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GUIDELINES CLINICAL PRACTICE

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New technologies to investigate the brain-gut axis

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Abstract

Functional gastrointestinal disorders are commonly encountered in clinical practice, and pain is their commonest presenting symptom. In addition, patients with these disorders often demonstrate a heightened sensitivity to experimental visceral stimulation, termed visceral pain hypersensitivity that is likely to be important in their pathophysiology. Knowledge of how the brain processes sensory information from visceral structures is still in its infancy. However, our understanding has been propelled by technological imaging advances such as functional Magnetic Resonance Imaging, Positron Emission Tomography, Magnetoencephalography, and Electroencephalography (EEG). Numerous human studies have non-invasively demonstrated the complexity involved in functional pain processing, and highlighted a number of subcortical and cortical regions involved. This review will focus on the neurophysiological pathways (primary afferents, spinal and supraspinal transmission), brain-imaging techniques and the influence of endogenous and psychological processes in healthy controls and patients suffering from functional gastrointestinal disorders. Special attention will be paid to the newer EEG source analysis techniques. Understanding the phenotypic differences that determine an individual's response to injurious stimuli could be the key to understanding why some patients develop pain and hyperalgesia in response to inflammation/injury while others do not. For future studies, an integrated approach is required incorporating an individual's psychological, autonomic, neuroendocrine, neurophysiological, and genetic profile to define phenotypic traits that may be at greater risk of developing sensitised states in response to gut inflammation or injury.

Key words: Brain-gut axis; Central processing; Neuraxis; Neurophysiology

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INTRODUCTION

Pain is a complex multidimensional experience comprising sensory-discriminative, affective-motivational and cognitive-evaluative components[1]. The sensory-discriminative component represents the ability to localise pain, and assess its intensity whereas the affective-motivational component qualifies its unpleasantness and gives rise to emotional aspects such as fear and distress. The cognitive-evaluative component allows the evaluation and interpretation of the pain experience and is involved in attention, anticipation and memory of the experience[2].

Pain is an extremely common symptom in clinical practice[3] and often emanates from the intra-abdominal viscera. Visceral pain can be the manifestation of a myriad of underlying pathologies, occur with varying intensities ranging from mild discomfort to severe pain, be acute or chronic, and be referred to a variety of locations such as the chest, pelvis and skin. Understanding the complex mechanisms leading to the development and maintenance of visceral pain, in particular that which arises from

the gastrointestinal tract, requires an appreciation of the neuroanatomical structures and neurophysiological processes involved. The gastrointestinal (GI) tract has a complex innervation including sensory neurones (afferents), and the rich neuronal innervation closely regulates visceral function as well as providing sensory information to higher structures. The ability to dissociate specific neurophysiological mechanisms of aberrant gastrointestinal sensory processing has been the aspiration of an increasing number of gastrointestinal researchers. Improved access to brain imaging techniques has vastly increased our understanding of the central processing of gastrointestinal sensation and pain in both healthy volunteers as well as in patients suffering from functional gastro-intestinal disorders (FGID).

So how far are we now? As the episodical gastrointestinal pain still exploits different non-investigated aspects, the question is whether the newer brain-imaging techniques have provided the scientists with further understanding of the underlying pathophysiology and mechanisms in FGID? This review will focus specifically on the sensory pathways (peripheral, spinal and supraspinal) involved in these pain mechanisms and highlight the newer techniques in electroencephalogram (EEG) source analysis.

SENSORY INNERVATION OF THE GASTROINTESTINAL TRACT

The gastrointestinal (GI) tract has a complex innervation with sensory neurones (afferents). As well as receiving dual sensory innervation from the central nervous system (CNS) referred to as extrinsic afferents, it has its own integrated network of intrinsic afferents (the enteric nervous system, ENS), that project locally. This rich neuronal innervation closely regulates visceral function as well as providing sensory information to higher structures.

Intrinsic sensory innervation (enteric afferent neurones)

The hollow intra-abdominal viscera have a rich sensory innervation with locally projecting afferent neurones, forming the enteric nervous system, whose cell bodies are located in the myenteric or submucosal plexuses[4]. This network of neurones and interneurones has a structural complexity and functional heterogeneity similar to that of the CNS, but mainly regulates local functions and reflexes such as secretion, motility, mucosal transport and blood flow[5,6]. Motor neurones located within the ganglia of the ENS coordinate these functions largely by regulation from local sensory neurones, although some also receive inputs from the CNS *via* autonomic (both sympathetic & parasympathetic) pathways[7]. Although the majority of enteric afferent axons are confined to the gut wall, some can project to the pre-vertebral ganglia of the sympathetic nervous system[8].

Extrinsic sensory innervation (primary afferent neurones)

The gastrointestinal tract has a dual sensory innervation from the CNS. In humans, visceral afferents project to the CNS mainly *via* the vagus nerve to the brainstem (vagal afferents) or through splanchnic nerves to the spinal cord (spinal afferents), and are described below.

