IRRITABLE BOWEL SYNDROME

Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls

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Background and aims: Many patients with irritable bowel syndrome (IBS) show intestinal hypersensitivity to distension and sensitisation after repeated intestinal distensions. Abnormalities in endogenous pain inhibitory mechanisms, such as diffuse noxious inhibitory controls (DNIC), may be implicated and were investigated during brain functional magnetic resonance imaging (fMRI).

Patients and methods: fMRI was performed in 10 female patients with IBS (five constipated (IBS-C) and five with diarrhoea (IBS-D)) and 10 female healthy controls during rectal balloon distension alone or during activation of DNIC by painful heterotopic stimulation of the foot with ice water. Rectal pain was scored with and without heterotopic stimulation (0 = none, 10 = maximal).

Results: Heterotopic stimulation decreased median rectal pain scores significantly in healthy controls (−1.5 (interquartile range −2 to −1); p = 0.001) but not in IBS-C (−0.7 (−1 to 0.5)), IBS-D (−0.5 (−1.5 to 0.5)), or in all IBS patients (0 (−1.5 to 1.3)). Brain activation changes during heterotopic stimulation differed highly significantly between IBS-C, IBS-D, and controls. The main centres affected were the amygdala, anterior cingulate cortex, hippocampus, insula, periaqueductal gray, and prefrontal cortex, which form part of the matrix controlling emotional, autonomic, and descending modulatory responses to pain.

Conclusions: IBS-C and IBS-D appear to have differing abnormal endogenous pain inhibitory mechanisms, involving DNIC and other supraspinal modulatory pathways.

Irritable bowel syndrome (IBS) is characterised by abdominal discomfort or pain accompanied by changes in gastrointestinal motility. The underlying mechanisms remain unclear but visceral hypersensitivity is apparent in many patients with IBS. Rectal distension studies have shown up to 60% of IBS patients have lower sensory thresholds compared with healthy controls, depending on the selection of patients and the distension protocol. Differences in pain scores or somatic referral areas during distension are inconclusive when comparing IBS patients and controls. Sensitisation to repeated intestinal distension has been considered specific for IBS, as healthy subjects did not develop significant rectal sensitisation of discomfort ratings after sigmoid conditioning. These data imply altered sensory processing in IBS, possibly involving central and spinal sensory modulation.

Major endogenous pain modulating mechanisms include the periaqueductal grey (PAG)-rostroventral medulla (RVM) network and the spino-bulbo-spinal diffuse noxious inhibitory controls (DNIC). They are central in regulating pain perception and have been shown to be abnormal in several conditions associated with IBS, such as fibromyalgia. Assessment of DNIC using the counterirritation technique is well validated and widespread in pain research. DNIC activation is quantified by the perceptual modulation of a painful stimulus by a secondary heterotopically applied nociceptive, generally thermal or electrical stimulus.

Hypersensitivity may be secondary to peripheral or central changes in the nervous systems. Peripheral sensitisation with subsequent spinal and supraspinal sensitisation provides a suitable mechanism for lowered sensory thresholds. The minimal inflammatory changes demonstrated in subgroups of IBS patients may represent an adequate stimulus. Hypervigilance, increased selective attention to potentially threatening stimuli, is a putative central component of sensitisation leading to overrating of stimulation intensities. Brain imaging techniques have demonstrated abnormal central processing of rectal pain in areas dealing with affective and emotional sensory processing in IBS. These areas are intricately involved in the integration of autonomic responses and in supraspinal afferent input modulation. No published data exist on imaging of brain function during activation of endogenous antinociception and DNIC during visceral pain and, although malfunctioning modulation has been repeatedly postulated, little is known about the functional intactness of endogenous pain modulation in IBS.

The aim of this study was to examine DNIC in IBS subgroups and healthy controls during functional magnetic resonance imaging (fMRI), by modulation of visceral pain with heterotopic noxious stimulation.

