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Review

Neuroimaging of the human visceral pain system—A methodological review

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ABSTRACT

During the last decades there has been a tremendous development of non-invasive methods for assessment of brain activity following visceral pain. Improved methods for neurophysiological and brain imaging techniques have vastly increased our understanding of the central processing of gastrointestinal sensation and pain in both healthy volunteers as well as in patients suffering from gastrointestinal disorders. The techniques used are functional magnetic resonance imaging (fMRI), positron emission tomography (PET), electroencephalography (EEG)/evoked brain potentials (EPs), magnetoencephalography (MEG), single photon emission computed tomography (SPECT), and the multimodal combinations of these techniques. The use of these techniques has brought new insight into the complex brain processes underlying pain perception, including a number of subcortical and cortical regions, and paved new ways in our understanding of acute and chronic pain. The pathways are dynamic with a delicate balance between facilitatory and inhibitory pain mechanisms, and with modulation of the response to internal or external stressors with a high degree of plasticity. Hence, the ultimate goal in imaging of pain is to follow the stimulus response throughout the neuraxis.

Brain activity measured by fMRI is based on subtracting regional changes in blood oxygenation during a resting condition from the signal during a stimulus condition, and has high spatial resolution but low temporal resolution. SPECT and PET are nuclear imaging techniques where radiolabeled molecules are injected with visualization of the distribution, density and activity of receptors in the brain allowing not only assessment of brain activity but also study of receptor sites. EEG is based on assessment of electrical activity in the brain, and recordings of the resting EEG and evoked potentials following an external stimulus are used to study normal visceral pain processing, alterations of pain processing in different patient groups and the effect of pharmacological intervention. EEG has high temporal resolution, but relative poor spatial resolution, which however to some extent can be overcome by applying inverse modelling algorithms and signal decomposition procedures. MEG is based on recording the magnetic fields produced by electrical currents in the brain, has high spatial resolution and is especially suitable for the study cortical activation.

The treatment of chronic abdominal pain is often ineffective and dissapointing, which leads to search for optimized treatment achieved on the basis of a better understanding of underlying pain mechanisms. Application of the recent improvements in neuroimaging on the visceral pain system may likely in near future contribute substantially to our understanding of the functional and structural pathophysiology underlying chronic visceral pain disorders, and pave the road for optimized individual and mechanism based treatments.

The purpose of this review is to give a state-of-the-art overview of these methods, with focus on EEG, and especially the advantages and limitations of the single methods in clinical gastrointestinal pain research including examples from relevant studies.

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Contents

1. Introduction

During the last decades there has been a tremendous development of non-invasive methods for assessment of brain activity. The use of these techniques has brought new insight into the complex brain processes underlying pain perception and paved new ways in our understanding of acute and chronic pain. The techniques most extensively used are functional magnetic resonance imaging (fMRI), positron emission tomography (PET), electroencephalography (EEG), evoked brain potentials (EPs), magnetoencephalography (MEG), single photon emission computed tomography (SPECT), and the multimodal combinations of these techniques.

Painful sensations from the human gastrointestinal (GI) tract are common symptoms in the clinic but the underlying aetiology is often difficult to identify [1]. In clinical practice, it is most likely that this is due to heterogeneity of the patient groups and the many confounding factors such as sedation, nausea and general malaise that all can affect the pain experience [2]. Also the relationship between disease severity and symptoms are ambiguous [3]. Hence, objective methods for pain assessment are needed in order to improve our understanding of GI pain and to facilitate the development of new therapeutic targets. The information on visceral sensory processing has traditionally been based on animal studies, but during the last decades our understanding has been facilitated by the advances in non-invasive technologies. These techniques have vastly increased our understanding of central processing of GI sensation and pain in both healthy volunteers and patients. The methods have demonstrated the complexity involved in pain processing and revealed a number of subcortical and cortical regions involved in this process. As pain is a conscious feeling, the ultimate goal in pain-imaging is to follow the pain stimulus throughout the neuraxis. The pathways are dynamic with modulation of the response to internal or external stressors with a high degree of plasticity. The ultimate outcome of pain perception is brought about by a delicate balance between facilitatory and inhibitory pain mechanisms, which is also influenced by sensory-discriminative, affective-motivational and cognitive-evaluative components. For a more detailed summary of the normal GI sensation see Section 2 below and recent reviews [4–8].