Vagal afferent neurones

The vagus nerve innervates the majority of the GI tract apart from the distal third of the colon[9]. 70%-90% of the fibres in the vagal trunks are unmyelinated C-fibre neurones with their cell bodies located in the nodose ganglia situated just below the jugular foramen, although a minority lie more proximally within the jugular ganglia and contain afferents primarily from the oesophagus[10]. Around 80%-85% of nerve fibres in the vagus are afferent and project viscerotopically to the medial division of the nucleus of the solitary tract (NTS). Second-order neurones project from the NTS to sites in the brainstem, hypothalamus and amygdala including the vagal motor nuclei, the rostral areas of the ventrolateral medulla and the parabrachial nuclei[11,12]. Cortical projections from the brainstem include the orbitofrontal, infralimbic anterior cingulate and insula cortex, the latter having reciprocal connections with the secondary somatosensory cortex.

Vagal afferents are classically believed to mediate non-noxious physiological sensations such as satiety and nausea due to their low response thresholds and saturation characteristics that are within the physiological range[13-15]. However, animal experiments have suggested that vagal afferents may be involved in the central inhibitory modulation of pain. For instance, electrical stimulation of cervical vagal afferents inhibits the responsiveness of spinothalamic tract neurones to noxious stimuli[16].

Spinal afferent neurones

Spinal afferent neurones project from the viscera through the splanchnic nerves to the thoracic, upper lumbar and sacral spinal cord with their cell bodies located in the dorsal root ganglia (DRG). They constitute only 5%-10% of all afferent fibres in the thoracic and lumbar dorsal nerve roots with the majority traversing the pre- and paravertebral ganglia en route to the spinal cord. Collaterals to the prevertebral ganglia may mediate local autonomic reflexes[7].

Spinal afferents are contained within the cardiac (superior, middle and inferior) and splanchnic (thoracic, greater and lesser) nerves. These pass through the white rami to join spinal nerves before entering the DRG. The oesophagus is innervated craniocaudally by afferents from the DRG located between the first cervical and third lumbar segments. Retrograde labelling studies have shown the maximum distribution of spinal sensory neurons to be in the following DRG: C1-T8 (striated muscle); C5-L2 (smooth muscle), and T1-L3 (lower oesophageal sphincter)[17].

SPINAL PAIN PROCESSING

From the cell bodies within the DRG, spinal visceral afferents enter the spinal cord and ascend or descend one or two spinal levels in the dorsolateral fasciculus (Lissauer's tract) before terminating within the grey matter. In the

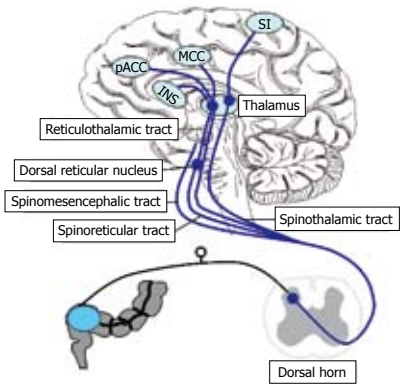

Figure 1 The principal visceral projections from the spinal cord to subcortial and cortical structures (blue lines). The spinothalamic tract terminates in the medial and posterior thalamus. Thalamocortical fibres then project to the primary somatosensory cortex. The spinoreticular tract terminates in the reticular formation to the medial thalamus. The spinomesencephalic tract projects to various regions in the brainstem, including the periaqueductal grey, locus coeruleus, and dorsal reticular nucleus in the medulla. Thalamocortical projections from the medial thalamus project to the cingulate cortex and insula which are involved in processing noxious visceral and somatic information. The brain regions innervated by these pathways that respond to painful visceral stimuli include the thalamus, insula, amygdala and anterior cingulate cortex (ACC). The ACC is comprised of two components, the perigenual ACC (pACC) involved in affect and the mid cingulate cortex (MCC) with behavioural response modification. Other pathways for transmission of noxious visceral stimuli (such as the dorsal column pathway), exist, but are not shown here.

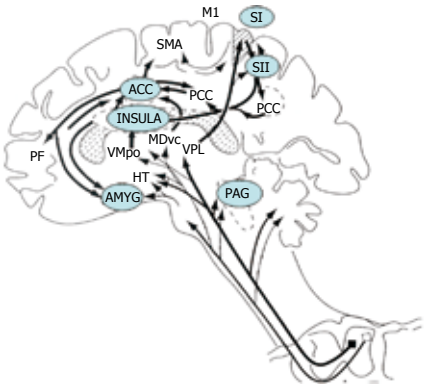

Figure 2 The subcortical and cortical structures that have been shown to be activated in response to visceral pain. PAG: Periaqueductal grey; PB: Parabrachial nucleus of the dorsolateral pons; VMpo: Ventromedial part of the posterior thalamic nuclear complex; MDvc: Ventrocaudal part of the medial thalamic dorsal nucleus; VPL: Ventroposterior lateral thalamic nucleus; ACC: Anterior cingulate cortex; PCC: Posterior cingulate cortex; HT: Hypothalamus; S1, S2: First and second somatosensory cortical areas, respectively; PPC: Posterior parietal complex; SMA: Supplementary motor area; AMYG: Amygdala; PF: Prefrontal cortex; M1: Motor cortex. (Adapted from Price DD. Psychological and neural mechanisms of the affective dimension of pain. *Science* 2000; 288: 1769-1772).