METHODS
Twenty right handed females, aged 20–50 years, were recruited for this trial. Ten patients with IBS (Rome 2 criteria) as their main complaint and a weekly average pain score greater than 30 mm on a 100 mm horizontal visual analogue scale.
analogue scale (VAS: 0 = none, 100 = unbearable) during a one week run-in period were recruited from our outpatient clinic. Five patients were constipated (IBS-C: <1 hard motion every third day, strong defecatory straining) and five had diarrhoea (IBS-D: >3 soft or liquid motions per day). The healthy control group consisted of 10 females recruited by word of mouth, with no gastrointestinal pathology or history of abdominal pain, bloating, discomfort, constipation, or diarrhoea more than twice a week in the last three months. Exclusion criteria in all groups were clinically relevant gastrointestinal, hepatic, or other systemic disease, lactose intolerance, bowel resections (except appendectomy) or abdominal operations, any medication, treatment with any investigational drug during the preceding 30 days, pregnancy, lactation, and inability to give written informed consent. The University of Berne ethics committee approved the study.

Whole brain fMRI scans were acquired during rectal distension with and without heterotopic somatic stimulation for assessment of brain activation due to DNIC. Before imaging, a lubricated latex balloon with virtually infinite compliance up to a distension volume of 800 ml and attached to the end of a catheter was introduced into the rectum with subjects in the left lateral position, inflated to 200 ml, and gently pulled outwards until slight resistance was felt. The balloon was tested for leakage before insertion and the catheter attached to a hand held syringe. Rectal distension thresholds for first sensation (first slight fullness), defecatory urge (“begin to look for toilet”), pain detection (first feeling of pain), and pain tolerance (“would deflate balloon because of strong pain”) were titrated twice with subjects in a comfortable supine position using a manually controlled continuous ramped inflation rate of 25 ml/s. Pain detection threshold volumes from the second determination plus 20% were used during fMRI. Subsequently, the heterotopic cold pressor test was performed by immersing the left foot in an ice water bath maintained at 4°C and pain intensity rated after 120 seconds on a 100 mm anchored horizontal visual analogue scale. This test was subsequently used for somatic heterotopic stimulation during rectal distensions.

After a resting period, subjects were positioned inside the MRI scanner, head motion was minimalised by use of a head frame and a bite plate, and subjects kept their eyes closed. Ear plugs were used to protect against gradient noise. MR imaging was performed on a commercial whole body 1.5 T system equipped with a circular polarised head coil (Siemens Magnetom Vision, Erlangen, Germany). A preliminary three plane scout image was acquired before initiation of the echo planar fMRI sequences for whole-brain T2* imaging (Tr 6 seconds; TE 84 ms; flip angle 90°). A total of 30 slices with a 128 pixel matrix, 5 mm thickness, 4 mm slice distance between slice centres (1 mm overlap, interleaved acquisition), and 68 measurements were performed per experimental run. Each run was performed twice with each subject, resulting in a total of 136 whole brain measurements per subject. There was a break of 10 minutes between runs to ensure re-warming of the ice water cooled foot. Slices were placed parallel to a line connecting the genu corporis callosi and confluens sinuum, in order to minimise echo planar induced susceptibility artefacts and to cover the whole brain with a minimum of slices. To minimise effects due to anticipation of pain, most likely at the transitions between rest and painful stimuli, two sample volumes (12 seconds) were excluded from analysis at each time point of change (balloon inflation/pressure release).

Eight rectal distensions of 48 seconds duration each were performed, separated by 48 second rest periods with complete balloon deflation. Heterotopic stimulation was performed during the third, fourth, seventh, and eighth distensions and extended into the immediately following baseline rest periods. The resulting 2×2 design allowed assessment and comparison of activation due to balloon distension versus rest both during and without heterotopic stimulation. To avoid motion artefacts due to leg movements, the ice water was filled into and then drained from a water bath positioned before the start of fMRI. Subjects reported rectal pain ratings (verbal rating scale: 0 = none, 10 = unbearable) after each pair of distensions (1st and 2nd, 3rd and 4th, 5th and 6th, and 7th and 8th).

