic neurotransmission, is associated with differences in the capacity to activate the brain  $\mu$ -opioid system in response to sustained pain stimuli (see below) (195).  $\mu$ -Opioid receptor regulation of noradrenaline release has also been described in the dorsal ACC and amygdala. Endogenous opioids and dopamine are believed to be one of the neurobiological mechanisms of the placebo effect on mood and behaviour by modulating the HPA system (194-199). Chronic activation of HPA axis and locus ceruleus/noradrenergic system may deplete the opioid-induced analgesia leading to lower pain thresholds for visceral pain (138). For example, proinflammatory cytokines which stimulate CRH production in the CNS have been associated with hyperalgesia (200). In addition, glucocorticoids via GRs raise and MRs lower thresholds for sensory stimuli and dysfunctional GRs might be involved in hampered filtering of visceral afferent signals (172).

There are sex differences in the activation of the  $\mu$ -opioid system. Males have higher activity in the anterior thalamus, ventral basal ganglia and amygdala whereas females have lower activity in the nucleus accumbens (an area associated with hyperalgesia) (201), which may partly explain the higher prevalence of chronic pain syndromes in women (202).

## **Val158Met Catechol-o-methyltransferase (COMT) polymorphism**

Catechol-o-methyltransferase (COMT) is a key regulatory enzyme in the degradation of catecholamines (dopamine, noradrenaline and adrenaline). A polymorphism in the gene coding for COMT is responsible for a variation of function of these neurotransmitters among individuals. A low COMT activity leads to high levels of dopamine and chronic activation of dopaminergic neurons which results in lower neuronal content of enkephalin and a decreased endogenous pain inhibitory system. The opposite is true with a high COMT activity (195). A common polymorphism in the COMT gene is val158met, which causes a valine (val) substitution to methionine (met) at codon 158. The val/val genotype exhibits a three- to fourfold higher activity of the COMT enzyme than the met/met genotype, and the heterozygous val/met genotype shows intermediate activity (195).

Chronic overactivity of catecholamines is associated to pain and blocking COMT in rats increases pain sensitivity possible by increased activation of  $\beta$ 2- and  $\beta$ 3-adrenergic receptors (203). Zubieta et al showed that individuals with the COMT met/met genotype have diminished regional  $\mu$ -opioid receptor activity, higher sensory and affective ratings of pain in response to painful stimuli (hypertonic saline infusion into the masseter muscle) compared to the val/val individuals (195). However, cancer patients with met/met genotype seem to require lower doses of morphine than val/val individuals. This might be explained by an increased amount of regional  $\mu$ -opioid receptors, compensatory upregulated in response to lower content of enkephalin within the peripheral neurons of these individuals (204).

Low COMT activity has been associated to chronic pain conditions such as facial pain (205-206), fibromyalgia (207) and women with non-migraineous headache (208). However, there have also been some negative results. In a sample of a normal population with different musculoskeletal symptoms (209) and in patients with neuropathic pain (210) no association to the val158met COMT polymorphism was found. Also, a human experimental study with application of short cutaneous thermal stimuli, did not show any predictive influence on pain sensitivity by the COMT genotype (211).

Diatchenko et al suggested that the val158met SNPs (single nucleotide polymorphism) mostly play a role in centrally mediated temporal summation of pain and that other SNPs in the COMT gene is responsible for variation in resting nociceptive sensitivity (212).

An association between COMT and HPA axis function has also been postulated. One study on healthy volunteers showed that individuals with the COMT met/met genotype had a more pronounced ACTH and cortisol response after naloxone administration than individuals with the val/val genotype (213).

Polymorphism in COMT val158met has also been associated to anxiety disorders but only restricted to female patients (214-215). Interestingly, there are also different effects within different ethnical groups. For example, Asian

females with panic disorders were associated with the met allele whereas Caucasian females were associated to the val allele (214).

So far, there are no studies aimed at investigating a potential association between the val158met COMT polymorphism and gastrointestinal symptoms except for a Norwegian study of a normal population that did not find any association between chest/abdominal pain and the val158met COMT genotype (209). Gastrointestinal symptoms such as nausea, diarrhea, abdominal pain and constipation are common side effects of the COMT inhibitors tolcapone and entacapone (216-217) and the concomitant feature of stress (CRH and catecholamines) and visceral hypersensitivity in patients with functional gastrointestinal symptoms (26, 170) may suggest a link between COMT and gastrointestinal symptoms.

## **AIMS**

The general aim of this work is to explore the influence of the emotional motor system (EMS) on gastrointestinal symptoms in humans.

### **Specific aims:**

To elucidate factors which are associated to gastrointestinal symptoms in patients with an established diagnosis of unipolar depression.

To test the function of the autonomic nervous system (ANS) by spectral analysis of heart rate variability, in patients with IBS compared to healthy controls.

To test if abnormal HPA axis suppression is associated to gastrointestinal symptoms in a sample representing a normal population

To explore the impact of the val158met COMT polymorphism on IBS/IBS-like symptoms.



## PATIENTS AND METHODS

### Study cohorts (paper I-IV)

The subjects who participated were patients with an established diagnosis of unipolar depression (I), patients with IBS (II, IV) and controls (I-IV) (Table I)

The study cohort of patients with depression consisted of 95 subjects, all with an established diagnosis of unipolar depression according to DSM-IV criteria (218) and without other major psychiatric co-morbidity or abuse. They were diagnosed by a semistructured interview by a trained psychiatrist, with at least two previous verified and medically treated major depressive episodes. The mean age at onset of depression was 38 +/- 15 years, and the majority of the patients had suffered from three or more major depressive episodes. All patients were on stable medical treatment for the last three months.

Two different groups of patients with IBS participated, all with a diagnosis of IBS according to Rome II (paper II) or Rome III (paper IV) criteria. All were recruited from our gastroenterology clinic. In paper II 18 IBS patients (14 women, 4 men; mean age, 31.6 years) were included and in paper IV 78 (67 women, 11 men; mean age, 33.4 years). In paper II the majority of the IBS patients had refractory symptoms and was on the waiting list for hypnotherapy, whereas in paper IV both refractory patients and first consulters for IBS were included.

Table I. Participants in the different papers.

Paper	Patients with unipolar depression	Patients with IBS (Group 1)	Patients with IBS (Group 2)	Betula controls	Healthy volunteers
I	X			X	
II		X			X
III				X	
IV			X	X	

The control subjects in the val158met COMT polymorphism study (paper IV) (n=308), the sex and age matched controls (n=190) to the patients with depression (paper I) and the subjects in paper III (n=157) were part of the Betula study, which is a prospective study in a normal population investigating memory, health and aging (219-222). The participants of the Betula study were recruited by random selection through a population register. The representativity towards the normal population of the region of Umeå, Sweden of the sample of

the Betula study has been demonstrated to be excellent. The sex and age matched subjects to the patients with IBS in study II were recruited from healthy volunteers, mostly hospital staff.

All subjects who were included in paper I, III-IV were interviewed for present medications and a routine clinical chemistry laboratory screen was performed. All subjects with abdominal surgery within three months before and after the survey, and all with established gastrointestinal diseases were excluded. However, patients with gastroesophageal reflux, functional dyspepsia and IBS were not excluded. All subjects on peroral or inhalation steroid therapy (only in paper III) or antibiotics (not in paper IV) were excluded as well as participants with cognitive dysfunction or dementia. Subjects on beta-blockers, calcium antagonists, statins, antidepressants, pain medication including non-steroidal anti-inflammatory medications were not excluded.