The purpose of this review is to give a state-of-the-art overview of the methods used for assessment of visceral pain in the neuraxis with focus on EEG and especially the advantages and limitations in GI pain research (see Table 1), including examples from relevant studies.

2. The visceral pain system

The GI tract has a complex dual innervation with both intrinsic and extrinsic sensory neurones (the latter is referred to as visceral afferents). Fig. 1 illustrates the principal visceral projections including the subcortical and cortical structures involved in the response to visceral pain. Intrinsic afferents mainly project locally in the wall of the gut and are involved in regulation of the visceral functions, such as secretion, motility, mucosal transport and blood flow [9,10]. Extrinsic afferents project to the central nervous system (CNS) via the vagal nerve to the brainstem or through splanchnic nerves to the spinal cord [11].

Most *afferent vagal fibres* are unmyelinated C-fibres that project viscerotopically to the medial division of the nucleus of the solitary tract. Second-order neurones project to sites in the brainstem, hypothalamus and amygdala including the vagal motor nuclei, the rostral areas of the ventrolateral medulla and the parabrachial nuclei [12]. Cortical projections from these brainstem areas include the orbitofrontal, infralimbic anterior cingulate and insula cortex, the latter having reciprocal connections with the secondary somatosensory cortex (SII). The vagal afferents are believed to mediate non-noxious physiological sensations such as satiety and nausea (low response thresholds) [13–15]. Also, vagal afferents may be involved in the central inhibitory modulation of pain [16]. On the contrary, visceral pain is mainly mediated through the *spinal afferents* in the splanchnic nerves which pass the dorsal root ganglion and project to distinct laminae of the spinal cord dorsal horn (mainly laminae I and V, and occasionally to contralateral laminae V and X). These afferent projections are organized in a segmental manner, but distributed over several spinal segments in both rostral

Table 1
Overview of the main advantages and limitation of the methods used in the assessment of brain activity and neurophysiological mechanisms behind visceral pain.

Modality	Temporal resolution	Spatial resolution	Receptor studies	Qualitative (waveform, coherence)	Feasibility and costs
EEG	+++	+	–	++	++
MEG	+++	(++)	–	(+)	+
fMRI	+	+++	–	(+, connectivity)	+++
PET/SPECT	+	+++	+	–	++

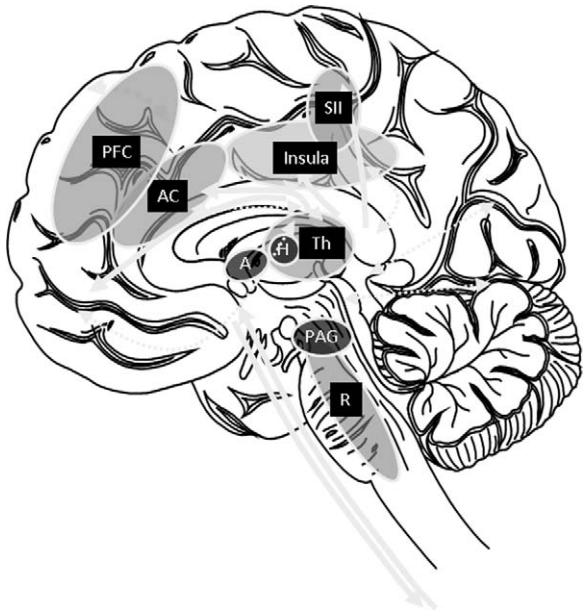

Fig. 1. Illustration of the principal visceral projections including the subcortical and cortical structures involved in the response to visceral pain. The thalamus (Th) functions as a relay station, which conveys the information to various parts of the brain. Third order neurons ascend to the secondary somatosensory cortex (SII), the limbic system including anterior cingulate cortex (AC), insula, and the prefrontal cortex (PFC). The dotted lines illustrate connections to other cortical centres simultaneously activated, see text for more information. Amygdala (A), hypothalamus (H), periaquaductal gray (PAG), reticular formation (R).