A structural high resolution spin-echo-train inversion recovery magnitude sequence was then run to acquire anatomical data for localisation and a high resolution three dimensional MPRAGE sequence for future co-registration. Images were exported to a Sun workstation (Sun Microsystems, Palo Alto, USA) for post-processing. Both quantitative volume of interest (VOI) analysis and visualisation with colour coded z score maps were performed using purpose written software. For quantitative assessment of brain activation in predefined cortical areas with respect to interindividual variability of anatomical details, we used a modified anatomically defined parcellation scheme, as described by Rademacher et al, and subsequently adapted for fMRI. Due to the high resolution, each subject's own echo planar imaging data were suitable for both blood oxygen level dependent (BOLD) fMRI and for structural anatomical definition of VOIs. The following VOIs were defined and individually delineated: paracentral gyri (sensormotor cortex S1 and M1), mesiotemporal region (amygdala, hippocampus), anterior cingulum, posterior cingulum, anterior insula, posterior insula, PAG, supramarginal gyrus (secondary sensory cortex S-II), orbitofrontal cortex, inferior dorsolateral prefrontal cortex (along inferior frontal sulcus), superior dorsolateral prefrontal cortex (along superior frontal sulcus), anterior, posterior, medial, and lateral quadrants of the thalamus, and the occipital visual cortex (control area). VOIs were assessed separately in each hemisphere. The time series was analysed for each VOI (see below).

Independent of VOI analysis, voxel based z score images were generated for visualisation according to standardised procedures. Cortical activation in response to balloon distension was separately evaluated in both cases for the phases during and without heterotopic stimulation. Activation was defined as a BOLD signal increase relative to baseline and deactivation as a BOLD signal decrease. One time sample point of each VOI consisted of the average signal intensity of all pixels in that VOI at the specific whole brain measurement time point, resulting in a time series of 136 measurements per VOI. Excluding two time samples (12 seconds) at each transition to allow for inflation or deflation of the balloon as well as for the cerebral haemodynamic response, the means and variances of each of the four phases (with and without balloon distension, each with and without heterotopic stimulation) were determined for each VOI, allowing statistical comparisons with the parametric z test for normal distribution of large samples. Z score limits for a given p value were Bonferroni corrected for the number of VOIs measured. Because VOIs were individually delineated and anatomical regions corresponded, VOI data could be reliably pooled for each group.

Per cent BOLD signal intensity changes due to balloon inflation were compared with the respective baseline and calculated separately for phases without and with heterotopic stimulation. Furthermore, the difference between this balloon inflation induced activation (per cent change) during heterotopic stimulation, minus the analogue balloon inflation induced activation (per cent change) without heterotopic stimulation, was calculated as the effect of the heterotopic modulation on balloon induced activation related to each VOI. This is possible when comparing per cent signal changes but
not when using z scores (for extensive statistical discussion see Nirkko and colleagues and Jaeggi and colleagues). Z test for large samples was used to assess statistical significance of the signal changes with respect to baseline, between conditions (using appropriately combined variances) and between groups. The z score limits for a given p value were based on two tailed analysis, and Bonferroni correction was made for the number of VOIs measured in the VOI analysis. Likewise, for voxel based visualisation, clusters were included with at least one voxel with a z score above 5.0, which corresponds to a full Bonferroni correction for a p<0.05 for all voxels of the complete brain. Additionally, the extent of such clusters was displayed with different colours coded down to a z score of 2.0, which correspond to an uncorrected p<0.05.

Statistical analysis of group data
All continuous group data are shown as means (95% confidence intervals (CI)). Analysis of variance (ANOVA) testing was used for comparisons within and between groups. Tukey’s honest significance difference test was used for confirmative testing. Non-parametric data were expressed as medians and analysed using the Kruskal-Wallis and Mann-Whitney U tests. A significance level of p = 0.05 was applied throughout.