Table II. Mean age and sex distribution for the available subjects in the different study groups.

	<b>Unipolar Depression (I) (n=73)</b>	<b>Controls (I) (n=144)</b>	<b>IBS (II) (n=18)</b>	<b>Controls (II) (n=36)</b>	<b>Controls (III) (n=124)</b>	<b>IBS (IV) (n=78)</b>	<b>Controls (IV) (n=308)</b>
<b>Mean age</b>	63.6	59.9	31.6	31.4	55.8	33.4	55.6
<b>Women</b>	60 %	58 %	78 %	78 %	48 %	86 %	51 %

### **Questionnaires (paper I, III-IV)**

The Gastrointestinal Symptom Rating Scale-IBS (GSRS-IBS) is a validated self-assessment instrument to assess symptoms of IBS (223). The GSRS-IBS questionnaire includes 13 items, each using a Likert scale (0-6 points). The items are grouped into symptom clusters; abdominal pain (two items), bloating (three items), constipation (two items), diarrhea (four items) and satiety (two items). There are not yet a defined cut-off level for having IBS when applying the GSRS-IBS questionnaire, therefore we used the median total GSRS-IBS score (the sum of all 13 items score) to investigate factors associated with IBS-like symptoms in patients with unipolar depression. The upper quartile of total GSRS-IBS score was used as cut-off level for control subjects. In addition, but not included in the total GSRS-IBS score, we used five questions from the former Gastrointestinal Symptom Rating Scale (GSRS), concerning symptoms of gastroesophageal reflux (two items) and dyspepsia (three items) (224). Moreover, the patients with IBS in paper IV prospectively filled in a two week validated IBS-symptom diary (225).

The Hospital Anxiety and Depression Scale (HADS), developed by Zigmond and Snaith in 1983 (226) is a highly sensitive instrument to screen for symptoms

of anxiety and depression among patients with somatic diseases (227-228). It consists of 7 items each for anxiety and depression, each using a 4-point Likert scale (0-3 points). We used the HADS scale because it has high sensitivity in detecting symptoms of anxiety and depression; it is well validated; and is simple to fill in which facilitates a higher response rate. The cut off level of 15 points of total HADS score and 8 points in HADS-D (HADS-Depression) is used to separate symptoms of possible anxiety/depression and was used to define patients with unipolar depression in remission (HADS-D <8).

### **Medical records (paper I, III-IV)**

After informed consent from the subjects records of primary care, clinics of surgery (including endoscopy unit), infectious diseases and psychiatry (not controls) were searched twice for exclusion criteria. In the area of Umeå, Sweden, all patients who attend gastroenterology out-patient clinic are referred by a general physician. Blinded by the result of GSRS-IBS and HADS questionnaires, the records of primary care health centers were investigated twice and ten years back in time to define consulters for functional bowel disorders, chronic pain and symptoms of fatigue and insomnia. Consulters for gastrointestinal symptoms were defined by diagnosis of a functional bowel disorder judged by their general physician or symptoms according to Rome III criteria (3). Consulters for chronic pain were defined as pain more than six months, both explained (for example arthrosis) and unexplained pain was included. Ten areas of pain were defined: Head, neck, thoracal and lumbar spine, shoulder, arm, hand, hip, knee and foot. Consulters for fatigue and insomnia were defined by recurrent consulting (two or more times) or chronic symptoms (more than three months).

### **Functional test on HPA axis suppression (paper III)**

To measure HPA axis suppression we used a weight adjusted low dose dexamethasone suppression test (DST) (III). Dexamethasone and synthetic steroids predominantly block stress-induced HPA activity at the pituitary level (157). On visit one, serum cortisol was measured between 0800 h and 0900 h. Visit two was scheduled 7 days (+/- 6 days) after visit one. The night before visit two at 2300 h, all participants ingested at home, 3.5 microgram per kg body weight of dexamethasone phosphate (229). The post-dexamethasone serum cortisol was drawn between 0800 h and 0900 h the following day. Serum cortisol levels were analysed in one accredited laboratory by Roche Elecsys reagents on Modular E170 analyzer (total coefficient of variation <10%). Moreover, in 86 healthy subjects also a plasma dexamethasone sample was taken at visit two. The plasma dexamethasone was analyzed to ensure compliance (unpublished observations).

In the premenopausal female participants within the studies (21 subjects in paper III), blood cortisol samples were randomly taken during the menstruation cycle. The post-dexamethasone cortisol was used for studying HPA axis suppression. To explore a non-linear relationship between post-dexamethasone morning cortisol and gastrointestinal symptoms, we subdivided subjects into the upper and lower 25<sup>th</sup> percentile based on their post-dexamethasone cortisol levels and compared those two groups with the subjects exhibiting intermediate post-dexamethasone cortisol levels.

### **Test of ANS function : Short term heart rate variability (paper II)**

Spectral analysis of short term heart rate variability (HRV) was used to test ANS function. The patients and controls, who did not have neurologic disorder or cardiac arrhythmia, were told not to exercise, not to smoke, drink coffee or tea, and to avoid drugs that might affect ANS function on the day of study. After 10 min of rest, an electrocardiogram (ECG) was taken, and blood pressure and respiratory rate were measured while the patients were lying supine on a tilt table, initially breathing normally. The ECG recording then continued when the patients breathed following a metronome at 12 breath per minute in supine position, and then during and for 3 min after passive tilt to a 70 degrees head-up position, at which point blood pressure was taken and ECG recording stopped. ECG data were digitized at 500 sample/seconds on a computer. R waves were detected by using an algorithm on the first differences on ECG (230). The immediate heart rate series were calculated as reciprocals of R-R intervals. The heart rate was interpolated using cubic splines (231), and the resulting wave form was resampled at 2 Hz. All recordings were visually inspected and corrected for detected errors such as spurious or undetected R-waves. Segments with durations of 120 sec and without artefacts and ectopic beats were selected for spectral analysis. The power spectral density was estimated by auto-regressive modeling of heart rate data after the mean value had been removed. The 30 auto-regressive variables were selected in accordance with the modified covariance model (232). This means that each sample was modelled as the weighted combination of the 30 previous samples.

The high-frequency (HF) band (0.15-0.45 Hz) of heart rate variability, which at normal breathing is related to respiratory rate (233), mirrors parasympathetic activity. The mid-frequency (MF) band (0.04-0.15 Hz) of heart rate variability, which is considered to be related to baroreceptor-mediated blood pressure control, provides a useful marker of sympathetic activity (233), especially its magnitude after postural change from supine to head-up tilt position. The low-frequency band (0-0.04 Hz) of heart rate variability, which is attributed to several physiologic variables, such as changes in thermo-regulation and in the renin-angiotensin system, was not considered to be of interest in our study.

### **Val158Met COMT polymorphism (paper IV)**

Genotyping of the functional polymorphism for COMT was performed by pyrosequencing on a PSQHS96 pyrosequencer (Pyrosequencing AB, Uppsala, Sweden). Biotinylated polymerase chain reaction (PCR) products were immobilized onto streptavidin-coated sepharose beads (American Biosciences, Uppsala, Sweden). Biotinylated single strand DNA was obtained by incubating the immobilized PCR products in 0.5 mol/L NaOH followed by two sequential washes in 10 mmol/L Trisacetate, pH 7.6. Primer annealing was performed by incubation at 80 degrees Celcius for 2 minutes and then at room temperature for 5 minutes (234). There was in total 12 (2 IBS and 10 controls) missing values during the work-up (Figure 1).

