



## TOPIC HIGHLIGHT

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# Experimental human pain models in gastro-esophageal reflux disease and unexplained chest pain

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## Abstract

Methods related to experimental human pain research aim at activating different nociceptors, evoke pain from different organs and activate specific pathways and mechanisms. The different possibilities for using mechanical, electrical, thermal and chemical methods in visceral pain research are discussed with emphasis of combinations (e.g., the multimodal approach). The methods have been used widely in assessment of pain mechanisms in the esophagus and have contributed to our understanding of the symptoms reported in these patients. Hence abnormal activation and plastic changes of central pain pathways seem to play a major role in the symptoms in some patients with gastro-esophageal reflux disease and in patients with functional chest pain of esophageal origin. These findings may lead to an alternative approach for treatment in patients that does not respond to conventional medical or surgical therapy.

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**Key words:** Pain; Gut; Experimental; Chest pain; Esophagitis

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## INTRODUCTION

Pain is probably the most prevalent symptom in medicine and characterization of pain is of major importance in the diagnosis and choice of treatment<sup>[1]</sup>. Abdominal pain is frequently occurring, even in the normal population<sup>[2]</sup>, and abdominal discomfort and pain are among the most common symptoms responsible for patients consulting the health care system<sup>[3]</sup>. Consequently, understanding and characterization of gut pain is an important issue in the diagnosis and assessment of organ dysfunction. Research leading to a better insight into pain mechanisms in the gastrointestinal (GI) tract will invariably improve the treatment of the patients<sup>[1]</sup>. However, in practice, assessment of clinical pain is difficult as the symptoms of the underlying diseases confound the description of pain. These confounders may include complaints relating to psychological, cognitive and social aspects of the illness, as well as systemic reactions such as fever and general malaise<sup>[4]</sup>. Treatment with analgesics may also cause sedation and other side effects. Finally, abdominal pain is much more diffuse than somatic pain with autonomic symptoms, referred pain and hyperalgesia in remote somatic tissues. These factors will invariably bias the clinical evaluation. For example in the evaluation of analgesics the patients often tend to interpret other effects of the medication-such as an effect on the anxiety and depression relating to the disease-as a relief of pain<sup>[5]</sup>. To encompass these bias experimental pain models are increasingly used, especially in animal studies. In these experiments, the neuronal nociceptive activity can be recorded or behavior can be assessed<sup>[6]</sup>. However, neuronal recordings or reactions do not reveal all aspects of pain being the net effect of complex multidimensional mechanisms that involve most parts of the central nervous system<sup>[7]</sup>. Furthermore, major species differences in pain mechanisms often influence the findings. Therefore, although nociceptive reflexes or electrophysiological recordings from selected pathways in the animal nervous system are important in basic research, the central pain mechanisms and associated complex reactions are typically suppressed. Thus, animal experiments can only to some degree reflect the experience of clinical pain in humans. Because of these factors, experimental human pain models are advantageous to assess basic pain mechanisms and to investigate abnormal pain responses in functional and organic diseases of the GI system. Methods related to

experimental human pain research aim to activate different nociceptors, evoke pain from different organs and activate specific pathways and mechanisms. Assessment of the output from these pain models can be based on psychophysical or neurophysiologic methods<sup>[8]</sup>. Using these models, the investigator can control the experimentally induced pain (including the nature, localization, intensity, frequency and duration of the stimulus), and provide quantitative measures of the psychophysical, behavioral, or neurophysiologic responses. Methods for imaging can also be added and some studies used a combination of these assessments. In this paper, however, the focus will be the methods for pain induction. The reader is referred to<sup>[4]</sup> for further detail regarding assessment of GI pain

## EXPERIMENTAL PAIN MODELS

The ultimate goal of advanced human experimental pain research is to obtain a better understanding of the mechanisms involved in pain transduction, transmission, and perception under normal and pathophysiological conditions. Experimental approaches can be applied in the laboratory for basic studies and in the clinic to characterize patients with sensory dysfunction and/or pain in organic and functional diseases<sup>[4,9]</sup>. Depending on the experimental model, different central mechanisms and conditions mimicking pathological pain such as increased sensation to normal physiologic/non-painful and painful stimuli (allodynia and hyperalgesia) can be studied. However, most previous studies have relied on relatively simple mechanical or electrical stimuli. These methods are easy to apply but as discussed below they have special considerations and limitations. Therefore, new controlled and reproducible models and protocols are highly warranted.

The ideal experimental stimulus to elicit gut pain in man should be natural, minimally invasive, reliable in test-retest experiments and quantifiable. Preferably the pain should mimic the observations in diseased organs by evoking phenomena such as allodynia and hyperalgesia<sup>[10,11]</sup>. The different methods for pain stimulation of the human GI tract are described below and in Table 1. Some of the existing stimulation methods seem to fulfill the above requirements but most laboratories use their own stimulation paradigms, often without the necessary standardization.

### Electrical stimulation

Depolarization of the nerve afferents by electrical current has been widely used as an experimental stimulus in humans for many years-for review see<sup>[9]</sup>. The electrical stimuli have proved to be safe in all parts of the gastrointestinal system<sup>[12-15]</sup>. Various electrical stimulator devices connected to electrodes applied to the mucosa of the gut can evoke electrical stimulation. Stimulator devices can deliver different stimulation patterns, e.g., different waveforms, frequencies, and duration of the stimulus. This activates with some selectivity different afferents and nervous structures, and hence evokes different kinds of pain<sup>[7]</sup>. The electrical stimulus intensity to evoke pain as well as the size of the referred pain area is reliable and