RESULTS

Subject characteristics
Mean ages (95% CI) did not differ significantly between healthy controls, IBS-C, and IBS-D: 31 years (24–38), 35 years (25–45), and 40 years (24–57), respectively. Mean weight and height in the respective groups were 61 kg (55–66) and 168 cm (164–171), 58 kg (53–63) and 166 cm (161–171), and 61 kg (50–72) and 167 cm (155–179). IBS had been present for 2–5 years in two patients and for over five years in three patients in both IBS groups.

Rectal distension threshold
Rectal distension threshold balloon volumes were significantly different between groups (ANOVA group effect p = 0.03) (table 1). Pain detection and tolerance volumes were significantly higher in IBS-C than in healthy controls or IBS-D (p<0.05). Distension resulted in similar pain and discomfort ratings after 120 seconds in all subject groups (table 1).

Distension pain ratings without and with heterotopic stimulation of the first and second series were pooled as there were no significant differences between the two series. Rectal pain ratings without and with heterotopic stimulation were significantly different in healthy subjects (p = 0.001) but not in IBS-C or IBS-D. The decrease in rectal pain scores with heterotopic stimulation was significantly greater in healthy subjects than in IBS-C and IBS-D (p<0.01) (fig 1). Mean pain scores during the cold pressor test alone were not significantly different between subject groups.

Table 1 Rectal balloon distension thresholds and pain/discomfort during tonic rectal distension in 10 controls and 10 irritable bowel syndrome

<table>
<thead>
<tr>
<th></th>
<th>1st sensation (ml)</th>
<th>Defecation urge (ml)</th>
<th>Pain detection (ml)</th>
<th>Pain tolerance (ml)</th>
<th>Tonic distension during fMRI (ml)</th>
<th>Distension: pain (VAS 0–100)</th>
<th>Distension: discomfort (VAS 0–100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>41 (24–58)</td>
<td>118 (93–143)</td>
<td>195 (142–248)</td>
<td>270 (204–335)</td>
<td>227 (170–284)</td>
<td>52 (36–68)</td>
<td>48 (32–64)</td>
</tr>
<tr>
<td>IBS-C</td>
<td>63 (14–100)</td>
<td>183 (101–265)</td>
<td>322 (153–491)</td>
<td>443 (239–647)</td>
<td>386 (183–590)</td>
<td>47 (30–65)</td>
<td>47 (20–74)</td>
</tr>
</tbody>
</table>

Means (95% confidence intervals) are shown. IBS-C: five patients with IBS with constipation; IBS-D: five patients with IBS with diarrhoea; VAS, visual analogue scale (0 = none, 100 = unbearable); fMRI, functional magnetic resonance imaging.

*p<0.05, IBS-C versus healthy controls and IBS-D; †p = 0.03, IBS-C versus healthy controls.

DISCUSSION
Differences in activation of endogenous noxious inhibitory pathways were clearly demonstrated between healthy controls and subgroups of IBS patients using a validated
method of counterirritation during rectal distension pain. This was evident in clinical pain measures as well as in brain activity by fMRI. Additionally, previously reported differences in brain activation during visceral pain between IBS patients and controls were corroborated and extended.\(^{16,24–26,32}\) Marked group differences in pain processing between IBS patients and controls were corroborated and extended.\(^{16,24–26,32}\)