### **Statistics (paper I-IV)**

We used SPSS version 12 (paper I, III) and 15 (paper IV). Non-parametric test were used for comparing ordinal scales and continuous variables (Mann-Whitney test (paper I, IV), Kruskal-Wallis test (paper III, IV)) and for correlation analyses (Spearman test) (paper I, III-IV). The Chi square test was used for crosstable analyses (paper I, II-IV) and Student's t-test was used for comparisons between groups (paper II). A logistic regression was used for adjusting for possible confounders (paper III-IV). In the literature; age, sex, body mass index (BMI) and depression have been shown to influence serum cortisol and were used as covariates (paper III) (171, 235). After logit analyses, BMI and age was not appropriate for being a continuous variable and were instead categorized. Age was categorized into two groups in paper II, IV ("cut-off" 65 years and 50 years respectively), and post-dexamethasone cortisol in three groups in paper III ("cut-off" lower and upper quartile). BMI was categorized into two groups ("cut-off" for overweight: BMI 25). HADS was treated as a continuous variable. A p-value less than 0.05 was regarded significant. Means and standard deviations were used for continuous variables and medians for ordinal variables. Bonferroni compensation was applied in paper I

### **Ethics (paper I-IV)**

All four studies were approved by the local committee for human ethics, Umeå University, and all patients and control subjects gave an informed consent to participate.

## RESULTS AND COMMENTS

### Anxiety/depression and gastrointestinal symptoms (paper I, IV)

The prevalence of anxiety and/or depression has been extensively studied in patients with IBS who attend gastroenterologic clinics, with prevalence figures in different studies between 33-59% (113-122). Therefore, not surprising the patients with IBS in our study had higher HADS scores (especially anxiety score) than controls (Table III). In patients with IBS there is evidence that symptoms of anxiety and depression present before or start at the same time as IBS symptoms evolves (62, 115, 119, 236-237). Whether the GI symptoms in patients with IBS resolves when symptoms of anxiety and depression disappear is not known. Anxiety and depression have instead been proposed to be associated with a more severe form of IBS with worse prognosis (121-125) even though anti-depressants in low doses have displayed effect on quality of life and IBS symptoms in some patients (136).

Table III. Patients with IBS and subjects with high scores for IBS-like symptoms (controls or patients with unipolar depression) all have higher HADS, HADS-anxiety and HADS-depression score. The patients with IBS and subjects with unipolar depression are compared to the total control group (n=270), whereas controls with high IBS-like symptoms are compared to controls with low IBS-like symptoms. Statistics: Mann-Whitney test.

	IBS (IV) (n=76)	Unipolar Depression High IBS score (I) (n=35)	Unipolar Depression Low IBS score (I) (n=38)	Controls High IBS score (IV) (n=67)	Controls Low IBS score (IV) (n=203)
Mean age	33.4	62.8	64.2	53.0	56.4
Women	86 %	69 %	53 %	60 %	48 %
Total GSRS-IBS score	34.5 (p<0.001)***	28.0 (p<0.001)***	3.0 (p=0.003)**	21.0	4.0
Total HADS score	12.0 (p<0.001)***	19.0 (p<0.001)***	4.5 ns	8.0 (p<0.001)***	5.0
HADS-Anxiety	8.0 (p<0.001)***	8.0 (p<0.001)***	3.0 ns	5.0 (p<0.001)***	3.0
HADS- Depression	4.0 (p=0.002)**	8.0 (p<0.001)***	2.5 ns	4.0 (p=0.001)**	2.0

On the other hand, it has also been argued that the higher prevalence of anxiety and depression in patients with IBS is due to an increased health seeking behaviour rather than a true association between IBS and anxiety/depression per se (126-127). There have been relatively few studies exploring gastrointestinal symptoms in patients with affective disorders, but there are data that points towards increased IBS-like symptoms in patients with anxiety, depression and dysthymia (128-132). In our study (paper I) we also found higher gastrointestinal symptom scores in patients with an established diagnosis of unipolar depression. But interestingly, the subjects with unipolar depression in remission, even though they had consulted primary care for IBS more frequent, did not differ from control subjects in IBS-like symptoms, indicating that when the depression resolves the IBS-like symptoms also resolves (Figure 6, 7).

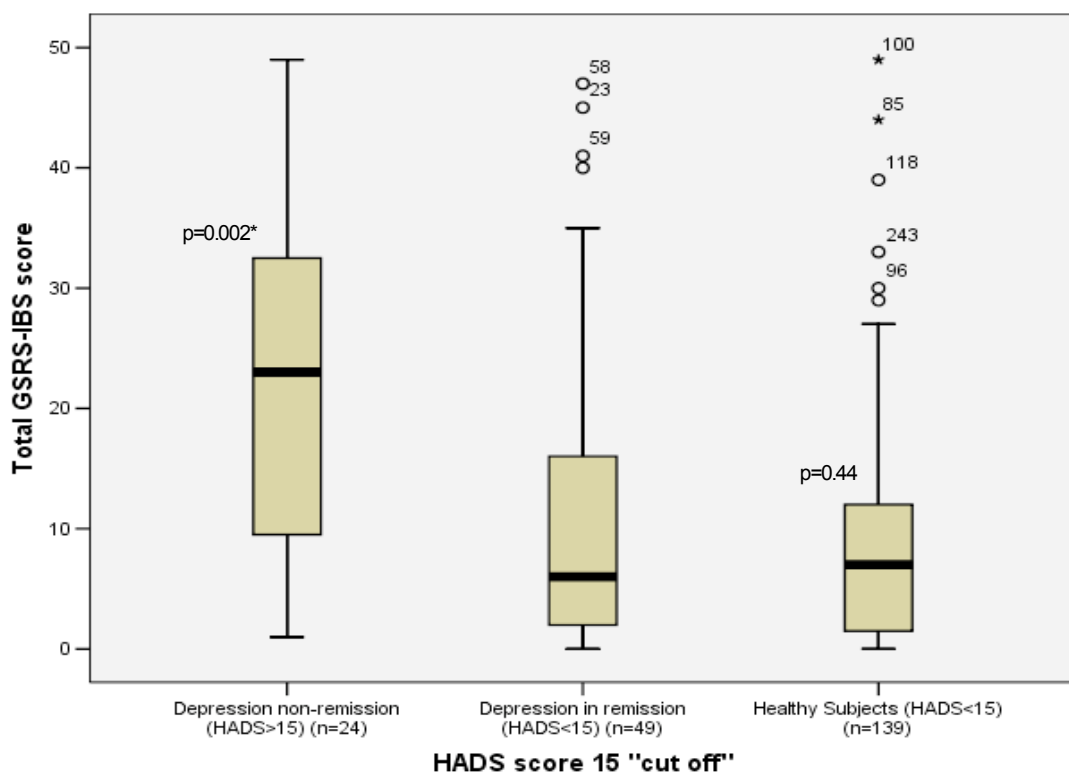


Figure 6. IBS-like symptoms in patients with unipolar depression in remission compared to patients with active depression and to control subjects.