and caudal directions [17]. This diffuse spinal termination pattern may explain the poor localization of visceral sensations often seen in clinical practice, and 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 (referred pain) [11,18]. From the spinal cord, pain transmits to the brain through diverse pathways. Most afferents travel in the spinothalamic tract to the thalamus. From the thalamus projections to the insula, hypothalamus, amygdala as well as to higher cortical levels such as cingulate and prefrontal cortices have been described. Insula has an important function for integrating the visceral sensory and motor activity together with limbic integration and is particularly important in pain perception from the gut [19]. The anterior cingulate cortices (ACC) and prefrontal cortices are a part of the medial pain system, which mediates the affective, emotional and cognitive components of pain experience [20,21]. In addition to the spinothalamic tract, some afferents ascend in the spinoreticular tract mediating arousal and autonomic responses through interaction with the reticular formation [22]. Finally, a population of afferents ascends in the spinomesencephalic tract, which relates to a complex neuronal network including the periaquaductal gray, rostroventral medulla (RVM) and dorsolateral pontine tegmentum. This network comprises the structural basis of descending pain control and possess a modulatory effect on the spinal pain processing through so called on- and off-cells in the rostroventral medulla, which are pro-nociceptive or anti-nociceptive, respectively [21,23].

2.1. Experimental visceral pain models

Several experimental methods have been developed to evoke visceral pain [24], and different criteria have been used to define the "ideal" experimental pain stimulus [25]. It is important that the noxious stimulus should be "natural" to the visceral organ, such as distension of the gut. The response to the stimulus should mimic the observations done in diseases of the stimulated organ. Moreover, the stimulus has to be exact, quantifiable and reliable in test–retest conditions. Additionally, especially when used in human experiments, it has to be minimally invasive and safe to use.

It is difficult to mimic the mechanisms responsible for different GI diseases in simple experimental protocols, since several mechanisms such as inflammation, distension, ischemia and chemical transmitters are often co-existing. While the pain in most visceral diseases lasts for hours, days, or even becomes chronic, the duration of an experimental stimulus is only limited to a few seconds or minutes. Peripheral and central neuronal mechanisms may therefore modulate the clinical sensation of the applied stimulus in a way that cannot be imitated by experiments. Visceral pain is probably a result of many components working together.

The different experimental stimulus modalities (mechanical, thermal, electrical and chemical stimulations) activate different pain mechanisms and evoke various sensory responses. A combination of these modalities – a multimodal approach – can mimic the clinical situation [2] and experimental multimodal pain protocols make it possible to investigate different aspects of the pain process individually and elucidate important mechanisms of clinical pain. For detailed review of the experimental visceral pain models, see [2].