Noxious input underlies multiple endogenous regulatory mechanisms at the spinal and supraspinal levels, which can be both facilitatory and inhibitory. Major modulation of pain perception is coordinated in the PAG-RVM network, with extensive input from the limbic forebrain, diencephalon and brainstem, and the spino-bulbo-spinal DNIC pathway.\(^{15,36,37}\) In the current study, heterotopic activation of the amygdala, hippocampus, and thalamus, and dysfunction in modulatory pathways has also been hypothesised in IBS but has yet to be demonstrated.\(^{8,13,14}\) Assessment of DNIC using the counterirritation technique is well validated and widespread in pain research. DNIC activation is quantified by perceptual modulation of a painful stimulus by a secondary heterotopically applied nociceptive stimulus by fMRI. Additionally, previously reported differences in brain activation during heterotopic stimulation were seen in the anterior cingulate cortex (ACC, Brodmann 24' and 32'), the insular cortex, and the prefrontal cortex, which have extensive connections to the PAG and RVM.\(^{11,36}\) Diminished activation during heterotopic stimulation in healthy controls in the anterior and posterior insula, the medial thalamus, and the PAG may reflect promotion of inhibitory feedback loops. PAG lies at a crossroads for neural circuits that coordinate rapid and profound somatic, autonomic, and antinociceptive modulation.\(^{29}\) The observed effects are not due to the cold pressor test itself, as all values were baseline corrected.

The absence of a significant inhibitory effect on visceral pain in either IBS subgroup suggests impaired activation of inhibitory controls, and brain activation patterns differed markedly from healthy controls, but also between subgroups. During heterotopic stimulation only IBS-C demonstrated significant activation of the amygdala, hippocampus, and thalamus, and also in the cingulate (Brodman 32') and prefrontal cortices.

### Table 3: Brain regions significantly activated by heterotopic stimulation during rectal pain (z score >3, Bonferroni-corrected, two tailed)

<table>
<thead>
<tr>
<th>Controls</th>
<th>IBS-C</th>
<th>IBS-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>+Bilateral(^*)</td>
<td>+Bilateral(^*)</td>
</tr>
<tr>
<td>Cingulate anterior</td>
<td>+Bilateral(^*)</td>
<td>+Bilateral(^*)</td>
</tr>
<tr>
<td>Cingulate posterior</td>
<td>+Bilateral</td>
<td>+Bilateral</td>
</tr>
<tr>
<td>Prefrontal cortex inferior</td>
<td>+Bilateral(^†)</td>
<td>+Bilateral(^†)</td>
</tr>
<tr>
<td>Prefrontal cortex superior</td>
<td>+Bilateral(^†)</td>
<td>+Bilateral(^†)</td>
</tr>
<tr>
<td>Insula anterior</td>
<td>+Bilateral(^†)</td>
<td>+Bilateral(^†)</td>
</tr>
<tr>
<td>Insula posterior</td>
<td>–Left</td>
<td>–Left</td>
</tr>
<tr>
<td>Thalamus lateral</td>
<td>–Left</td>
<td>–Left</td>
</tr>
<tr>
<td>Thalamus medial</td>
<td>–Right</td>
<td>–Bilateral(^†)</td>
</tr>
<tr>
<td>Thalamus anterior</td>
<td>–Bilateral(^†)</td>
<td>–Bilateral(^†)</td>
</tr>
<tr>
<td>S1</td>
<td>+Bilateral</td>
<td>+Bilateral</td>
</tr>
<tr>
<td>PAG</td>
<td>–Midline</td>
<td>–Midline</td>
</tr>
<tr>
<td>Orbitofrontal</td>
<td>+Bilateral(^†)</td>
<td>+Bilateral(^†)</td>
</tr>
<tr>
<td>Amygdala/hippocampus</td>
<td>+Bilateral(^†)</td>
<td>+Bilateral(^†)</td>
</tr>
</tbody>
</table>

\(\text{IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhoea.}\)

\(\text{+}, \text{Significant activation; --}, \text{significant deactivation; Bilateral, bilateral symmetrical activation changes; Right or Left, respective asymmetrical activation changes.}\)

\(\text{+ Increased versus IBS-D; \(-\text{decreased versus IBS-D; \(*\text{increased versus IBS-D; \***\text{increased versus healthy and IBS-D; \(\dagger\text{decreased versus healthy and IBS-D; \(\ddagger\text{increased versus healthy and IBS-D.}\)}}\)}\)