Also “primary care IBS patients” and “non-patient” IBS in our study (paper IV) had higher HADS score than subjects with low IBS-like symptoms which also is supportive of a “true relationship” between anxiety/depression and IBS. Some studies claim that psychological disturbances are more prevalent in patients with IBS attending a gastroenterologic clinic compared to non-patients or IBS patients attending primary care (238-241) but other studies suggest no differences in neuroticism and psychiatric comorbidity (242-245). It has also been argued that non-patient IBS show less psychological denial and better

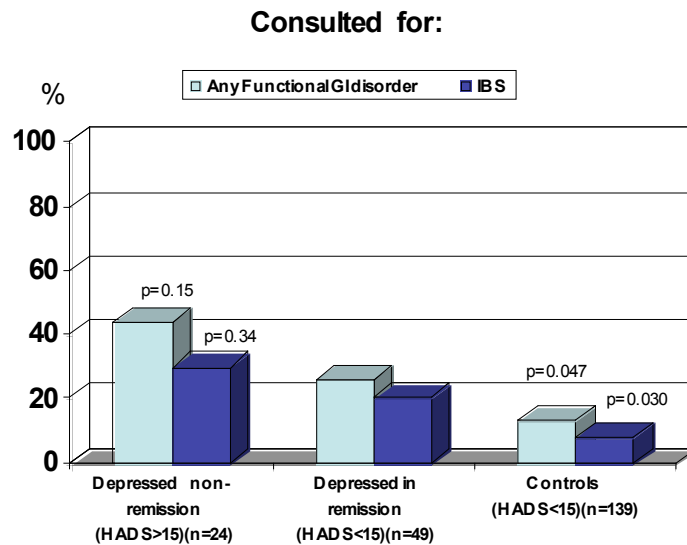


Figure 7. Consulting frequency for functional gastrointestinal disorders during a ten year period in patients with unipolar depression in remission compared to patients with active depression and to controls

ability to accept a link between psychological stress and the gut, whereas IBS patients at a gastroenterologic clinic attributed their complaints to somatic causes (238, 246).

We also investigated the correlation between anxiety/depression and different gastrointestinal symptoms (paper I) (Table IV). We found that HADS-score for patients with unipolar depression significantly correlated to total GSRS-IBS score in all symptom clusters, except constipation and gastroesophageal reflux. All gastrointestinal symptoms correlated slightly more with anxiety than depression except constipation which correlated more to depression. Also, patients with unipolar depression who used anxiolytics in our study tended to have more IBS-like symptoms. In addition, but not presented in the papers, there were highly significant correlations between HADS score and different gastrointestinal symptoms among the control subjects, which was most pronounced for anxiety. Being on the upper level of GSRS-IBS scale the correlations were understandably smaller among patients with IBS. However we found that abdominal pain and diarrhea correlated to anxiety (unpublished). In IBS patients there was also significant correlations between dyspepsia and anxiety as well as depression. The combination of IBS and functional dyspepsia is more common among women (247), is associated with increased visceral hypersensitivity (247), increased saliva cortisol (179) and in analogy with our results, also is associated to anxiety/depression (248-249).

To conclude, the overall strongest correlations in all the study groups were between anxiety and abdominal pain as well as between anxiety and diarrhea. A



possible common mechanism involved in the symptoms of anxiety and diarrhea/abdominal pain is direct and indirect effects (via ANS) of CRH (138) (see below). For example, CRH<sub>1</sub> receptors in amygdala mediate anxiety symptoms and the same receptor in colon mediates increased GI motility and visceral sensitivity (161). In patients with IBS LPS-induced TNF- $\alpha$  production (in vitro) correlates significantly with anxiety (55) and inflammation may also play a role in brain-gut interactions. Lastly, having abdominal pain, diarrhea or even worse, fecal incontinence have severe impact on psychosocial life (250), so gut function may deteriorate psychological status as well.

Table IV. Correlations between Hospital Anxiety and Depression Scale (HADS) score and different gastrointestinal symptoms in patients with recurrent depression (n=73), in patients with IBS (n=77) (**in bold**) and control subjects (n=283) (*in italic*). GSRs-IBS =Gastrointestinal Symptom Rating Scale for irritable bowel syndrome.

\*\*\* p<0.001, \*\* p<0.01, \*p<0.05

	HADS score $r_s$ (p-value)	HADS-Anxiety $r_s$ (p-value)	HADS-Depression $r_s$ (p-value)
Total GSRs-IBS score	0.537 (<0.001)*** <b>0.205 (0.074)</b> <i>0.400 (&lt;0.001)***</i>	0.518 (<0.001)*** <b>0.216 (0.059)</b> <i>0.422 (&lt;0.001)***</i>	0.470 (<0.001)*** <b>0.133 (0.247)</b> <i>0.260 (&lt;0.001)***</i>
Abdominal pain	0.463 (<0.001)*** <b>0.178 (0.122)</b> <i>0.301 (&lt;0.001)***</i>	0.479 (<0.001)*** <b>0.220 (0.055)</b> <i>0.378 (&lt;0.001)***</i>	0.382 (<0.001)*** <b>0.106 (0.360)</b> <i>0.170 (0.004)**</i>
Bloating	0.458 (<0.001)*** <b>0.127 (0.272)</b> <i>0.371 (&lt;0.001)***</i>	0.436 (<0.001)*** <b>0.134 (0.245)</b> <i>0.378 (&lt;0.001)***</i>	0.396 (<0.001)*** <b>0.076 (0.510)</b> <i>0.252 (&lt;0.001)***</i>
Constipation	0.281 (0.016)* <b>0.120 (0.298)</b> <i>0.235 (&lt;0.001)***</i>	0.184 (0.120) <b>0.076 (0.512)</b> <i>0.295 (&lt;0.001)***</i>	0.307 (0.008)** <b>0.164 (0.155)</b> <i>0.089 (0.135)</i>
Diarrhea	0.509 (<0.001)*** <b>0.131 (0.256)</b> <i>0.357 (&lt;0.001)***</i>	0.549 (<0.001)*** <b>0.210 (0.067)</b> <i>0.378 (&lt;0.001)***</i>	0.400 (<0.001)*** <b>-0.020 (0.865)</b> <i>0.226 (&lt;0.001)***</i>
Satiety	0.458 (<0.001)*** <b>0.174 (0.130)</b> <i>0.293 (&lt;0.001)***</i>	0.462 (<0.001)*** <b>0.123 (0.288)</b> <i>0.278 (&lt;0.001)***</i>	0.443 (<0.001)*** <b>0.179 (0.120)</b> <i>0.189 (0.001)**</i>
Gastroesophageal reflux	0.235 (0.047)* <b>0.195 (0.092)</b> <i>0.243 (&lt;0.001)***</i>	0.263 (0.026)* <b>0.147 (0.206)</b> <i>0.220 (&lt;0.001)***</i>	0.158 (0.158) <b>0.224 (0.052)</b> <i>0.190 (0.001)**</i>
Dyspepsia	0.456 (<0.001)*** <b>0.315 (0.006)**</b> <i>0.272 (&lt;0.001)***</i>	0.463 (<0.001)*** <b>0.315 (0.006)**</b> <i>0.273 (&lt;0.001)***</i>	0.412 (<0.001)*** <b>0.248 (0.031)*</b> <i>0.195 (0.001)**</i>