3. Magnetic resonance imaging

MRI allows imaging of both brain structure and activity. Brain activity measured by fMRI has most commonly been acquired by the blood oxygenation level dependent (BOLD) technique, which is based on different paramagnetic properties of oxy- and deoxyhemoglobin in the blood. The BOLD signal reflects simultaneously changes in local blood flow, volume and deoxyhemoglobin content, which derive from changes in neuronal activity [26]. Regions of activation are identified by subtracting regional BOLD signal during a control/resting condition from the signal during a stimulus condition. There is a rapid development in both hardware and software. Recently, other techniques such as arterial spin labeling (ASL) and signal enhancement by extravascular water protons (SEEP) have been applied. ASL allows the measurement of whole brain cerebral blood flow (CBF) in absolute units through the use of magnetically labeled endogenous water in blood acting as a diffusible tracer [27,28] allowing assessment of the temporal dynamics of the neural activation induced by pain. This has been used to detect changes in regional CBF associated with a standard cutaneous heat pain model [29] and infusion of hypertonic saline [30]. ASL is particularly suited to studies of prolonged pain since it becomes increasingly more sensitive than BOLD to changes in neural activation as the stimulus duration exceeds 1 min [31]. The SEEP technique has been used in fMRI of the spinal cord, which is essential in the complete mapping of the pain system, and spinal cord and brain stem sensory-related neural activity has been consistently observed in a number of studies [32–44]. Recently, structural information obtain by other MRI techniques, such as diffusion tensor imaging (DTI) including tractography, volumetry of brain structures and measurement of gray matter density, has been superimposed to gain more structural information on the neural structures and connections between the centres involved in pain processing [45–47]. Most neurophysiological research is performed at 3T field strength, but 7T scanners are now emerging with new possibilities for enhanced resolution.

Results obtained with fMRI have been found to be strongly correlated with those from PET-CBF in identical paradigms [48–50]. So far, the 'pain responses' obtained using PET and fMRI methods have been very similar, but more comparisons of results using both techniques in the same population of subjects are needed.

Advantages: fMRI has an excellent spatial resolution (2–5 mm), especially in the more superficial layers, but limitations are seen in the deeper structures, such as the brainstem and thalamus, due to pulsation artefacts. fMRI has the possibility to take into account anatomy and other individual characteristics, which is a clear advantage over PET. The temporal resolution (ranges from a 300 ms theoretical value to a more realistic 1–3 s) in event-related fMRI studies with echo-planar systems is another advantage compared to PET. fMRI appears as an intermediate solution between the temporal resolution of PET (tens of seconds) and electrophysiology (tens of milliseconds). A clear advantage of fMRI is that it operates in a non-invasive and non-radioactive environment, allowing subjects to be studied repetitively. Single-subject analysis of fMRI will also be an important gain in pain studies, since pain is notoriously dependent upon individual factors.

Limitations: The temporal resolution of fMRI is clearly inferior to EEG/MEG meaning that fMRI is not a specific tool for investigating the primary neuronal activity directly related to the painful stimuli (first few hundred ms post-stimulus). Furthermore, the fMRI activity to gastrointestinal stimulation is not stimulus-specific, anticipation of stimuli can trigger similar activity and repeated activation can result in habituation [51,52]. fMRI remains currently mainly limited to "activation" studies, but recently also resting state fMRI has been applied in pain research including connectivity analysis between multiple brain network [53]. In contrast to PET studies a limitation in fMRI studies is the lack of information regarding neurotransmitters or involved receptors. Furthermore, MR scanners are expensive, and the time allowed for research studies in clinical departments is often limited.

3.1. Examples from previous studies in patients with irritable bowel syndrome

fMRI has been used in several studies for demonstrating abnormal brain processing in functional gastrointestinal disorders (FGID), such as irritable bowel syndrome (IBS). Kwan et al. identified abnormal event-related sensations in five brain regions following rectal distensions in IBS [54,55]. In the primary somatosensory cortex (SI), urge-related responses in the IBS group were seen compared to the control group. This could be interpreted as up regulated afferent input underlying visceral hypersensitivity or "visceral allodynia". In the IBS group pain-related responses were seen in the medial thalamus and hippocampus, but not in the control group. However, pronounced urge- and pain-related activations were present in the right anterior insula and the right ACC in the control group, but not the IBS group. Finally, lack of activation in right anterior insula was found in IBS patients, interpreted by the authors as either a ceiling effect or a dysfunction in interoceptive processing or control of visceromotor responses. In a similar study, Bonaz et al. 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 [56].

Seminowicz et al. [57] found decreased gray matter density in IBS patients in widespread areas of the brain, including medial prefrontal and ventrolateral prefrontal cortex, posterior parietal cortex, ventral striatum, and thalamus. Furthermore, increased gray matter density was seen in IBS in the pregenual ACC and the orbitofrontal cortex. These changes in density of gray matter in regions involved in cognitive/evaluative functions are specifically observed in patients with IBS, indicating neuroplastic changes.