1950's Rexed divided the spinal grey matter into a system of ten laminae (LI-LX) which in turn divides the grey matter into four regions: the dorsal horn (LI-VI), the intermediate zone (LVII), the ventral horn (LVIII and IX) and the region of the central canal (LX)[18]. Second order neurones in the afferent pathway have a cell body in the dorsal horn of the spinal cord and relay signals to the brain *via* a number of ascending tracts.

The central pathways for processing nociceptive information begin at the level of the spinal cord dorsal horn. Spinal afferent projections terminate in distinct laminae of the spinal cord dorsal horn (mainly I and V, and occasionally to the contralateral laminae V and X) where they are organised in a segmental manner, but distributed over several spinal segments[19]. This diffuse termination pattern may explain the poor localisation of visceral sensation often seen in clinical practice, whereas the convergence of visceral and spinal afferents in the spinal dorsal horn may explain the phenomenon of viscerosomatic convergence, whereby visceral pain is often referred to nearby somatic structures[20,21].

ASCENDING SPINAL PATHWAYS

The ascending spinal tracts that convey sensory information to supraspinal structures are contained within the anterior lateral and posterior tract systems. The anterior lateral system comprises the spinothalamic, spinoreticular, spinomesencephalic, and spino-limbic tracts, illustrated in Figure 1. The medial and lateral subdivisions of the spinothalamic tract project to the medial/intralaminar and ventral/ventral posterior lateral (VPL) nuclei of the thalamus. respectively[22]. Third-order thalamocortical fibres then project to the somatosensory, insula and medial prefrontal cortices[23]. The spinothalamic tracts mediate sensations of pain, cold, warmth and touch and are also important for sensory discrimination and localisation of visceral and somatic stimuli[24,25].

The spinoreticular tract conducts sensory information from the spinal cord to the reticular formation in the brainstem. The reticular formation is mainly involved in the reflexive, affective and motivational properties of such stimulation[26]. Third-order reticulothalamic tract neurons project from the dorsal and caudal medullary reticular formation to the medial and intralaminar nuclei of the thalamus. From the intralaminar nuclei, ascending pain signals spread bilaterally to the prefrontal cortex (PFC), including the anterior cingulate cortex (ACC)[25]. The spinomesencephalic tract ascends the spinal cord with fibres to various regions in the brain stem, including the periaqueductal grey (PAG), locus coeruleus (LC), and dorsal reticular nucleus in the medulla[25].

The spinolimbic tracts project to areas such as the amygdala, medial thalamus, hypothalamus and other limbic structures, and are also believed to be important in mediating the motivational aspects of pain[25]. See Figure 2.

The posterior system comprises three synapsing tracts: first order dorsal column neurones, the post-synaptic dorsal column (PSDC) pathway and the spinocervical tract. These pathways were not believed to convey nociceptive information; however, recent studies have highlighted the importance of the dorsal column in viscerosensory processing. Al-Chaer demonstrated in primates that the responsiveness of neurones in the ventral posterior lateral nucleus of the thalamus to colorectal distension could be significantly attenuated by dorsal column lesions[27]. Lesions of other tracts had no consistent effects, thus, supporting the role of the dorsal column in conveying visceral nociceptive input to the thalamus.

PAIN PROCESSING IN THE BRAIN

Knowledge of how the brain processes sensory information from visceral structures is still in its infancy; however, our understanding has been propelled by technological imaging advances such as functional Magnetic Resonance Imaging (f-MRI), Magnetoencephalography (MEG), Positron Emission Tomography (PET), and EEG. Human studies have non-invasively demonstrated the complexity involved in pain processing, and highlighted a number of subcortical and cortical regions involved.

The pathways involved in the perception of visceral pain are highly complex. In addition, these pathways are dynamic and amenable to change in response to internal or external stressors. Numerous mechanisms can be engaged in response to stressors from the primary afferent level right up to the cerebral cortices, resulting in a high degree of plasticity in the nervous system. The ultimate outcome of pain perception is brought about by a delicate balance between facilitatory and inhibitory mechanisms. As pain is a conscious feeling, the ultimate goal in pain-imaging is to follow the pain stimulus throughout the neuraxis.

Imaging studies have been performed to explore normal brain processes involved in visceral perception, whether liminal or subliminal and its modulation by attention, conditioning and emotion[22,28-31]. Several studies have also looked at the role of visceral perception in emotions and cognitive processes such as learning[32,33].

Visceral pain has been contrasted with pain arising from superficial skin structures[34,35]. Recent reviews have summarized imaging findings in normal GI sensation[36-38].

Recently, a number of new technologies have emerged within imaging of the brain-gut axis, and in this review we focus on the EEG techniques where signal analyses have made it possible to follow the early and pain specific pathways to the brain with high temporal and spatial resolution.