## **The autonomic nervous system (ANS) and IBS (paper II)**

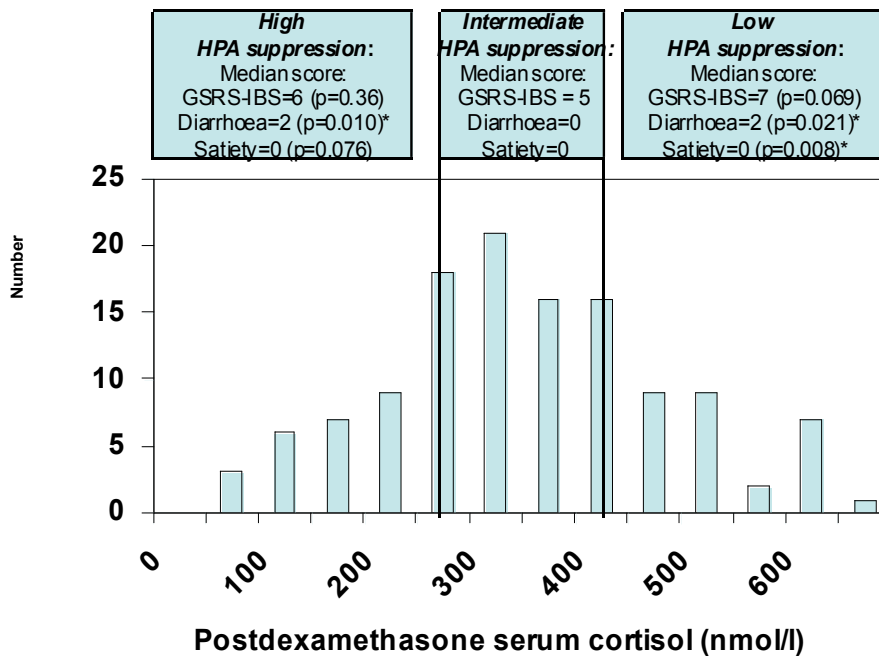
Stress has been associated to disturbances in gut motility and sensitivity and ANS plays a central role in the influence of the bodily response to stress and reacts within seconds after a possible threat to the homeostasis (138, 140). As early as 1928 Bockus et al suggested that IBS is caused by an imbalance of the autonomic nervous system (166). In the 1980s and 1990s several methods have been used to measure ANS function in patients with IBS and showed that both adrenergic and cholinergic dysfunction may be associated to IBS (22, 167-169). These studies involve techniques such as beat-to-beat heart rate and blood pressure variations in response to different stimuli (cold stress, postural changes, valsalva maneuver, deep breathing) and the collection of urinary catecholamines. However, there has been some criticism regarding lack of standardization, for being dependent on active co-operation of patients and that the tests are not representing ANS influence on gastrointestinal function (251). Modern computerized techniques have permitted the development of spectral heart rate variability (HRV) which is considered a reliable selective test of sympathetic and parasympathetic function (252). We presented the first study using short term spectral HRV on 18 patients with IBS in 1998 (paper II), and found in patients with IBS, in supine position at rest and after tilting, a significant increased MF band indicating increased sympathetic activity. 8 of 18 patients exceeded the upper 90<sup>th</sup> percentile of the control subjects for the MF band in supine position at rest. Following our study, there have been several studies using spectral analysis of HRV, both short term HRV and long term HRV in patients with IBS (180, 253-265). The majority of these studies has shown an increased sympathetic/parasympathetic ratio in patients with IBS which is in line with the result in our study (180, 253, 255-256, 258, 260-265). Vagal dysfunction was the most profound feature in these studies (180, 253, 258-259, 261-262, 265) which also earlier studies on ANS function in IBS patients have shown (169). IBS patients also demonstrate an increased postprandial MF/HF ratio but a decreased MF/HF ratio after colonic distension compared to controls (265). Also when testing gut-specific autonomic function, using rectal mucosal blood flow, both patients and controls have reduced blood flow to psychological and physical stress. But compared to controls, IBS patients showed delayed recovery in blood flow after these stressors (266). Using finger top blood flow IBS patients displayed evidence of increased sympathetic activity (267). Aggraval et al suggested that vagal dysfunction is specially associated with constipation predominant IBS and sympathetic adrenergic dysfunction associated with diarrhea predominant IBS (168). Elsenbruch et al found that ANS imbalance was most evident among diarrhea predominant IBS patients (180). However, other studies have displayed conflicting results (260, 267). In our study, the subgroups of predominant diarrhea respectively predominant constipation IBS were too small to enable comparison. A similar neurocardiac imbalance of ANS characterized by a low

cardiovagal modulation, a raised sympathetic nerve activity and a high resting heart rate is seen in patients with major depression (268). Jarret et al found that the presence of symptoms of anxiety and depression was the cause of ANS dysfunction in patients with IBS (261). But, Robert et al could not detect differences in autonomic activity during presleep period or sleep stages between IBS patients with and without depressive symptoms (257). We did not in our study evaluate the presence and influence of affective disorders or symptoms in our IBS patients, but one can assume that the presence of affective symptoms was high in this group of IBS patients referred to a tertiary centre. Recently short term HRV has been criticized for not showing accurate reproducibility and reliability, and the authors argued in favor of long term HRV (269). However, the fact that in our study we found increased sympathetic activity in both unstimulated and stimulated situations, and that later studies have shown concordant results support our HRV data. It is not known how relevant cardiac autonomic function reflects gut autonomic function. However, when studying diabetic autonomic neuropathy, there is close concordance between cardiovagal and abdominovagal dysfunction (181).

Overall, the data from our study and other studies indicates that patients with IBS have impaired ANS function. Whether it is increased sympathetic or decreased parasympathetic activity, or secondary to anxiety/depression is still unclear.

### **Hippocampal-HPA axis and IBS (paper III)**

HPA axis or Hippocampal-HPA axis play a central role in defending an individual against exteroceptive or interoceptive stressors (140, 158). It has slower onset but are more persistent than the ANS activation to stress (140). A pathological stress insult can result in dysfunction of the hippocampal-HPA axis with either an excessive CRH drive leading to hypercortisolism (i.e. in anxiety and major depression) or an “exhausted” HPA axis which leads to hypocortisolism (i.e. in post-traumatic stress disorder) (138, 159). Both CRH and stress have been associated to decreased upper gastrointestinal motility, increased lower gastrointestinal motility and increased visceral hypersensitivity (25-26), and IBS patients have been shown to have higher basal unstimulated saliva cortisol levels (179-182). We therefore explored the association between the degree of negative feedback sensitivity of the HPA axis and gastrointestinal symptoms in a control group representing a normal population. We used a weight adjusted low dose dexamethasone suppression test (DST) as a marker for the “CRH-drive”.