A combination of DTI and fMRI were used to investigate the anatomical relationships between areas involved in sensations to rectal distension in healthy subjects, giving insight into the neural network of visceral sensory perception [58]. DTI revealed direct connections between insula, and the ACC, thalamus, SI, SII and PFC, which were activated in this study.

Finally, fMRI has been used to evaluate the effect of the tricyclic antidepressant amitriptyline, which is believed to be of clinical benefit in IBS patients [59]. Amitriptyline reduced pain related cerebral activations in the pACC and the left posterior parietal cortex compared to placebo, but only during mental stress [60].

3.2. Functional significance of hemodynamic studies (fMRI and PET)

An everlasting problem associated to both fMRI and PET is the interpretation of blood flow changes in terms of "activation" of underlying cerebral structures. Substantial evidence supports that increased rCBF reflects increased synaptic activity [61,62], which may reflect either activating or inhibitory energy-consuming processes. The rate of blood flow increase is determined by firing rates in the synaptic terminals, and this independent of whether an excitatory or an inhibitory neurotransmitter is released. To quote Sokoloff: "To distinguish between the two, one must look one synapse downstream; if an inhibitory transmitter is released, one will observe reduced glucose utilisation in the next synapse. If an excitatory neurotransmitter is released, then glucose utilisation will increase at the next synapse" [63]. It is obviously impossible with current PET or fMRI technology to ascertain rCBF changes "in the next synapse" of a given region. Network analysis, which seeks for statistical correlation of dynamic flow changes between interconnected regions, is one promising strategy that might partially surmount this limitation [64–66] and may help significantly to interpret the functional significance of the observed changes.

4. Single photon emission computed tomography and positron emission tomography

SPECT and PET are nuclear imaging techniques that can trace radiolabeled molecules injected into the blood stream, whereby the distribution, density and activity of receptors in the brain can be visualized. This provides an insight into the organization of functional networks in the brain, which cannot be achieved by morphologic investigations or imaging of blood flow and metabolism [67]. Preclinical and clinical trials may therefore benefit greatly from such molecular imaging techniques [68]. In fact pharmaceutical compounds used as radiolabeled tracers can combined with kinetic models allow quantification of receptor sites and enzyme function in human subjects using SPECT or PET. The majority of studies have investigated opioids and their receptors, but recently other neurotransmitter systems, e.g. dopamine, have also been examined [69].

Advantages: An advantage with SPECT is that isotopes with a long half life are used, making it possible to widen the observational time window. This allows biomedical scientists to observe biological processes *in vivo* several hours or days after administration of the labeled compound [70]. PET has excellent spatial resolution (2–5 mm), which allows tagging 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. This tends to be the future main application of PET.

Limitations: SPECT exhibit lower sensitivity, i.e. ability to detect and record a higher percentage of the emitted events, compared to PET. However, use of specialized collimators is viewed as a technique to improve the sensitivity of SPECT without degrading image resolution [71]. The temporal resolution of SPECT and PET is poor (minutes) compared to especially EEG/MEG, and group analyses

are needed for meaningful results. However, PET has some intrinsic advantage over SPECT for dynamic studies [71]. Another major disadvantage is that the subject receives a considerable dose of radiation and the expense of a PET scanner.