**Table 1** The different methods for pain stimulation of the human GI tract

Stimulation modality	Stimulated structures	Advantages	Limitations
Electrical	Nerve fibers primarily in mucosa and muscle layers dependent of the stimulation intensity (not a specific activation of the nociceptors)	Excellent for repeated stimulation, suitable for neurophysiologic assessments of the pain	The electrical threshold depends on the fiber diameter, i.e. small-diameter nerves cannot be excited without exciting others. May induce arrhythmias in areas near the heart
Mechanical	Mechanoreceptors located in different layers	Imitates a bolus, reproducible stimulus	Problems with estimating the transmural pressure and change in circumference
Thermal	Thermal sensitive receptors preferentially in the luminal layers	Activation of unmyelinated afferents in the mucosa selectively	Temperature stimuli in the range that can be felt are normally only relevant for sensation in the upper GI tract.
Chemical	Chemo-sensitive receptors, primarily in the mucosa	Resembles clinical inflammation, chemical stimuli activate predominantly unmyelinated C-fibres	Require a relative long latency time to the onset of effects, and that they are often not reproducible when repeated

reproducible<sup>[12,13,16,17]</sup>. The well defined onset and offset of the stimulation eliminates the latency to stimulation of the afferents seen with other methods<sup>[7]</sup> and makes it suitable to study pain mechanisms related to time<sup>[16,18]</sup>. Thus, the method is very suitable for neurophysiologic assessments of the pain<sup>[18-20]</sup>. Furthermore, the stimulation field is usually not as large as in other models, and diseases with localized pathology such as ulcers may better be mimicked<sup>[13,18]</sup>. Central integration or temporal summation, being a human correlate to the initial phase of "wind-up", is a potent mechanism for generation of referred visceral pain and can easily be evoked by electrical stimulation<sup>[16,18]</sup>. The major shortcomings are that electrical stimulation bypasses the receptors and activates the nerve fibres directly, and the method is not a specific activation of the nociceptors. The electrical threshold is related to the fiber diameter and one cannot usually excite small-diameter nerves without additionally exciting others. Furthermore, electrical stimuli may induce arrhythmias when areas near the heart are stimulated<sup>[18,21]</sup> and therefore bipolar stimulation where the electrical field is more localized may be recommended.

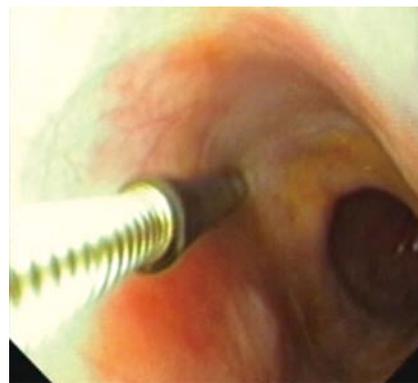
Previous methods have suffered from problems with insufficient contact between the electrodes and the gut, and in some studies pain was not evoked in all

subjects. We have improved the method by integrating the electrodes on the biopsy forceps for the endoscopes, which makes it possible to stimulate well defined areas in the esophagus, stomach, duodenum, terminal ileum and colon<sup>[13,14,16,18]</sup>. Recently we used a 6 mm nasal endoscope for the stimulations. Apart from a little unpleasantness associated with the intubations, all subjects were able to lie comfortably and none complained of unpleasantness due to the endoscope for up to 4 h. An example of stimulation of the esophagus is shown in Figure 1. The advantage of this modification is that the position of the electrodes is controllable and can be changed in case of stimulation of nearby somatic structures and nerves. The contact with the organ wall is secured and evoked motor phenomena such as secondary contractions can be studied. However, endoscopic stimulation demands special equipment and facilities and improvement of methods for “blind stimulation” is highly warranted.

### Mechanical stimulation

In the last decade several studies have addressed the mechanical and sensory function of the GI tract by means of mechanical distension. The mechanical properties of the GI tract are important for its function as a digestive organ and the gut contains mechanoreceptors at various locations in the wall, mainly in the muscle layers<sup>[6]</sup>. Mechanical GI tract stimulation, in particular balloon distension, has been widely used also to study GI smooth muscle tone<sup>[22-24]</sup>, distensibility<sup>[22,25,26]</sup>, peristaltic reflex responses<sup>[27]</sup>, functional and organic disorders<sup>[28-32]</sup>, referred pain and cerebral activation patterns<sup>[33]</sup> and the screening of new analgesics in healthy subjects and in patients with gut disorders<sup>[30,34-37]</sup>. There are several ways to stimulate the GI tract mechanically. Simple and physiological methods for distension of the gut such as ingestion of well-defined meals may be useful in clinical studies<sup>[38]</sup>. Balloon distension is, however, much more used than ingestion as mechanical stimulation method and easier to control. Therefore, the following sections will focus on controlled balloon distension methods used to evoke non-painful sensations and pain in the gut.