**Figure 8: Association Between GI Symptoms and degree of HPA-axis suppression in a sample of a normal population (paper III)**

Our data show, to our knowledge for the first time, a link between HPA suppression and gastrointestinal symptoms. Among individuals from a normal population, higher post-dexamethasone morning cortisol levels, indicating a low HPA suppression, were significantly associated to more diarrhea (adjusted OR 5.7) and early satiety (adjusted OR 6.7) symptoms compared to those subjects with intermediate post-dexamethasone cortisol levels (Figure 8). Symptoms of diarrhea and satiety have been associated with the stress response (138) and the low HPA suppression in these individuals might indicate an increased CRH drive leading to a direct or indirect influence on gastrointestinal motility. Moreover, patients with IBS-D (diarrhea predominant) have higher postprandial cortisol levels than IBS-C and controls (180). Interestingly, CRH<sub>1</sub> receptor stimulation has been demonstrated to increase colonic motility (151), but CRH<sub>1</sub> receptors are also important in mediating anxiety-like behaviour in response to stress (161). In concordance to this, the patients with unipolar disorder (paper I), patients with IBS and control subjects in our studies showed a high correlation between anxiety score and diarrhea scores. However, the control subjects in our study (paper III) when applying multivariate analysis displayed an effect of HPA suppression on diarrhea and satiety symptoms which was independent of

the influence of anxiety/depression. Therefore, it seems that excess HPA-axis activity has an independent effect on these gastrointestinal symptoms.

A maladaptive stress response, when the negative feedback action via the PVN and neural inhibitory circuits from hippocampus do not succeed to override the excitatory extrahypothalamic influences on the HPA axis, may explain the excess CRH drive and deficient HPA suppression (159, 171). A maladaptive stress response, with deficient HPA suppression/increased “CRH-drive” can be seen in various chronic disease states such as psychiatric, endocrine/metabolic, and/or autoimmune diseases or vulnerability to such diseases (138, 140). Other possible mechanisms for diminished HPA suppression could be differences in glucocorticoid receptor function (271). Chronic physical and psychological stressors have shown to produce tolerance to elevated glucocorticoids by down-regulation of GRs in the CRH/Vasopressin neurons of the PVN (272-273), leading to an increased HPA activity and hypercortisolism.

Unexpectedly, we also found a higher prevalence of low post-dexamethasone serum cortisol levels (indicating an enhanced suppression of the HPA axis) in subjects with high IBS-like symptoms (Figure 8). Low post-dexamethasone cortisol levels in our subjects could reflect low morning serum cortisol levels, with or without a higher clearance of cortisol in liver and fat tissue (235) and/or an increased inhibition of dexamethasone at the pituitary level (176). Hypersuppression of HPA axis have also been seen in atypical depression, chronic fatigue syndrome, fibromyalgia and post traumatic stress syndrome (138, 175-176); syndromes which partly are associated both to depression and IBS (191, 202). The cause of the phenomenon of HPA axis hypersuppression is not known. There may be different mechanisms of HPA axis hypofunction in different disorders (159). Synergy of GRs with intracellular signalling mechanism in the PVN, a deficit in CRH drive, down-regulation of pituitary CRH receptors or an altered sympathetic outflow diminishing adrenal sensitivity to ACTH have been suggested (159, 175, 200). A hypo- and hypersuppressive dysfunction of the HPA axis might also share the same etiology. For example, long standing or chronic repetitive stress has in rats been associated with a gradual decrease in basal corticosterone levels implicating a time course in the development in HPA axis dysfunction with initially hyperactivity and later hypoactivity (175).

Recently, studies using CRH challenge test to evaluate HPA function in patients with IBS have been presented. They show evidence of disturbances in HPA axis response but the results are conflicting (127, 178). Böhmelt et al found that patients with functional gastrointestinal disease had both attenuated unstimulated salivary cortisol levels and blunted ACTH and salivary cortisol levels after a CRH challenge test compared to healthy controls indicating a blunted HPA axis (178). Contrary, Fukudo et al and Dinan et al found both exaggerated ACTH and serum cortisol response to a CRH challenge test (25, 127). These conflicting results may reflect differences in the inclusion of

postinfectious IBS or comorbid disorders such as anxiety, depression or other functional somatic syndromes in the IBS patients recruited for the studies. For example, Dinan et al excluded patients who fulfilled DSM-IV criteria for psychiatric diagnoses, whereas Böhmelt et al did not (127, 178). However, in analogy to our results, these conflicting data may reflect a dual pathophysiology in HPA axis function in patients with IBS or a “u-shaped” relationship between HPA axis function and IBS. Also studies analysing basal unstimulated cortisol levels have shown conflicting results with both high (179, 181-182, 249) and low levels (274) in patients with IBS.

Both Böhmelt et al (178) and Dinan et al (127) also investigated the HPA axis by DST but none of the studies showed any differences in post-dexamethasone cortisol levels between patients with IBS and controls. These studies also included non-diarrhea IBS patients and might be under-powered (n=30 and n=27) to detect a non-linear relationship between post-dexamethasone cortisol levels and diarrhea symptoms. The lack of weight adjusted doses of dexamethasone, controlling post-dexamethasone cortisol levels for BMI, and differences in analyses techniques might have influenced the results (e.g. post-dexamethasone cortisol blood samples were not drawn in the morning (127) and higher dexamethasone doses (127, 178) were used).

A low-dose DST has the advantages of being both a test of hyper- and hypofunction of the negative feedback sensitivity of the HPA axis since you receive a graded/quantitative suppression in contrast to the response of complete or no suppression exhibited by the conventional high dose DST:s. Whereas the low-dose DST used in our study is more sensitive in detecting hypersuppression in conditions with enhanced feedback sensitivity (275), the combined dexamethasone/CRH test has higher sensitivity than a conventional DST in detecting a hyperactive HPA system (271, 276). Perhaps, using a combined dexamethasone/CRH test in our study we might have detected a more pronounced increased HPA drive in the individuals with IBS-like symptoms but on the other hand we might have failed to detect subjects with hypersuppression of the HPA axis. Also the higher doses of dexamethasone used in previous studies might have failed to detect individuals with HPA hypersuppression (127, 178). For future implications perhaps a combination of a weight adjusted low dose DST (3.5 µg/kg body weight) followed by a higher dose DST (for example 35 µg/kg body weight ) would be appropriate in order to first detect HPA hypersuppressors and then HPA hyposuppressor.

To conclude, we found in subjects representing a normal population associations between both a high and a low degree of HPA-axis suppression and symptoms of diarrhea and satiety, whereas intermediate HPA-axis suppression seems to be protective.

## Catechol-O-Methyltransferase (COMT) and IBS (paper IV)

Catecholamines (adrenaline, noradrenaline, dopamine) are important neurotransmitters in the stress system (i.e ANS) and in the modulation of pain (140). COMT is the first enzyme and a key rate-limiting step in the degradation of catecholamines and there are genetic individual differences in the activity of the enzyme (195). These differences have been shown to influence the experience of pain (195) and psychological symptoms like anxiety (214) and recognition of negative stimuli (277). Stress, pain and psychological features are implicated in the etiology and expression of IBS. Therefore we aimed to study the polymorphism val158met COMT in patients with IBS and in control subjects, a polymorphism with significant impact on COMT function. The val/val genotype leads to a three- to fourfold higher activity of the COMT enzyme than the met/met genotype, and the heterozygous val/met genotype shows intermediate activity (195).

We present in this thesis to our knowledge the first study on val158met COMT polymorphism and its association to IBS/IBS-like symptoms. We could not detect a significant difference between patients with IBS (n=76) and control subjects (n=298) but there was a trend for a lower frequency of the heterozygous val/met genotype compared to the other genotypes (p=0.15) (p=0.10 women only) in the IBS patients. However, in the control subjects we found that subjects with high IBS-like symptoms (defined by the upper quartile in total GSRS-IBS score) had a higher occurrence of the met/met genotype (adjusted OR: 1.92 (CI: 0.99-3.83)) and significantly lower frequency of the heterozygous val/met genotype (adjusted OR: 0.51 (CI: 0.27-0.93)) compared to individuals with low IBS-like symptoms (Table V). The heterozygous val/met genotype remained significant protective when analyzing the total study population (patients with IBS + controls) (adjusted OR: 0.54 (CI: 0.31-0.93)).