IMAGING TECHNIQUES

Most commonly f-MRI is based on a technique using different paramagnetic properties of oxy- and deoxyhaemoglobin in the blood. These regional changes in blood flow, volume and oxygenation of haemoglobin derive from changes in neuronal activity and, thus, regions of activation may be identified by subtracting regional cerebral blood flow during a control condition from blood flow during a stimulus condition or by correlating regional blood flow with the intensity or time course of a stimulus or its perception[2]. A major advantage of f-MRI is that it is non-invasive and non-cumulative, allowing subjects to be studied repetitively. f-MRI has an excellent spatial resolution (2-5 mm), especially in the more superficial layers. Limitations are seen in the deeper structures, such as the brainstem and thalamus, due to pulsation artefacts. The temporal resolution is poor (1-3 s) and therefore f-MRI is not a specific tool for investigating the neuronal activity directly related to the painful stimuli. Since the exogenous brain activity takes place within the first 150 ms post stimulus, the response may miss the fast occurring activity and model, instead, the endogenous activity rather than brain responses due to pain. In contrast to PET studies a limitation in f-MRI studies is the lack of information regarding neurotransmitters or involved receptors[39]. A comparison between localization of visceral and somatic regions of the oesophagus in healthy subjects using fMRI has been done[40]. Distension of the distal oesophagus was represented bilaterally at the junction of SI and SII. Different activation patterns were also observed in the ACC, prefrontal cortex and cerebellum. Another recent study was carried out to determine whether behavioural differences are due to differences in the central processing of visceral and somatic pain[30]. It was demonstrated that visceral stimuli induced deactivation of the perigenual cingulate bilaterally with a relatively greater activation of the right anterior insula i.e. regions encoding affect. Kwan *et al*[41] used f-MRI as a diagnostic tool for demonstrating abnormal brain processing in Irritable Bowel Syndrome (IBS). They identified abnormal event-related sensations in five brain regions following rectal distensions. In the primary sensory cortex, there were urge-related responses in the IBS, but not the control group. In the medial thalamus and hippocampus, there were pain-related responses in the IBS, but not the control group. However, pronounced urge- and pain-related activations were present in the right anterior insula and the right anterior cingulate cortex in the control group, but not the IBS group. These findings conflict with the findings of Bonaz *et al*[42], who demonstrated significant deactivations within the right insula, the right amygdala, and the right striatum following rectal stimulations in patients suffering from IBS compared to healthy subjects.

PET

PET measures the cerebral blood flow after injection of a radioisotope. The most commonly used in gastrointestinal research is $H_2{}^{15}O$ labelled water. PET has excellent spatial resolution (2-5 mm) and allows the operator to tag important biological molecules that bind to targeted receptor groups or glucose metabolism in active neuronal tissue. PET is superior in imaging radiopharmaceuticals and/or other ligands as it offers the ability to study receptor distribution and explore the site of action[2]. However, the temporal resolution is poor (minutes), and as the subject receives a considerable dose of radiation, group analyses are needed for meaningful results, interpreting endogenous brain activity following pain rather than exogenous brain activity following painful stimulation. Another major disadvantage is the expense of a PET scanner.

Silverman *et al*[43] characterized the cerebral processing of visceral noxious events, by measuring the changes in regional cerebral blood flow. Healthy controls demonstrated a significant increase in anterior cingulate cortex activity following noxious stimuli, whereas no activity was seen in response to non-painful stimuli. In patients suffering from IBS, the ACC failed to respond to the same stimuli, whereas significant activation of the left prefrontal cortex was seen. In contrast, another study