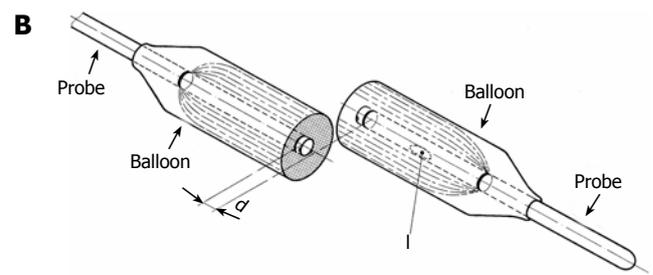
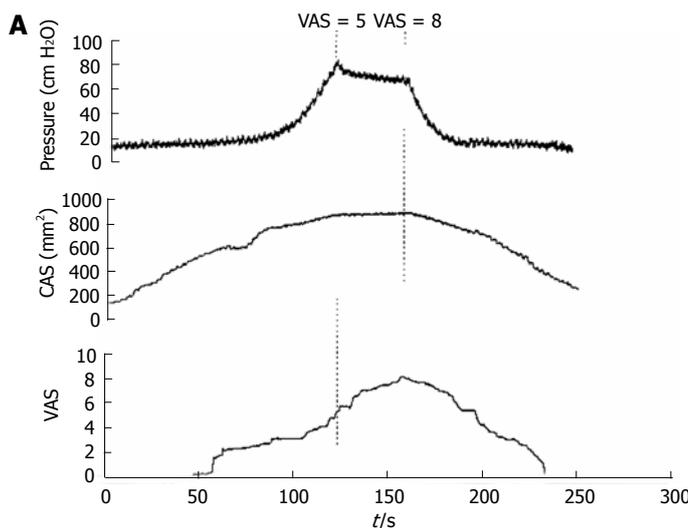
Most recent studies have used a method of measuring changes in volume of air in a balloon at constant pressure levels named “the Barostat”. Several protocols and stimulation paradigms have been recommended for the Barostat, such as for example “phasic and tonic distensions”. The stimulation paradigms have been thoroughly discussed recently and will not be described here-for review see<sup>[30,39]</sup>. The great advantage of the Barostat system and similar pressure-volume based methods are the relatively low cost, especially if the system is self-made. Furthermore, they are easy to use for routine purposes. Such systems have been used for determination of sensory and pain thresholds, and under different conditions attempts have been done to calculate the compliance and tension of the organs<sup>[26,30,39,40]</sup>. However, there are drawbacks with these simple methods. For example using the Barostat device, the data must be corrected for compressibility of air. The four major concerns, however, relate to (1) elongation of the balloon



**Figure 1** The stimulation electrode visualized in the distal esophagus using the nasal endoscope.

during distension; (2) the use of very long balloons; (3) the use of erroneous assumptions; and (4) the improper use of distension protocols. In more detail: (1) Balloon distension in a tube will cause the balloon to elongate to some degree due to the resistance to deformation in radial direction<sup>[41]</sup>. Volume is therefore not an accurate measure for the distension of the organ; (2) The use of long balloons is problematic because phasic contractions can be misinterpreted as muscle tone<sup>[42]</sup>. In addition relaxation in one end of the balloon and simultaneous contraction in the other end may not be registered as a change in volume at all; (3) Most previous studies have not validated the assumptions the data were based on. The most obvious and common mistake is to consider the fundus of the stomach to be spherical. From an anatomical and geometric point of view the stomach is not spherical and the wall structure with muscle layers in various directions indicates complex (anisotropic) properties. Distending a balloon in the fundus will deform the balloon into the corpus and antrum, resulting in a highly complex geometry; (4) No previous pressure-volume studies have considered the strain softening effect<sup>[43]</sup> and therefore it is highly questionable whether the results are reproducible. The tissue needs to be preconditioned before proper testing

The first three of the points mentioned above makes it impossible to compute the radius from the measured volumes. Consequently, estimation of wall tension using Laplace's law and computation of deformation in terms of strain measures will be invalid. It has also been a common mistake in gastrointestinal distension studies to consider the mechanoreceptors to be pressure receptors, volume receptors or tension receptors. Circumferential strain or stress are more likely candidates as the direct receptor stimulus, because the tensile stress and strain in distensible biological tubes are largest in the circumferential direction during distension<sup>[44]</sup>. Considering mechanical and receptor kinematic properties, strain is a more relevant parameter than stress (and tension). This is partly due to the fact that strain is a non-dimensional parameter that is independent of the original size of the organ and directly associated with tissue deformation. Recent studies clearly demonstrated that circumferential strain is an important determinant of mechanoreceptor mediated responses<sup>[45-48]</sup>. Correspondingly, studies providing tension calculations from Barostat studies have shown conflicting results, e.g.



**Figure 2** Top corner: The impedance planimetric probe for mechanical stimulation of the gastrointestinal tract. *l* and *d* denote infusion side for filling the balloon and the distance between two detection electrodes. Left: Recordings in the small intestine using the impedance planimetric probe with pressure, cross-sectional area (CSA) and VAS response as function of time during a ramp distension of the balloon up to the pain threshold where the bag volume is kept constant. It appears that the pressure decreased, the CSA was constant whereas the pain continued to increase.

in a recent study of the stomach the estimated tension seemed to correlate with the sensation<sup>[26,49]</sup>, whereas another study<sup>[35]</sup> showed a highly inter-individual variability in the sensation score to the applied tension, suggesting that other factors than wall tension influence the sensation. However, uncertainties in the assumptions given above, and lack of proper geometric and biomechanical considerations can also explain these findings<sup>[41]</sup>.

Newer methods based on impedance planimetry (Figure 2) allows recording of the luminal cross-sectional area directly and estimation of the radius in the distended segment of the esophagus or intestine<sup>[17,45,47,48,50,51]</sup>. From the radius it is possible to compute the circumferential wall tension and strain with more accuracy than from volume measurements<sup>[44]</sup>. Circularity must be assumed but this assumption has often been validated<sup>[44]</sup>. Finally, mechanical distension combined with ultrasound methods may offer the possibility for a better anatomical characterization and computation of stress in the GI tract<sup>[52]</sup>. For the stomach and other organs with complex geometry, imaging methods that provides data on the surface curvatures (3D-ultrasound, multislice CT-scanning or MR-scanning) in the whole organ may be used together with numerical mechanical analysis such as finite element analysis.