The absence of a significant inhibitory effect on visceral pain in either IBS subgroup suggests impaired activation of inhibitory controls, and brain activation patterns differed markedly from healthy controls, but also between subgroups. During heterotopic stimulation only IBS-C demonstrated significant activation of the amygdala, hippocampus, and thalamus, and also in the cingulate (Brodman 32') and prefrontal cortices.
IBS-D differed profoundly from IBS-C and controls in only showing decreased activation in the right anterior insula during heterotopic stimulation. The main divergent activations in the IBS subgroups were seen in the brain regions integrating emotions, attention, and cognition, implying dysfunction in the corresponding modulation of nociceptive input. It has been previously proposed by Villanueva et al, that DNIC may play a physiological role in the detection of nociceptive signals and also constitute both a filter, which allows extraction of the signal for pain, and an amplifier in the transmission system, which increases the potential alarm function of the nociceptive signals. Malfunction of this filter would explain several of the postulated sensory disorders in IBS.

Evidence for dysfunction in rectal pain processing without counterirritation has previously been presented in mixed groups of IBS patients. This study corroborates earlier data and adds additional information by separately considering IBS subgroups. In controls, significant bilateral activation compared with baseline was seen during painful rectal distension in most of the brain centres classically associated with pain perception and processing, such as the cingulate, insular, prefrontal cortices, the SI and SII regions, the thalamus, and limbic system. The regions activated are constituents of a complex matrix of connections involved in the primary sensory analysis and secondary integration of emotional, affective, memory, autonomic, and motor responses, as well as feedback and feed-forward regulation of nociceptive stimulation. Activation patterns in the IBS subgroups differed from each other as well as from controls, except for the sensory discriminative SI and SII pain centres, where patterns were similar between all groups, excluding the clear hemispheric lateralisation in both IBS groups. In contrast with healthy controls, both IBS groups showed no significant bilateral activation in the anterior perigenual cingulate, and prefrontal cortices during painful distension compared with baseline. The absence of ACC activation during rectal pain in IBS patients probably demonstrates a resetting of the gain of the secondary integrative pain processing system and a pre-existing saturation in the entire pain/anxiety network. An analogous seemingly paradoxical absence of cortical activation due to baseline saturation has been observed in other conditions, such as the Gilles de la Tourette syndrome. Decreased activation of the ACC has been previously demonstrated in IBS patients where it was interpreted as a diminished antinociceptive response to aversive visceral events, and also
Arousal and attention and of motor reactions and response selection appe...cortical and limbic centres in IBS.

Potential weaknesses of this study include, firstly, the small patient numbers due to splitting of the IBS patients into subgroups. However, pooling of the data was deemed inappropriate due to the marked differences between the groups. Highly significant and consistent effects were demonstrated but minor effects may have been insignificant due to inadequate statistical power. The current data need to be confirmed in larger groups of patients. Secondly, manual volume based distensions rather than barostat pressure controlled inflations were used due to difficulties with the use of a barostat in the MRI scanner room. This is unlikely to affect the results as titrations to individual perception levels were performed using a rapid phasic inflation paradigm. Pain threshold volumes were significantly higher in IBS-C than in IBS-D, as has previously been demonstrated for pressure thresholds. However, small numbers and the relative insensitivity of volume versus pressure thresholds preclude further speculation. Thirdly, because of the coexistence of inhibitory and excitatory neurones in many of the involved centres, a causal correlation between visualised activations and function is currently not possible using fMRI techniques.

In summary, activation of inhibitory noxious controls by counterirritation in healthy controls activated extensive supraspinal pathways, probably including the PAG-RVM pathways. Constipated and diarrhoeic IBS patients differed significantly from each other and healthy controls in their inappropriate brain activation responses to rectal pain without and with counterirritation. The centres affected were part of the matrix controlling emotional, autonomic, and descending modulatory responses to pain.

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REFERENCES