4.1. Examples from previous studies

Both SPECT and PET have been used in studies investigating which brain areas are activated during painful stimuli [72,73]. Nevertheless, it has not been used very widely in clinical pain studies. A study by Fukumoto et al. assessed regional cerebral blood flow of the contralateral thalamus in 10 patients with reflex sympathetic dystrophy syndrome [74], but has so far not been used in the investigation of visceral pain. PET has on the other hand been used widely in normal subjects and in clinical studies to detect regional brain activation during pain stimulation [69]. Several studies have used PET for investigating brain activation during visceral pain [75–77], but to our knowledge no studies of specific receptor systems have been conducted. The studies found activation to gastric distension in: postcentral gyrus, superior temporal gyrus, inferior frontal gyrus, midanterior cingulate gyrus, anterior insula, and cerebellar hemisphere [76]; and dorsal brain stem, inferior frontal gyrus, insula, subgenual, and ACC [77]; and thalami, insula, ACC, caudate nuclei, brain stem periaqueductal gray matter, cerebellum, and occipital cortex [75].

It should be stressed that existing PET and SPECT technologies benefit from improvements in image reconstruction software and from the potential of dual-tracer imaging. Here PET/CT has received wide clinical acceptance, and although SPECT/CT systems continues to increase, the evolution of SPECT/CT has not followed the same trend, probably due to costs [71]. A study performed by Peyron et al. used both PET and fMRI to investigate areas of activity during laser heat stimulation and found consistent responses for the two techniques [78]. However, to our knowledge, has this combination of techniques not been implemented in visceral pain, but may be very valuable in basic and clinical studies investigating, e.g. visceral pain.

5. Electroencephalography

EEG is a method to assess electrical activity in the brain generated by firing between neurons. The activity of the neural networks is usually randomly active in time, but can be synchronized and enhanced in response to an external event. Thus, the activity can be recorded in either the *resting state* (resting EEG) or as *evoked potentials* (EPs) following an external stimulus. Both types of recordings may be used to study normal visceral pain processing and to identify alterations of pain processing in different patient groups or due to pharmacological intervention. The *resting EEG* is typically used to study the pathophysiology of pain in chronic pain patients, while the *EPs* are used to study the nociceptive pain response including the sequential brain activation following GI stimulations [79].

The resting EEG is often assessed by time–frequency analysis (see section below), while several analysis methods such as sophisticated time–frequency analysis and visual inspection exist for evaluation of EPs. The EPs can be analyzed either as single-sweeps or after an average process. The single-sweep analysis enables an evaluation of both synchronized and unsynchronized brain activity relative to the stimulus, which may provide additional information on abnormal pain processing in some patients [80,81]. The average process is performed over multiple stimuli which improve the signal-to-noise ratio and hereby reveals less prominent peaks. Averaging a sufficient number of sweeps enables visual inspection of the EPs to analyze the peaks in terms of amplitude and latency as illustrated in Fig. 2.

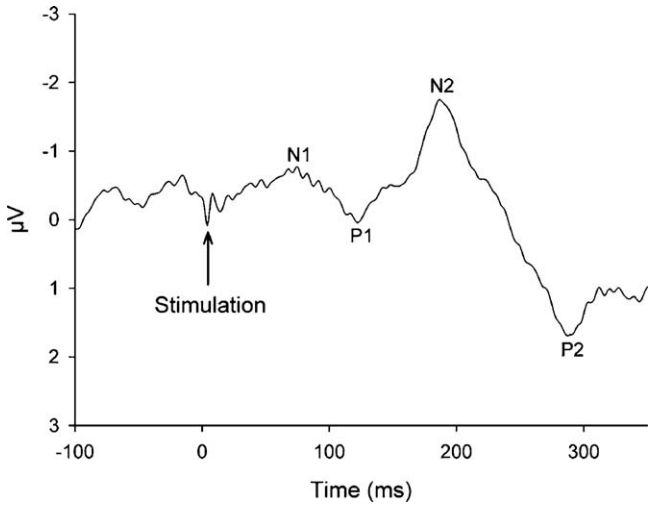

Fig. 2. A typical evoked potential (vertex-electrode) recorded after stimulation of the rectosigmoid junction in a patient with chronic pancreatitis. This is an average of the 50 electrical stimuli. Note the different peaks denoted N1, P1, N2 and P2, each defined by latency (ms) and amplitude (μV).