### Thermal stimulation

In the skin rapid heating activates first myelinated A $\delta$ -fiber, where the evoked sensation corresponds to the “first pain felt within 0.4 s after the heat stimulus. The first pain is followed by a C-fiber mediated second pain which is less well localized and of longer duration, being described as ‘throbbing, burning or swelling’<sup>[53]</sup>. Slow heating gives a preferential activation of the unmyelinated C-fibers and the best evaluation of second pain<sup>[54]</sup>. Short lasting thermal stimuli have also been used in the human GI tract. Here they are believed to activate unmyelinated afferents in the mucosa selectively as opposed to mechanical and electrical stimuli, which activate afferents in both the superficial and deeper layers<sup>[6]</sup>. Thermosensitive mucosal afferents throughout the gut have been demonstrated in animal studies<sup>[6]</sup>, and in the human esophagus, stomach and rectum<sup>[6,17,55-57]</sup>. The major limitation of these models

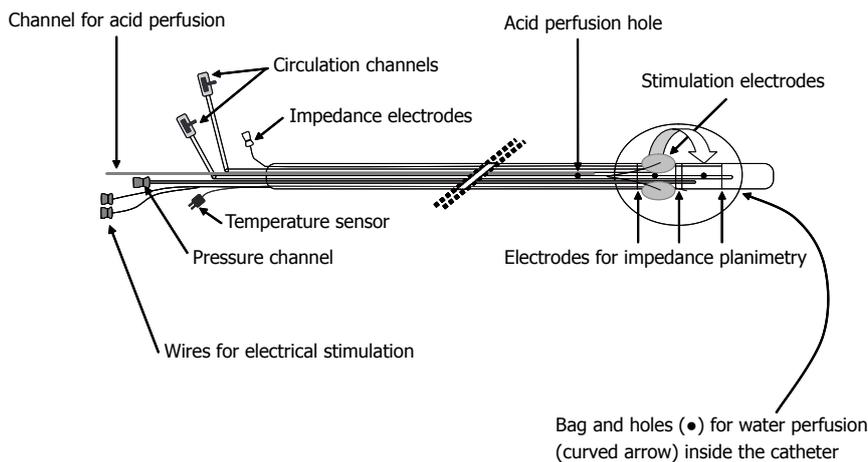
is that temperature stimuli in the range that can be felt are normally only relevant for sensation in the upper GI tract. On the other hand, although temperature receptors may have less clinical relevance, characterization of the response to stimuli in the most superficial layers of the gut may be of major importance to develop a comprehensive sensory evaluation<sup>[4]</sup>. In recent studies the temperature of re-circulating water was continuously measured inside a balloon positioned in the esophagus<sup>[17,58]</sup>. The temperature stimuli showed a nearly linear stimulus-response relationship, demonstrating the validity of the receptor activation.

### Chemical stimulation

Chemical stimulation of the GI tract more closely resembles clinical inflammation and is believed to approach the ideal experimental visceral pain stimulus<sup>[9]</sup>. Such stimuli have successfully been applied to the skin and muscles<sup>[11,59,60]</sup>. In contrast to electrical stimuli, chemical stimuli activate predominantly unmyelinated C-fibres<sup>[61]</sup> and this may be an advantage in basic studies of differentiated nerve populations. Acid stimulation of the esophagus is the most used method to sensitise the gut<sup>[59,62-65]</sup> but chemical stimulation of the gut with alcohol, bradykinin, glycerol, capsaicin and hypertonic saline were also done in humans<sup>[18,55,58,66-69]</sup>. For details see<sup>[4]</sup>. The major disadvantage of chemical stimuli is that they require a relative long latency time to the onset of effects, and that they are often not reproducible when repeated<sup>[9]</sup>. Thus, although chemical stimulation may evoke pain, the major relevance of the model is in sensitization of the visceral afferents to subsequent experimental stimulation.

### Ischemic stimulation

Ischemia has been widely used for induction of muscle pain. Ischemia is important in many diseases such as mesenteric ischemia and ischemic colitis. Ischemia has been used in animal models of visceral pain<sup>[9]</sup>. Such studies are not acceptable in humans and unfortunately no human experimental gut models exist. However, distension of the gut may result in diminished blood flow. Accordingly, Öhman *et al.*<sup>[70]</sup> observed a significant drop in blood supply during distension of balloons in the small intestine in rats.



**Figure 3** Schematic illustration of the probe used for multi-modal (electrical, mechanical, cold and warmth stimuli) of the esophagus. The stimulations can be combined with sensitization using acid perfusion of the distal esophagus.

Very recently, Hoff and coworkers presented a multimodal probe that selectively was developed for differentiation between mechanical and ischemic pain mechanism<sup>[71]</sup>.

### Multimodal stimulation of the GI tract

As pain is a multidimensional perception it is obvious that the reaction to a single stimulus of a given modality can represent only a limited fraction of the entire pain experience. The possibility for combining different methods for stimulation and assessment will approximate the clinical situation where many different nerves and pathways are activated. Thus, the method will give a more comprehensive and differentiated information about the nociceptive system compared to stimuli using single or a few modalities<sup>[72]</sup>. For comprehensive experimental studies mimicking the clinical situation, a multimodal testing approach must therefore be used, where the test battery will increase the probability for activation of a range of relevant nervous mechanisms. Such sophisticated methods will be able to select the best test procedures to explore different basic aspects of pain as well as pharmacological modulations. Multimodal models have shown their value in somatic pain models, where single modality models have been inadequate to test for example pathophysiological changes and effects of specific drugs<sup>[73,74]</sup>. Especially if the stimulation includes modalities known to evoke peripheral as well as central sensitization, the likelihood that a model will mimic clinical pain is high despite the non-harmful nature of the stimulation<sup>[73,75]</sup>. In somatic models it is possible to give many different stimuli and evoke central phenomena (e.g. central integration of summated stimuli) of clinical relevance (for review see<sup>[11,59]</sup>). In the GI tract, however, difficulties with access to the organs and technical limitations of the currently available models have until now made such a multimodal stimulation approach difficult. Some authors have combined mechanical and electrical stimuli<sup>[12,75,76]</sup> or mechanical and chemical stimuli<sup>[77,78]</sup>, and in a recent study in the esophagus mechanical and electrical stimuli were combined with sensitization to acid<sup>[79]</sup>. We recently introduced a multimodal model with combined mechanical, electrical, cold and warmth stimuli of the esophagus together with acid sensitization<sup>[17,58]</sup> (Figure 3). The multimodal approach gives the possibility for a differentiated stimulation of receptors in the

superficial and deep layers of the gut. The possibility for induction of hyperalgesia with e.g., acid perfusion and evoking central phenomena such as summation, allodynia and referred pain makes the model clinically relevant with respect to increase the knowledge of peripheral and central pain mechanisms<sup>[64,65,80]</sup>. The pain assessment should ideally also be multimodal and for example include quantitative and qualitative sensation, assessment of referred pain and neurophysiologic measurements. For details see<sup>[4]</sup>. However, it will typically not be feasible with too many stimulation and assessment parameters.