Table V. Val158met COMT polymorphism and IBS-like symptoms in a normal population. Odds ratio (OR) adjusted for age, gender, HADS-anxiety, HADS-depression and chronic pain. \* Statistically significant

	<b>met158met</b>	<b>val158met</b>	<b>val158val</b>
<b>High IBS-like symptoms (n=67)</b>	22 (33 %)  Adjusted OR: 1.92 (0.99-3.83)	30 (45 %)  Adjusted OR: 0.51 (0.27-0.93)*	15 (22 %)  Adjusted OR: 1.27 (0.61-2.65)
<b>Low IBS-like symptoms (n=203)</b>	45 (22 %)	113 (56 %)	45 (22 %)

The met/met genotype has been associated to a reduction in the activity of the pain inhibitory system and increased chronic somatic pain (195, 205-208) but to our surprise abdominal pain had very little influence on the results, whereas diarrhea and satiety were the symptoms which were significantly associated with the met158met COMT polymorphism in our study. Opposite to the hypothesis that the met/met genotype is associated to pain, we could not detect a higher health seeking behaviour for chronic somatic pain among the subjects with the met/met genotype.

In our subanalysis, the IBS patients with the val/val genotype had more IBS-D like symptoms such as increased bowel frequency and looser stool consistency. As previously discussed, the symptoms of satiety and diarrhea are consistent with the stress response (138), and our data point towards that both high and low levels of catecholamines may be a vulnerability factor to this response and indicating a “u-shaped” relationship between gastrointestinal symptoms and COMT function. A possible “U-shaped” relationship between COMT activity and outcomes has also been demonstrated in other studies. For example, antidepressant treatment is more effective in the patients with major depression with the heterozygous val/met genotype compared to the patients with met/met and val/val genotypes (278-279). Also, our results on HPA suppression (paper III), another limb in the EMS, demonstrate a “u-shaped” relationship and might indicate that “being intermediate” is favourable.

The val/val genotype has been associated to anxiety (in women) (214), to faster and better recognition of negative facial expressions (277), and a reduction in the ability to experience rewards (280). However the subjects with the val/val genotype in our study did not show increased anxiety scores and in the multivariate analysis COMT had an independent influence on gastrointestinal symptoms.

We conclude that the heterozygous val/met genotype is protective against IBS-like symptoms in a sample of a normal population and also patients with IBS tended to have a lower frequency of this genotype. Also, the met/met genotype seems to be more common among control subjects with IBS-like symptoms. In patients with IBS the val/val genotype is associated to diarrhea symptoms.

Finally, the val158met COMT is just one polymorphism together with many others which influence COMT activity (206), and further studies also need to address the effect of other “COMT polymorphisms” on functional gastrointestinal disorders.

### **Other somatic symptoms and IBS (paper I, IV)**

Patients with IBS also report a higher frequency of chronic non-gastrointestinal somatic complaints and have more frequent health-care admissions (16-17). Consistent with these observations, we found in our study that patients with IBS had in general more visits in primary care and an increased number of visits for symptoms of chronic pain and fatigue (Table VI). There is lack of studies



Table VI. Patients with IBS and subjects with high IBS-like symptoms (patients with unipolar depression or controls) do more often visit a primary care doctor for somatic complaints. Statistics: Mann-Whitney and Chi<sup>2</sup> test. Patients with IBS and patients with unipolar depression are compared to total control subjects. The controls with high IBS-like symptoms are compared to those with low symptoms.

	IBS (IV) (n=74)	Unipolar Depression High IBS score (I) (n=35)	Unipolar Depression Low IBS score (I) (n=38)	Controls High IBS score (IV) (n=67)	Controls Low IBS score (IV) (n=203)
Visit per year in primary care	1.45 (p<0.001)***	1.62 (p<0.001)***	1.09 (p=0.049)*	1.25 (p<0.001)***	0.86
Consulter for functional GI disorder	100 %	50 % (p<0.001)***	14 % ns	32 % (p<0.001)***	9 %
Consulter for IBS	100 %	45 % (p<0.001)***	3 % ns	15 % (p=0.011)*	5 %
Two or more parts in the body with pain	41 % (p=0.003)**	69 % (p<0.001)***	26 % ns	35 % (p=0.035)*	22 %
Chronic pain					
Headache	20 % (p=0.002)**	18 % (p=0.046)*	3 % ns	14 % (p=0.064)(*)	6 %
Neck pain	25 % ns	41 % (p<0.007)**	9 % ns	29 % (p=0.034)*	17 %
Lumbago	28 % (p=0.018)*	41 % (p=0.001)**	31 % (p=0.064)(*)	27 % (p=0.004)**	12 %
Fatigue	24 % (p<0.001)***	19 % ns	20 % (p=0.010)*	17 % (p=0.004)**	5 %
Insomnia	13 % ns	61 % (p<0.001)***	53 % (p<0.001)***	18 % P=0.069(*)	10 %

regarding frequency of other somatic symptoms in non-consulters with IBS. However, recently a Finnish study showed that also IBS non-consulters demonstrate high rates of comorbidity (99). We can also in our study (paper IV) show consistent data and we found that control subjects with high IBS-like symptoms had visited a general practitioner more often, they reported more widespread pain (more than two areas of pain), more headache, more neck pain, more lumbago, more fatigue, and more problems with insomnia in comparison to control subjects with low rate of IBS-like symptoms (Table VI). Also patients with unipolar depression and a high degree of IBS-like symptoms (paper I) showed a higher health seeking behaviour in primary care compared to patients with recurrent depression and low IBS-like symptoms, and reported a higher frequency of wide spread chronic somatic pain, more chronic headache, more neck pain and more lumbago compared to the depressed subjects with low IBS-

like symptoms (Table VI). However, insomnia and fatigue did not differ between the high and low IBS-like symptom unipolar depression groups. Therefore we claim that having IBS-like symptoms (whether diagnosed or not), the odds for visiting a general practitioner for other somatic complaints (mainly pain) is increased. What are the possible links between IBS/IBS-like symptoms and other somatic symptoms? There is no straightforward answer to this question, but possible mechanisms could be an enhanced perception of bodily symptoms in general due to disturbances in pain inhibition or sensitization of pain both at peripheral and central levels (142, 190, 281). Genetic vulnerability, learned behaviour to react to bodily symptoms (282), or dysfunction due to pathological stress (140) may be possible factors involved. In our studies we could not find any association or interaction between having multiple pain and val158met COMT polymorphism on IBS symptoms, neither could we detect any association to HPA suppression (unpublished data).

In patients with unipolar depression the co-occurrence of pain has been associated with a greater severity of depression and patients with depression who fail to reach remission often have somatic residual symptoms (134, 283). Although having a more rapid effect, somatic symptoms may not be as responsive to antidepressant medication as are psychological symptoms (112, 284). Antidepressants with dual serotonergic and noradrenergic activity appear to be most effective in reducing pain syndromes (284) indicating that dysfunction in both descending serotonergic and noradrenergic monoamine pathways may be involved in pain in depression (190). Also in patients with IBS antidepressants with dual activity have shown effectiveness (285). The patients with depression in our study who were on SSRI treatment tended to have more IBS symptoms (paper I). This could be related to side effects of SSRI but also to an insufficient treatment effect on possible dysfunction of noradrenergic pathways.