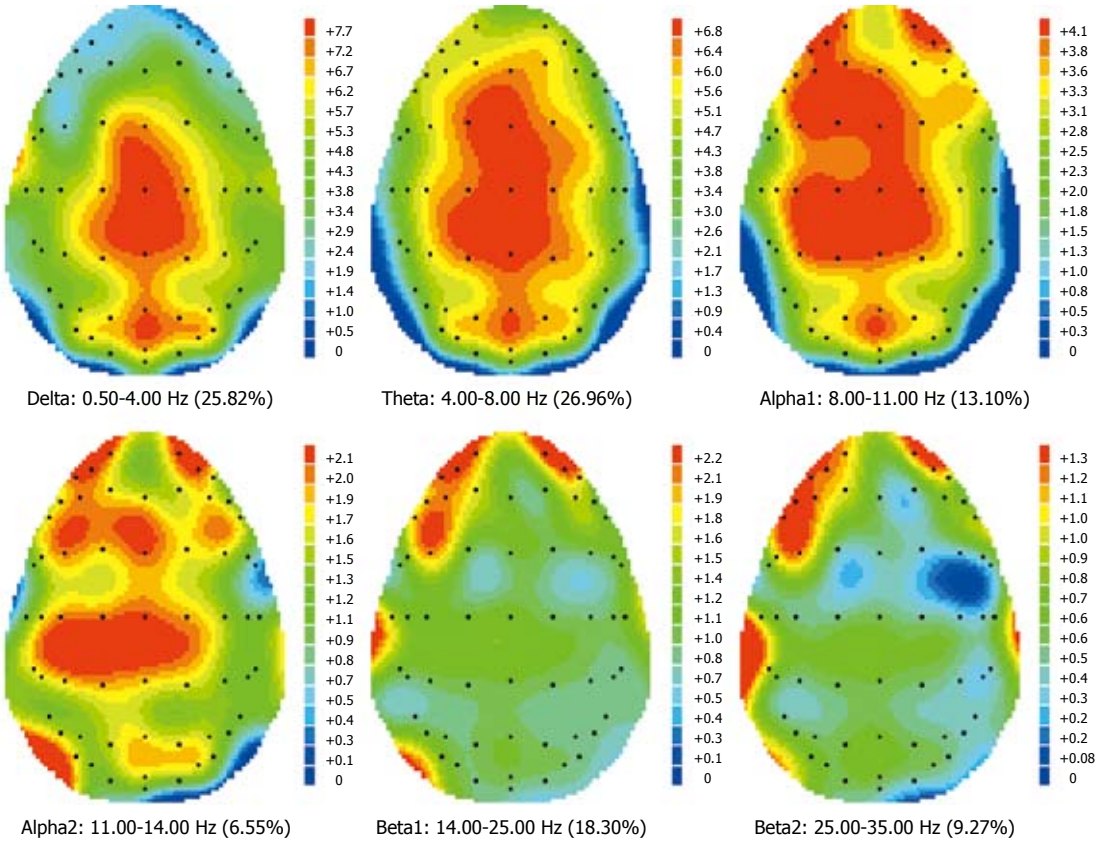

Figure 3 Example from painful CEP from the gut performed in a healthy volunteer. The figure shows the topographies at different frequency bands from one subject, and the percentage of the presence of each frequency band in the overall signal. The black dots represent electrodes. The colours represent how much power a particular frequency band holds at each electrode. The scales describing the colours are to the right of the topographies.

compared healthy controls and patients suffering from IBS, and found no group differences in anterior insula and dorsal anterior cingulate cortex (dACC) activity, two regions consistently activated by painful intestinal stimuli[44]. However, IBS patients showed greater activation of the amygdala, rostroventral ACC, and dorsomedial frontal cortical regions.

MEG

MEG is a non-invasive brain imaging tool, which allows detection of cortical neuromagnetic activity as opposed to metabolic changes, which are secondary. The spatial resolution is comparable to f-MRI and PET; however, MEG also has millisecond temporal resolution, and is suitable for both individual and group studies. MEG is not widely available; systems are only present in specialist centres. The technical limitation of MEG is that it is less able to resolve the radial current, and is not sensitive to deep sources; but it is especially sensitive to the tangential activity in the cortex.

EEG

EEG measures direct electrical brain activity, through non-invasive scalp electrodes. This electrophysiological tool is widely used. EEG can be used to investigate the activity in both health and disease, as it is non-invasive and completely harmless. While f-MRI and PET brain imaging techniques have excellent spatial resolution, their time resolution is poor. Thus, these methods do not directly show brain activity in time. The EEG signal is divided into five frequency bands: Delta: < 4 Hz, Theta: 4-8 Hz, Alpha 8-12 Hz, Beta: 13-30 Hz, and Gamma: greater than 30 Hz. Figure 3 shows an example of a presentation of different frequency bands present in a painful cortical evoked potential (CEP) in the oesophagus. Analyses like this can be used to compare frequency alterations and topographical appearance between different subject groups. Drewes *et al*[45] found significant differences in theta and delta bands in CEPs between healthy controls and patients with chronic pancreatitis (CP) following painful stimulation in the gut. The patients showed higher activity in the theta band and the main theta band components oscillated by 4.4 Hz in patients and by 5.5 Hz in controls. Furthermore, the energy in the delta band was higher in the controls, whereas patients only showed scattered delta activity.

EEG recordings can be used for CEP, which detect brain activity in real time, with temporal resolution on the millisecond scale. CEP is an electrical response in the brainstem or cerebral cortex following a stimulus, i.e. painful stimulation in the gut. CEP amplitudes are typically lower than the amplitudes of spontaneous EEG (less than a microvolt to several microvolts, compared to tens of microvolts for EEG); but, since the CEPs are time-locked to the stimulus and the background activity occurs randomly, the CEP amplitudes become higher during the averaging process, and most of the background noise cancels out. In order to extract the CEPs with a

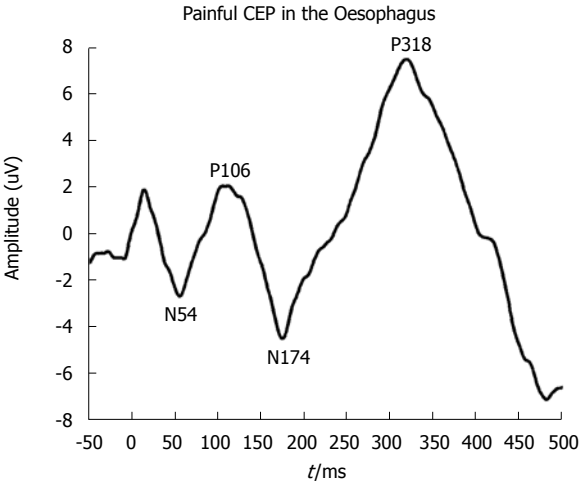

Figure 4 CEP at Cz electrode from a healthy volunteer. The subject was electrically stimulated in the oesophagus through a 6-mm nasal endoscope. Electrical stimulation was at the pain threshold and this CEP is an average of 35 such stimulations.