## METHODS FOR AMPLIFICATION OF THE CENTRAL NEURONAL RESPONSE

Chronic pain is characterized by modifications of the central nervous system such as central sensitization<sup>[81]</sup>. Hence, decreased thresholds to normal stimuli such as stools and air in the GI tract seem to contribute to many of the symptoms reported by patients with inflammatory and functional diseases in the gut<sup>[82,83]</sup>. Phenomena relating to sensitization of the central nervous system (CNS) are enhanced responses to repeated stimulation, allodynia/hyperalgesia and increase in the referred pain area<sup>[4]</sup>. Repeated electrical or mechanical stimuli to the small and large intestine<sup>[16,84,85]</sup> may cause integration of the neuronal response and thus pain, and this may be used as a model for enhanced central gain (Figure 4). Munakata *et al* showed the importance of central mechanisms in functional gut disorders<sup>[86]</sup>. In their study the patients developed rectal hyperalgesia following repetitive sigmoid distensions. Induction of hyperalgesia in one area of the GI tract following conditioning of the more proximal parts most probably results from central sensitization. Chemical stimulation may also evoke sensitization. Acid stimulation of the esophagus has been used as a chemogenic stimulus by several authors<sup>[76-79,87]</sup>. Increased response to mechanical, electrical and thermal stimuli after the chemical sensitization have been demonstrated<sup>[58,76,78,79]</sup>, although previous studies using latex balloons were not as consistent. This may be related to methodological problems using latex balloons, where the distension data need to be corrected for the balloons intrinsic mechanical properties *etc.*<sup>[44,78]</sup>. Sarkar *et al*<sup>[79]</sup> demonstrated the relevance

of central mechanisms in the gut, as acid exposed to the esophagus not only resulted in local allodynia, but also sensitized the proximal, non-acid exposed part of the organ to electrical stimuli. Capsaicin also increases the sensation in the esophagus to acid reflux<sup>[88,89]</sup> and when capsaicin was applied to the small intestine, consistent hyperalgesia to mechanical stimuli was seen<sup>[18]</sup>. Lipid infusion in the duodenum has also been shown to induce allodynia and increase the referred pain area to distension in functional disorders<sup>[90,91]</sup>. As fat is a natural stimulus to the gut, such studies may increase the understanding of the postprandial symptoms in these patients.

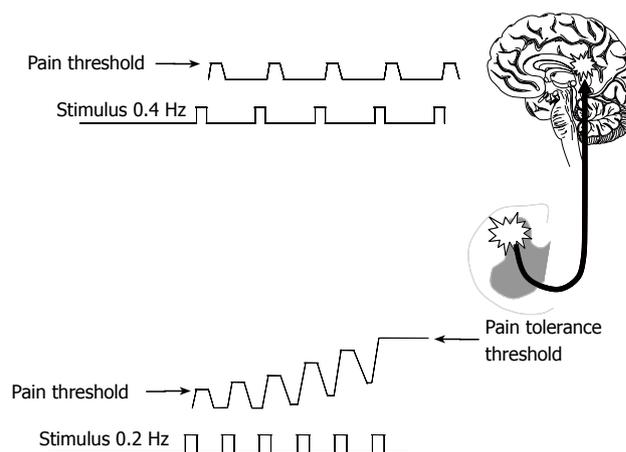
## EXPERIMENTAL PAIN AND GASTRO-ESOPHAGEAL REFLUX DISEASE

Gastro-esophageal reflux disease (GERD) is defined as chronic mucosal damage or typical symptoms, which reduce quality of life by the abnormal reflux of gastric contents into the esophagus. GERD is very common in the population with up to 30% of the European population reporting heartburn and/or acid regurgitation during the previous 12 mo<sup>[92]</sup>. Recent studies revealed that up to 70% of the GERD patients have non-erosive reflux disease (NERD) according to endoscopy, 24-h pH profile and symptom-reflux association indices. However, in patients with NERD, the quality of life impairment is comparable to that in patients with erosive esophagitis<sup>[93]</sup>. The symptoms in reflux disease are highly variable and poorly understood. Thus, in patients with GERD no simple relation seems to exist between the symptoms and severity of the disease<sup>[94-96]</sup>. Although treatment with proton pump inhibitors (PPI) is very effective, many patients continue to have symptoms despite treatment<sup>[97,98]</sup>, and in a recent study 50% of the patients continue to have pathologic reflux despite effective symptom control with PPI<sup>[99]</sup>. Furthermore, it is estimated that 30%-60% of patients with NERD will have normal ambulatory 24-h esophageal pH monitoring and thus no effect of PPI treatment<sup>[93]</sup>. Although some of these patients may have reflux of non-acid gastric contents, it is still not clear what causes the symptoms in many patients.

Experimental pain methods have contributed to the understanding of the symptoms in reflux disease. In an animal study Garrison *et al.*<sup>[100]</sup> demonstrated that spinal neurons in the cat receiving input from the distal esophagus also received convergent input from the thoracic wall and the heart. When the esophagus was sensitized with turpentine, the neurons responded to a smaller mechanical stimulus from the different sites. Such data gives evidence that central mechanisms may explain the symptoms in a substantial proportion of the patients. In humans, however, relative few studies have been done to explore the pain mechanisms in reflux disease.