Whitehead et al suggested that there may be a specific subgroup of IBS patients that have widespread pain syndromes and that these individuals have a central mechanism of their symptoms which are also associated to deeper psychological disturbances (16). Most studies on patients with IBS include a wide spectrum of patients, i.e. those with multiple pain, those with anxiety/depression and those without. In the future there may be a need to characterize subjects according to their comorbidity in order to be able to appropriately evaluate different treatment regimes.

### **Concluding remarks (paper I-IV)**

There is a broad consensus in that brain-gut interactions play a role in the genesis of many unexplained gastrointestinal symptoms and in functional gastrointestinal disorders such as IBS. However, the mechanism of this interplay is mainly unknown and we are still waiting for major breakthroughs in psychosomatic research. The model of an emotional motor system (EMS) which

reacts similar to all threats to the homeostasis of an individual, including psychological (i.e. anxiety) or intrinsic (i.e. infection) stressors has been proposed. The ANS, catecholamines, HPA axis and endogenous pain inhibition, regarded as “the peripheral limb of EMS” are important systems which communicates central stress-processing to the body. The aim of this thesis was to explore the role of EMS and its “peripheral limbs” in IBS. We could detect impairments in all these systems in subjects with IBS/IBS-like symptoms. Patients with IBS have an increased sympathetic tone at rest as well as after tilting and individuals with IBS-like symptoms is associated to lower degradation of catecholamines (low COMT activity) compared to individuals with low IBS-like symptoms. The degree of negative feedback sensitivity of the HPA-axis is associated to diarrhea and satiety symptoms in individuals representing a normal population. Intermediate HPA suppression, and intermediate COMT activity (the heterozygous val/met genotype) seems to be protective against IBS-like symptoms and we propose that in further research of these systems a search for “u-shaped” relationships should be encouraged. Multiple somatic pain and anxiety/depression are more common among patients with IBS, subjects with IBS-like symptoms and patients with recurrent depression who have IBS-like symptoms. We found that patients with recurrent depression who reached remission had no more IBS-like symptoms than control subjects, indicating that when a psychological stressor ceases the gastrointestinal symptoms seem to decrease.

## CONCLUSIONS

- There is a strong association between symptoms of IBS and symptoms of anxiety/depression in patients with unipolar depression. However, depressive patients in remission do not have more IBS symptoms than controls. Multiple pain symptoms, higher health seeking behaviour and selective-serotonin-reuptake inhibitor intake were associated to IBS-like symptoms in patients with unipolar depression.
- Patients with IBS show, in short term spectral analysis of heart rate variability, an increased sympathetic activity at rest and after tilting compared to healthy controls.
- A trend toward a non-linear relationship between total IBS-like symptoms and HPA suppression, and a significant non-linear relationship between the symptoms of diarrhea/satiety and HPA suppression was demonstrated in a sample representing a normal population. Intermediate HPA suppression seems to be protective against IBS-like symptoms.
- The heterozygous val/met COMT genotype seems to have a protective effect against IBS-like symptoms with a significantly lower occurrence in subjects with IBS-like symptoms representing a normal population and a trend of a lower occurrence in the patients with IBS.

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.

## POPULÄRVETENSKAPLIG SAMMANFATTNING

Att mänskliga känslor kan förnimmas i magen har länge varit känt av människor, det stöds av uttryck som ”fjärilar i magen”, ”magkänsla” och ”stressmage”, och motsvarigheter finns även i andra kulturer. Vi vet idag att symtom från magen är vanliga vid affektiva sjukdomar som ångest och depression. Hos flertalet av de patienter som söker läkare för problem med magen finner man inget avvikande på blod och vävnadsprover och de får en diagnos inom gruppen funktionella tarmsjukdomar (till exempel irritable bowel syndrome (IBS)). Inte sällan finner man även hos dessa patienter ångest och depressionsproblematik. Interaktionen mellan hjärna och tarm är komplex och är till stor del okänd. De senare årens forskning har dock kunnat visa på möjliga mekanismer varför magen reagerar på stress. Kroppen verkar reagera likvärdigt på yttre (t ex livskriser) och inre (t ex infektioner) stress. Att reagera på stress är livsnödvändigt men det är också viktigt att när ”faran är över” kunna stänga av stresssystemen. Det emotionella motor systemet (EMS) är centralt i hanteringen av stress. Det består av centra i hjärnan som integrerar signaler från yttre världen med den inre världen, och ger oss rätt uppmärksamhet på faror, rätt känslouttryck samt rätt signaler ut i kroppen. EMS verkar ut i kroppen via det autonoma nervsystemet (ANS), hypothalamus-hypofys-binjure (HPA) axeln samt även via ett smärtlindringssystem. ANS är det nervsystem som vi inte kan påverka och som styr t ex hjärta och tarm. HPA axeln ser till att stresshormonet kortisol bildas lagom mycket och det endogena smärt-lindringssystemet påverkar vår smärtröskel (uppåt respektive nedåt).

I denna avhandling frågar vi oss om magsymtom är relaterade till avvikelser i EMS. Avhandling undersöker hur magsymtom är relaterade till viktiga beståndsdelar i EMS som ANS, HPA axeln, COMT genen (en gen som styr nedbrytningen av stresshormoner och som har betydelse för smärtlindring), samt hur magsymtom uttrycker sig vid olika grader av ångest och depression hos patienter med känd depression sjukdom.

Vi fann att patienter med känd depressionssjukdom hade jämfört med en kontroll grupp ökad förekomst av IBS symtom när de mådde psykiskt dåligt men när de kommit ur sin depressionsperiod så fanns ingen skillnad avseende magsymtom längre. Vår slutsats blev att om man behandlar depressionen så försvinner förmodligen även magsymtomen.

I vår andra studie så undersökte vi ANS med hjärtfrekvensvariabilitet. Vi fann att patienter med IBS hade en ökad sympatikusaktivitet jämfört med en kontroll grupp.

I vår tredje studie fann vi hos ett urval av befolkningen en störning i förmågan att ”stänga av” HPA axeln hos individer som hade symtom på tidig mättnad och diarréer. Intressant nog fanns denna association hos både de individer som hade svårt att ”stänga av” och hos de individer som hade ovanligt lätt att ”stänga av” HPA axeln.

I fjärde studien undersökte vi en så kallad variant i COMT genen som finns i tre former i befolkningen (met/met, val/met, val/val). De med met/met har en långsam nedbrytning av katekolaminer (stresshormoner), de med val/val en snabb nedbrytning och de med val/met en ”lagom” nedbrytning. Vi fann även här att ”lagom är bäst” då de individer som bar på val/met varianten hade mindre IBS symtom en de med de övriga formerna. Även patienter med IBS tenderar oftare att bära på val/val och met/met. Framförallt diarré symtom var hos dessa patienter relaterade till val/val varianten.

Denna avhandling visar således upp samband mellan IBS symtom och symtom på ångest/depression, på funktion i ANS och HPA axeln, och i förmågan att bryta ner stresshormoner (COMT genen). Sammantaget stödjer det att det emotionella motor systemet (EMS) har betydelse för uttryck av magsymtom (IBS symtom).

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