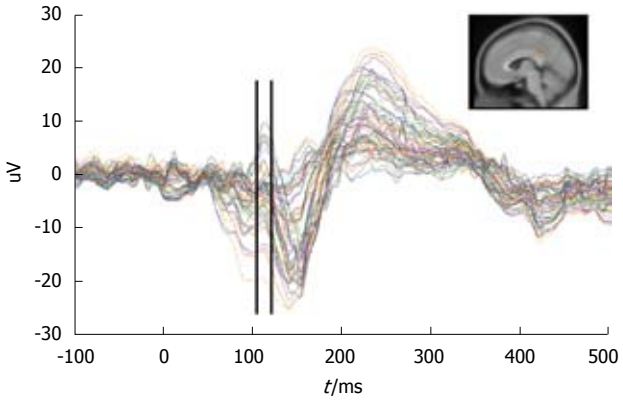

Figure 5 This is a butterfly plot of 62 channels (data of all 62 channels superimposed on each other). The vertical lines mark the time course of the peak that was used for analysis. The red dot on the MRI image on the top right corner represents where the activity was calculated to be by MUSIC.

good signal-to-noise ratio, a number of stimulations are presented at a certain frequency, and these stimulation trials are then cleaned for artefacts and averaged. An example of an averaged painful CEP from electrical stimulation of the gut is shown in Figure 4. Each peak in the CEP represents a synaptic event associated with the synchronous transmission of afferent information from one group of neurons to another. Several studies have examined the amplitudes and latencies of painful CEPs in the gut, and compared the results between a control group, and a study group (i.e. patients suffering from chronic pancreatitis, non-cardiac chest pain or patients treated with analgesics)[46-51]. Dimcevski *et al*[46] showed decreased early CEP latencies in patients with CP compared to healthy controls. Sami *et al*[48] showed decreased latencies in the first two positive peaks (P1 and P2) of CEPs following painful stimulation in the oesophagus after acid perfusion. Rossel *at al*[47] found that P1 had a shorter latency and smaller amplitude in patients with IBS compared to healthy controls. Furthermore, the group showed that the controls had a mid-latency positive component after 100 ms, which was absent in the patient group, and the healthy controls had a single late positive component (> 150 ms) whereas the IBS group had a late component which was biphasic. The demonstrated changes in latencies and frequencies most likely explain neuronal changes, such as plasticity, in the CNS.

INVERSE MODELLING OF CORTICAL EVOKED POTENTIALS

EEG is a mixture of signals from all over the brain due to the current generated by groups of neurons not only being produced at the source location, but also flowing to the surrounding tissue *via* volume conduction. Thus, by the time the signal arrives at the scalp electrodes it is distorted. Therefore, while CEPs have excellent time resolution on the millisecond scale, the spatial resolution is limited, and it is impossible to predict which sources in the brain are generating these potentials. However, methods using advanced mathematics and signal analysis to address these problems exist. This is known as "inverse modelling." Inverse modelling is based on the idea that groups of neurons generating the potentials at the scalp can be modelled by equivalent current dipoles. From multiple-channel recordings of CEPs, it is possible to mathematically calculate the locations of these dipoles. In order to do this, freeware and commercial software such as [EEGLAB, BrainStorm, Statistical Parametric Mapping (SPM), BESA, ASA and CURRY] are available. Some studies have performed inverse modelling on CEPs following painful stimulation in the gut. Dimcevski *at al*[46] found that dipolar activities corresponding to the early CEPs were located consistently in the bilateral insula, in the anterior cingulate gyrus, and in the bilateral secondary somatosensory area. Furthermore, they showed that in a CP patient group, the bilateral insular dipoles were localized more medial than in the healthy control group. They also showed changes in the cingulate cortex where the neuronal source was more posterior in patients than in controls. Drewes *et al*[52] showed two dipoles in the bilateral insular cortex, one dipole in the anterior cingulate gyrus and two dipoles in the bilateral secondary somatosensory area post the painful stimulus. Moreover, they found the anterior cingulate dipole to have a more posterior position in IBS patients than in healthy controls[53]. Inverse modelling algorithms, such as low-resolution brain electromagnetic tomography (LORETA) and multiple signal classification (MUSIC) have usually been applied to instantaneous CEP data by selecting a certain time frame in the data and calculating the location of dipole(s) generating the CEP at this time, see Figure 5.

Different inverse modelling algorithms and the ideas behind them are discussed in detail elsewhere[54-57]. The disadvantage of performing inverse modelling on instantaneous CEPs is the instability of algorithms when multiple sources are active and the interference of background electrical and physiological noise. For this reason, different signal decomposition methods have been used in order to separate the signal into a sum of