### Non-erosive reflux disease

**Peripheral pain mechanisms:** Assessment of mechanosensitivity using intra-esophageal balloon distension has yielded contradictory results. In NERD patients Rodriguez-Stanley *et al.*<sup>[101]</sup> reported a decrease



**Figure 4** Central integration of repeated stimulation is seen when the stimulation frequency exceeds 0.3 Hz (bottom picture), whereas slower stimulation does not change the central gain (top picture).

in sensation and pain thresholds to distension compared with a historic control group. Trimble *et al.*<sup>[102]</sup> studied NERD patients without excessive reflux and found that these patients were most sensitive to esophageal balloon distension, whereas patients with excessive reflux had a level of sensitivity similar to that of healthy control subjects. In another study using esophageal balloon distension delivered by an electronic barostat, patients with NERD and patients with erosive esophagitis did not demonstrate an increase in mechanosensitivity when compared to normal controls<sup>[87]</sup>. The previous experimental pain studies used mechanical stimulations based on recording of volume and pressure. As stated above these studies may lead to errors relating to the deformation field and erroneous conclusions<sup>[47]</sup>. It has been suggested that chronic esophageal exposure to excess acid affects chemosensitive but not mechanosensitive afferent pathways<sup>[87]</sup> and that the key abnormality in NERD patients is that they are hypersensitive to acid reflux. Recently we conducted a study in patients with NERD where 50% had a positive 24-h pH measurement. Furthermore a multimodal approach was used. Our data showed that the patients had hyperalgesia to heat stimulation, whereas they were hyposensitive to mechanical stimulations. There was a difference in the NERD subgroups as patients with a pathological pH profile exhibited hyposensitivity to mechanical stimulations compared to both controls and patients with normal pH monitoring (Figure 5). Taken together with the above findings the results reflect that patients with pathological acid reflux may be less sensitive to mechanical stimulation and more sensitive to heat. The selective sensitization to heat may be related to specific receptor activation. Recently, we showed that acid perfusion of the esophagus in healthy subjects differentially sensitizes the esophagus to heat, but not cold stimuli<sup>[80]</sup>. The VRI receptor may be important for our findings. The receptor can be activated by a variety of stimuli, including acid (protons) and increases in temperature that reach the noxious range. Thus, it can be hypothesized that in patients with NERD the acid reflux and resulting peripheral sensitization

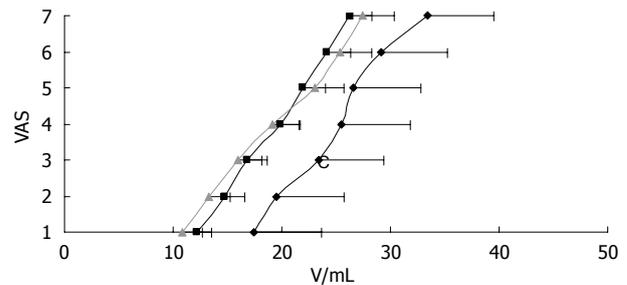
results in a significant change in the sensation to heat stimuli working on the same receptor as the acid. Further studies need to explore whether the VRI receptors are up-regulated as seen in patients with erosive disease of the esophagus<sup>[103]</sup>-see below.

**Central pain mechanisms:** In the studies described above we also found an increase in the referred pain areas for both mechanical and heat stimulation in NERD patients. Previously we showed that acid perfusion of the distal esophagus in healthy subjects resulted in an increase in the referred pain area to differentiated esophageal stimuli<sup>[58]</sup>. This is most likely related to central neuronal hyperexcitability after acid perfusion, and subsequent opening of latent connections between converging neurons from visceral and somatic structures in the CNS<sup>[81]</sup>. Thus, the larger and widespread localization of the referred pain area is thought to represent central hyperexcitability. Consistent with these findings Penagini *et al*<sup>[104]</sup> showed that patients with NERD had increased sensitivity to distension of the proximal stomach. This viscerovisceral hyperalgesia is also considered a central phenomenon<sup>[105]</sup>. Experimental acid perfusion of the esophagus in healthy subjects has also been shown to increase the amplitude of the polysynaptic nociceptive reflex working at the spinal level<sup>[58]</sup>. Sarkar *et al*<sup>[79,106]</sup> demonstrated that acid perfusion of the distal esophagus resulted in allodynia and shorter latencies of the evoked brain potentials to electrical stimulation of a more proximal segment of the esophagus. Accordingly, we recently found a backward shift of the early activity in the cingulate gyrus to esophageal pain stimuli after acid perfusion of the organ<sup>[107]</sup>. Thus, there is substantial evidence that exposure of acid in the esophagus such as in some NERD patients may result in central neuroplastic changes at spinal and supraspinal levels.

### Erosive GERD

**Peripheral pain mechanisms:** Patients with erosive disease of the esophagus may have a more severe disease than NERD patients, although erosive disease may also be a distinct entity<sup>[108]</sup>. In a study comparing patients with esophagitis with controls, Fass *et al*<sup>[87]</sup> demonstrated enhanced perception to acid perfusion, but the response to mechanical stimulation was normal. Such findings may point towards a differential effect on mechano-sensitive and chemo-sensitive pathways in esophagitis. Recently, we compared the sensory response in patients with grade B esophagitis and healthy controls using the multimodal approach described above. The patients had hyposensitivity to the mechanical stimulations, but had hyperalgesia to heat. These findings were comparable to those in NERD patients with abnormal pH profile and indicate that the pain mechanisms may be the same whenever erosions occur or not. A recent paper suggested that abnormal tissue resistance to acid may explain both the hyperalgesia and motor abnormalities seen in many patients with GERD and NERD<sup>[109]</sup>. In our studies the patients showed hyperalgesia to heat (but not cold) stimuli<sup>[110]</sup>. We believe that VR1 receptors sensitized by the acid reflux are important, and VR1 receptors have recently been shown to be up-regulated in esophagitis<sup>[103]</sup>.