Advantages: EEG is generally highly available, relatively easy to use and low-cost. The main advantage is the high temporal resolution which allows assessment of the primary pain processing, including sequential activation and analysis of coherence and cross talk between brain centres.

Limitations: The main limitation of EEG is the relative poor spatial resolution. However, various inverse modelling algorithms and signal decomposition procedures (see below) have overcome this limitation to some extent and ongoing research in this field holds promise for further improvements of the methods. While multichannel EEG, cerebral EPs and inverse modelling offers a non-invasive approach to study brain activity with time resolution on millisecond scale, it must not be overlooked that the position of the calculated dipolar sources does not represent the accurate position but rather the "centre of gravity" of brain activity.

5.1. Application of EEG in visceral pain studies

A number of studies addressing various patient groups have examined alterations in amplitudes/latencies in EPs following electrical stimulation of different gut segments. In IBS the EPs to painful stimuli of the gut showed decreased latency and reduced amplitude of the first positive peak (P1 in Fig. 2) [82]. In painful chronic pancreatitis decreased latencies of the early EP components (N1, P1 in Fig. 2) were found, which is thought to reflect changes in exogenous pain processing [83]. Diabetes patients with autonomic neuropathy and GI symptoms demonstrated increased latencies and reduced amplitudes of most peaks in the EP in response to oesophageal stimulations [84]. Overall these patient studies indicate differences in pain processing which could be explained by a possible functional reorganization of the central nervous pathways.

5.2. Time–frequency analysis in visceral pain studies

Continuously recorded signals for both resting EEG and EPs have a complex nature, which prevents immediate manual assessment of traces. In spite of the chaotic nature of the signals, it has been shown that the EEG oscillates in certain frequency bands associated with specific brain functions such as pain perception and attention to pain. The EEG frequency bands are typically defined as: delta (up to 4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (above 30 Hz) [85]. A number of algorithms to calculate fre-

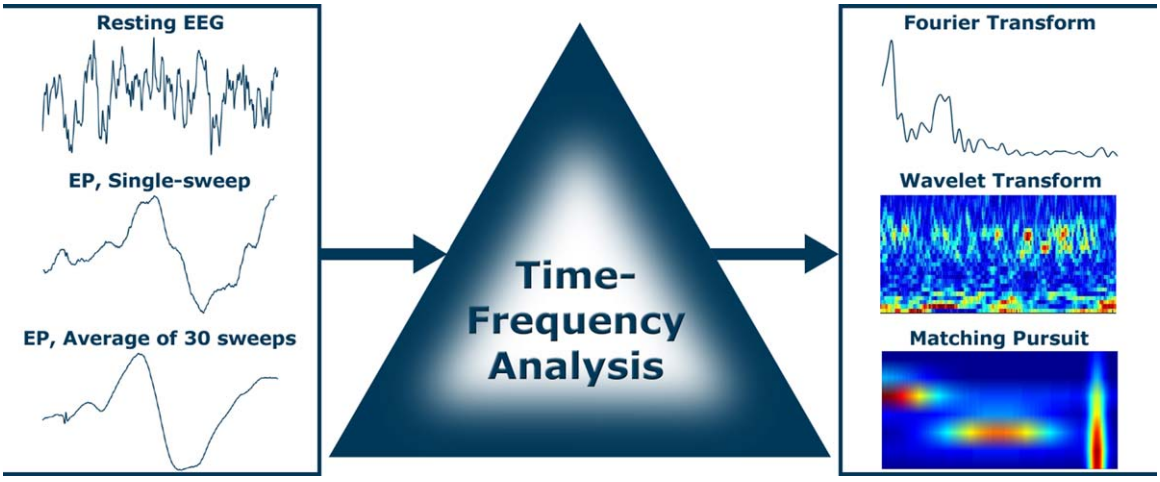

Fig. 3. Time–frequency analysis in visceral pain studies. Illustration of the different algorithms to calculate frequency distributions, including the Fourier transformation, wavelets, and matching pursuit.