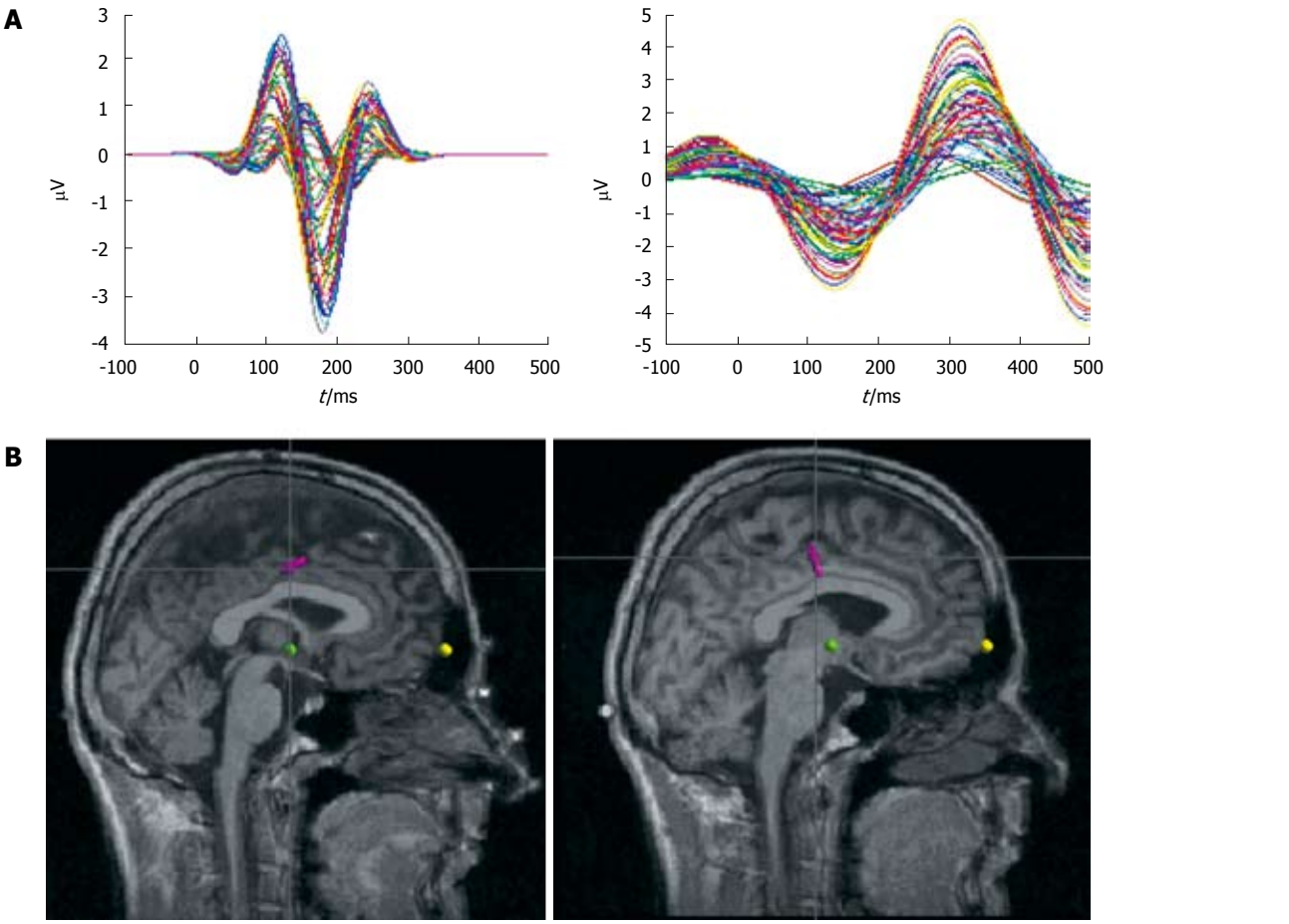

Figure 6 An example of two MMP atoms from painful CEPs in the oesophagus. A: Butterfly plots of the atoms; B: Dipole location of each atom.

waveforms, each having a single dipole generator. These methods make it possible to differentiate signals corresponding to brain activity from those corresponding to noise and artifacts. Once the signals are decomposed, inverse modelling can be completed on each waveform, and furthermore, it is possible to observe at which time and frequency this particular dipole is active, as shown in Figure 6.

Recently, Multichannel Matching Pursuit (MMP) was introduced, which decomposes the data into a sum of waveforms (usually termed atoms), each of them defined in time, frequency and space. We showed that decomposing the CEPs using MMP prior to inverse modelling (namely MUSIC) is superior to some blind source separation (BSS) methods, namely Independent Component Analysis (ICA) and Second-Order Blind Identification (SOBI), which are typically used for CEP signal decomposition prior to source analysis. These decomposition methods are described in detail elsewhere[58-66]. Additionally, we showed that MMP prior to MUSIC was much more accurate than MUSIC on the instantaneous data on both simulated and empirical CEPs[67]. MUSIC on MMP atoms was able to localize deep, superficial, and simultaneously active dipoles with high accuracy. The spatial resolution for MUSIC on MMP atoms was 3-20 mm compared to MUSIC on ICA components (5-27 mm for superficial dipoles, deep dipoles failed to localize), MUSIC on SOBI components (5-32 mm, deep dipoles failed to localize), and MUSIC on raw data (7-81 mm, simultaneously active dipoles typically did not localize correctly). Comparisons between different inverse modelling methods have been carried out in other studies[54,55]. We chose MUSIC because it has demonstrated an advanced ability to localize a restricted number of independent sources, and has the ability to reliably replicate temporal waveforms[57]. Furthermore, it is possible to combine an individual's MRI scan with the digitized locations of electrodes on their scalp in order to create a realistic head model, and use this head model to find the inverse solution for the individual's CEPs. These combinations of non-invasive methods allow us to study the sequence of cortical activations due to pain. Although combination of MMP, inverse modelling, and individual MRIs allows us to find new information regarding pain processing in the brain, one shortcoming of MMP is the lack of order in the atoms. This makes it difficult to compare between groups; hence, to distinguish which atoms from one subject correspond to the atoms of another subject and which atoms in one group are different/similar to the atoms in another group. For this reason, clustering of atoms/dipoles can be done. Delorme *et al*[58] have implemented such a method for clustering of ICA components and incorporated it into their EEGLAB toolbox. Currently, we are developing a toolbox to cluster MMP atoms based on time/frequency, topography, both time/frequency and topography, or dipoles. Furthermore, it is