**Central pain mechanisms:** Central changes are also



**Figure 5** Stimulus-response function (mean  $\pm$  SEM) to mechanical stimulation in patients with non-erosive gastro-esophageal reflux disease (NERD). The controls ( $\blacksquare$ ,  $n = 15$ ) and patients with a normal 24-h pH-profile ( $\blacktriangle$ ,  $n = 7$ ) had similar response to the mechanical stimulation, whereas patients with pathological pH-profile ( $\diamond$ ,  $n = 6$ ) showed hypoalgesia to the stimulations. The x-axis shows the volume of the balloon in the distal esophagus and the y-axis denotes the pain response on a visual analogue scale (VAS) with 5 as the pain threshold.

believed to be important in erosive disease. Although Fass *et al*<sup>[87]</sup> found a normal location of the referred pain we recently showed that the size of the referred pain area was larger than in controls. Thus, there is substantial evidence that exposition of acid in the esophagus in patients with esophagitis results in central neuroplastic changes. One can speculate that the reason for specific hyposensitivity to mechanical stimulations in patients with erosive disease may be related to well functioning counter-regulatory neural mechanisms acting from the brain stem at the spinal cord level. These may prevent the development of long-lasting sensitization of mechanosensitive afferent pathways<sup>[111]</sup>. The central pain modulating systems rely on a balance between facilitatory and inhibitory descending pathways and intrinsic spinal circuits and is not predictable in the individual patient<sup>[112,113]</sup>. Studies on animals have shown that the system is an important mechanism in the modulation of visceral stimuli<sup>[114]</sup> and these neuroplastic changes may result in increased referred pain on the one hand and dampening of the activity from mechanosensitive pathways on the other. In general chronic tissue injury and pain has been associated with higher thresholds to mechanical stimulation in different regions of the gastrointestinal tract. For example, chronic inflammation of the small bowel<sup>[115]</sup> in patients with inflammatory bowel disease is not associated with mechanical hyperalgesia of the rectum. This is contrasted by the pain in functional visceral disorders where hyperalgesia and allodynia to mechanical stimuli of the gut are typically found<sup>[86,115-118]</sup>. Hence, it can be speculated that a difference in the balance between noxious control systems arising in the brainstem may explain the findings in the different patient groups.

### Non-cardiac chest pain

Non-cardiac chest pain (NCCP) was a term invented by the cardiologists. It was defined as “angina-like” chest pain without demonstrable abnormalities in the coronary vasculature. The more broad term “unexplained esophageal chest pain” is probably a better definition in gastroenterology, and may be defined as chronic chest pain, which is most likely of esophageal origin, but where

conventional tests for cardiac, esophageal, pulmonary (pain can be evoked from the pleura only) and musculoskeletal disorders are negative. The pain can be angina-like, but can also mimic other chest pain disorders such as gastro-esophageal reflux disease and musculoskeletal disorders. However, in the following the term NCCP will be used as it is the most common description of the syndrome. NCCP is very common especially in the younger age groups and the prevalence ranged from 22%-30% in the general population<sup>[119,120]</sup>. However, many persons have only modest and intermittent complaints and such cases must be classified as intermittent chest pain that is of no major burden, and a rough estimate would be that severe symptoms are seen in 2%-3% of the population.

If the patients describe "heartburn" and "acid regurgitation" they are typically classified as having GERD. However, burning pain may also be evoked by e.g., experimentally distension or electrical stimulation of the stomach<sup>[121]</sup> and the value of the subjective description of chest pain is up to discussion. GERD is also the most frequent cause of NCCP<sup>[122,123]</sup>. In this case the chest pain is no longer unexplained and the overlap invalids the diagnostic criteria for NCCP. The reason for heartburn in GERD is also up to discussion. Fass *et al.*, have demonstrated that acid is not the only visceral stimulus that leads to heartburn<sup>[87]</sup>, and recently Balaban *et al.*, suggested that longitudinal muscle contractions of the esophagus as detected by high frequency intraluminal ultrasound, may be the motor equivalent of heartburn sensation<sup>[124]</sup>. Thus there is major overlap between the symptoms relating to the different chest structures.

### **The origin of chest pain**

The symptom description is consistent with the organization of the nerves supplying the esophagus and the chest. The visceral afferents converge on a large scale with neurons that receive input from superficial and deep somatic tissue as well as other viscera<sup>[105]</sup>. Accordingly, in the thoracic spinal cord more than 75% of all neurons receive both somatic and visceral information, being contrasted by the actual number of visceral afferents (5%-15% of the inflow) entering the dorsal roots<sup>[6,125]</sup>. The visceral afferent terminals are widely distributed in the spinal cord and some organs have a wide spinal representation spreading to several segmental levels. For example the esophagus is innervated cranio-caudally from the mid-cervical to the second lumbar segments<sup>[6]</sup>. Therefore a few visceral afferent fibers can activate many central neurons. Through the extensive functional convergence of somatic and visceral nerves on the same central neurons this wide activation of the CNS may explain the diffuse and unpleasant nature of visceral pain together with referred pain to somatic structures and other viscera<sup>[126]</sup>. In the clinic the above considerations typically leads to a very diffuse and heterogeneous description of the symptoms and experimental pain models may shed light over the pathogenesis of NCCP and related symptoms.