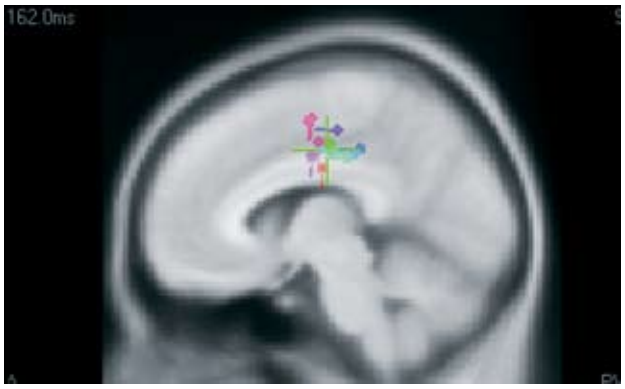

Figure 7 One of the clusters of cingulate dipoles generating CEPs following a painful stimulation in the gut in 10 subjects.

possible to take the Talairach coordinates of each dipole in individual clusters, and look up the anatomical location of the source in a Talairach atlas. For an example of clusters, see Figure 7. These dipoles were localized in the cingulate gyrus. In the future, we are aiming at clustering the dipoles after source localization has been performed using realistic head models for each individual (i.e. combining their individual MRI scans and digitized EEG electrode locations). These methods will allow for more precise source localization, automated separation of dominant sources during painful CEPs in different groups, and allow the study of the sequential order of activated centres post stimulus. These advancements will provide new insight into how different subject groups process pain.

THE BRAIN-GUT AXIS IN FUNCTIONAL GASTROINTESTINAL DISEASES

The ROME II multinational consensus has defined functional gastrointestinal disorders (FGID) as "a variable combination of chronic or recurrent gastrointestinal symptoms which are not explained by structural or biochemical abnormalities"[68]. Despite remarkable advances in our understanding and management of "organic" gastroenterological complaints such as peptic ulcer disease, IBS and even cancer over the last 30 years, our understanding of the mechanisms of pain in FGID patients remains far from complete. The lack of effective treatments for these disorders leads to chronic symptoms, recurrent attendances in hospital, poor patient satisfaction and significant morbidity. Health care costs are estimated to be around $34 billion in the 7 largest Western economies[69,70].

Although patients with FGID show marked heterogeneity in their clinical presentation and response to treatment, common features have become apparent as our knowledge of these disorders has increased. These patients often display a heightened sensitivity to experimental gut stimulation, termed visceral pain hypersensitivity (VPH) which is believed to be important in their pathophysiology and symptom generation. The hypersensitivity may be caused by peripheral and central factors relating to primary afferents as well as the autonomic and enteric nervous systems; however, in this review we will focus on the changes in the CNS which can be elucidated using the new imaging techniques described above.

Mayer *et al*[71] studied the perceptual responses to rectosigmoid distension in IBS patients and controls with functional brain imaging using $H_2{}^{15}O$ PET and found that following a train of repetitive sigmoid distensions, control subjects demonstrated greater activation of the PAG and thalamic regions compared to patients. This effect was seen both during actual rectal distension and during expectation of the stimulus, despite its absence. As has been outlined, the PAG is an important structure involved in the modulation of spinal pain processing, and the above finding suggests that a proportion of IBS patients have inadequate activation of brain regions involved with antinociception. Mayer *et al*[38] have recently reviewed imaging studies in FGID which has been critiqued by Hobson and Aziz[36,37,72].

"Visceral hypersensitivity" is a hallmark feature in IBS patients, who show an abnormal pattern of ACC activation during pain perception which is an interesting parallel to ACC activation relative to increasing pain perception in healthy subjects[43,73,74]; hemispheric preference, as well as cognitive style of information processing served as indicators of covert changes in brain functions in 21 adult IBS patients[75]; and abnormal cerebral processing of oesophageal stimuli was found in patients with noncardiac chest pain[50,51]. Drossman *et al*[76] found that alterations in brain activity were associated with resolution of emotional distress and pain in a case of severe IBS.

A recent longitudinal study in IBS found that there were significant decreases in amygdala, dACC and dorsal brainstem activation over a 12-mo period during anticipation for pain although pain-related activations and symptoms were stable[77]. Rectal pain induced significant activation of the perigenual ACC, right insula and right prefrontal cortex. Amitriptyline was associated with reduced pain-related cerebral activations in the perigenual ACC and the left posterior parietal cortex, but only during stress[78]. Taken together these findings strongly suggest that abnormalities in the brain-gut axis play a key role in our understanding of FGID, and future studies using the techniques described above will undoubtedly increase our understanding of these disorders.

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