### **Peripheral pain mechanisms**

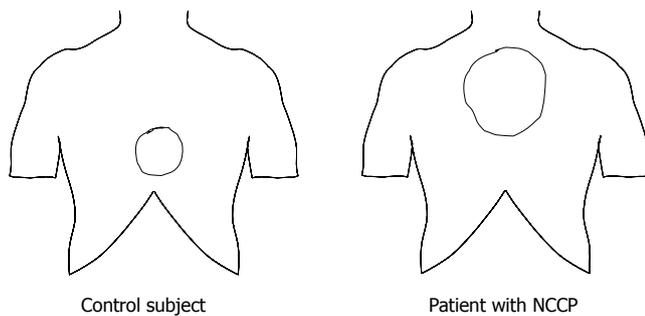
Experimental pain studies in patients as outlined in the

Introduction section have the possibility to control the stimulus and assessment parameters, and thus explore the abnormal pain system in further detail. Most studies found that the sensation and pain detection thresholds to distension, electrical and acid stimuli of the esophagus were lower in NCCP patients compared with thresholds found in healthy subjects<sup>[76,79,127-132]</sup>. However, the reliability of the test for diagnosing NCCP has been questioned<sup>[4]</sup>, and in some experiments only few patients had abnormal sensation<sup>[133]</sup>. There are, however, severe methodological problems relating to these studies. Early balloon distension studies were based on simple volume and pressure measurements using latex balloons. As stated in the section above "Mechanical stimulations" this caused large errors due to the deformability of latex and lack of control of the stimulation field. There are also limitations and sources of error with the systems based on volume and pressure. The use of methods based on impedance planimetry has encompassed many of these limitations. Rao *et al.*<sup>[134]</sup> used impedance planimetry and showed that patients with NCCP suffered from esophageal hyperalgesia. Furthermore the esophagus was more "irritable" than in controls, as the distensions resulted in more vigorous contractions at lower pressures. In more recent studies hyperalgesia was also found after relaxation of the smooth muscle with atropin<sup>[135]</sup>, and there was evidence that the lower segments of the esophagus exhibited a higher level of sensory dysfunction compared to higher segments<sup>[136]</sup>. These studies were based on phasic distensions, which did not take the strain softening effect into account. Hence, we recommend to preconditioning the tissue by several distensions until the stress-strain relationship becomes reproducible<sup>[17,45,47]</sup>.

### **Central pain mechanisms**

Paterson *et al.*<sup>[137]</sup> showed that repeated distensions conditioned the esophagus in NCCP resulting in higher pain scores. The effect of repeating stimulations has also been demonstrated in patients with irritable bowel syndrome, and probably this is a phenomenon relating to central hyperexcitability<sup>[86]</sup>. In a recent study we used a ramp distension protocol to distend the distal esophagus in patients with NCCP before and after sensitization with acid<sup>[138]</sup>. Patients with NCCP did not seem to be more vulnerable to develop esophageal hyperalgesia to the slowly increasing mechanical distensions as compared to controls. However, there was evidence for abnormal central pain processing as there was an increased and widespread referred pain area to the mechanical stimulations (Figure 6) and the patients were sensitive to repeated mechanical stimulation. Furthermore, after acid perfusion (believed to evoke hyperexcitability of central pathways mainly) there was a major sensitization to the distensions. Thus, it was concluded that NCCP patients showed facilitated central pain mechanisms, which may explain the character of their symptoms. This may have important implications for the diagnosis and treatment in these patients, where drugs with central effects should be used in the treatment.

Several studies have focused on brain activation to experimental pain stimuli in NCCP. The



**Figure 6** The localization and size of the referred pain area to mechanical stimulations of the distal esophagus in a typical control subject and a patient with non-cardiac chest pain (NCCP). The patient had an increased size and abnormal localization of the referred pain.

electroencephalography monitors the brain activity to external stimuli directly in real time. When a repetitive stimulus is applied and the cortical electrical activity is averaged (time-locked to the stimulus), the stimulus evoked cortical potential (EP) can be extracted from the background electrical activity and is shown in shape of a waveform with different peaks. Each peak in the EP represents a synaptic event associated with the transmission of afferent information from one group of neurons to another. The early peaks are supposed only to be influenced by the stimulation rate, intensity and localization, and they reflect to a major degree the brain loci that process the pain intensity and localization<sup>[139,140]</sup>. Three studies showed lower EP amplitudes to esophageal electrical and distension stimuli in patients with NCCP compared with healthy subjects<sup>[127-129]</sup>. Furthermore, Hollerbach *et al*<sup>[128]</sup> found shorter latencies of the EP peaks to electrical esophageal stimulation and more pronounced changes of the outflow of the autonomic nervous system in NCCP patients. Frobert *et al*<sup>[141]</sup> stimulated additionally the sternal skin (referred pain area) and showed shorter latencies and lower amplitudes of the EP to skin stimulation in NCCP patients. These findings suggest an increased central nervous system response to visceral stimuli in patients with NCCP comparable to those found in other groups with functional bowel disorders, e.g. patients with irritable bowel syndrome. Recently, Hobson and Aziz<sup>[142]</sup> suggested that different subgroups of patients with NCCP may exist: (1) Those with short latency of the early EP components having sensitization of gastrointestinal afferent pathways, and (2) those with long latencies and enhanced late responses reflecting hypervigilance and increased affective processing.

## CONCLUSION

Pain is the most prevalent symptom in gastroenterology but yet poorly understood. This is reflected in the treatment of visceral pain that is often very difficult and highly challenging. Methods to evoke and assess experimental pain in the GI tract have recently been improved and used to explain the pain mechanisms in health and disease. In diseases relating to the esophagus these methods have contributed to our understanding of the symptoms reported in these patients. Especially does

abnormal activation and plastic changes of central pain pathways seem to play a major role. These findings may lead to an alternative approach for treatment in patients that does not respond to conventional medical or surgical therapy, and probably analgesics with effect on the central nervous system such as tricyclic antidepressives, serotonin reuptake inhibitors, ion-channel blockers or opioids with additional effect on the N-methyl-D-aspartate (NMDA) receptor or kappa- opioids receptors should be used. Phase II trials where the experimental methods are used to evaluate these drugs will be a feasible way to obtain more knowledge, before more expensive large scale phase III studies in the clinic are initiated.

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