quency distributions exist, most common ones being the Fourier transformation, wavelets, and matching pursuit as illustrated in Fig. 3 [86]. Frequency analysis can be applied in order to study how EEG characteristics are altered in different patient groups suffering from acute and chronic visceral pain. Rickenbacher et al. found that bladder dysfunction was associated with long-term changes in brain activation characterized by decreased delta oscillations and prominent theta oscillations [87]. Tayama et al. compared 10 healthy controls and 10 IBS patients, and found a significant lower alpha power percentage and a higher percentage of beta power in patients compared to controls [88]. Subsequently, Drewes et al. used topography matching pursuit on EPs in a study of painful chronic pancreatitis in order to assess whether the pain could be of neuropathic origin [89]. They found persistent increased theta activity in the patients indicating pain in chronic pancreatitis is likely of neuropathic origin.

5.3. Pattern recognition

Most studies on brain processing of visceral pain have been based on assessment of differences between patients groups and healthy controls. The conclusions have been drawn upon statistical analysis, which is limited to give an estimate of the degree of difference between patients and controls. Hence, abnormalities have only been reported if common patterns were observed in the majority of patients, without giving an estimate of how well abnormalities from individual subjects matched those obtained from the remaining group of patients. However, detailed analysis of such individual characteristics might reveal predictors on progression of the disease, symptom patterns and how central pain processing is correlated to the analgesic effect of different drugs. An understanding of these predictors is believed to be very important in developing individualized and effective pain therapy for chronic pain patients.

One possibility to obtain this knowledge is by applying pattern recognition to features extracted from the individual EEG signals. Pattern recognition uses the input features to define a decision rule to separate subjects belonging to different classes as for example patients and healthy controls. One pattern recognition method is the support vector machine, which has demonstrated high classification performance in many biomedical applications [90]. The support vector machine has the advantage that it calculates a separating hyperplane which discriminates the classes in the most optimal way without requiring pre-determined models. As a consequence, patterns not known a priori to the test will still be detected by the system, which might help identifying new biomarkers for visceral pain patients. Graversen et al. used a support vector machine in a study of viscero-visceral hyperalgesia, and showed that sensitization of the oesophagus by acid and capsaicin induced a decrease in gamma or theta activity (or both) in EPs following stimulation in the rectosigmoid colon (unpublished data). In another study they investigated the analgesic effect of morphine in healthy volunteers. The study was conducted during oesophageal stimulation, and the EEG response before and after drug administration was classified, which revealed that two subjects had an abnormal EEG alteration compared to the remaining group of subjects. Interestingly, these two subjects displayed no alteration in painful sensation after morphine administration and were considered non-responders, while all other subjects had an analgesic effect of morphine [91].

5.4. Inverse modelling

The location of brain centres underlying EPs can be determined by multi-channel EEG recordings combined with inverse modelling. This method possesses the opportunity to study pain specific cortical activation dynamically, as it reflects the sequential activation of neuronal pain networks underlying the EPs. The signal recorded on the scalp is distorted by conduction through the scalp and is furthermore a mixture of multiple electrical sources inside the brain. This poor spatial resolution is, in contrast to the high temporal resolution, the main disadvantage of the EEG. There are a number of commercial software and freeware available for inverse modelling, most commonly used are EEGLAB (Matlab, The MathWorks Inc., Natick, MA, USA), BESA (MEGIS Software GmbH, Gräfelfing, Germany), and CURRY (Compumedics, El Paso, TX, USA).

In a study of healthy volunteers based on the BESA algorithm, EPs to electrical stimulation of the upper gut and sigmoid colon were explained by bilateral brain sources in the SII, insular cortices and a single dipole in the ACC [92]. Interestingly, a viscerotopic organization of the different gut segments was seen, thus revealing a “visceral homunculus” mimicking that seen for the somatic sensory system. In IBS patients the ACC dipole (BESA algorithm) following painful electrical stimulation of the sigmoid colon showed a more posterior position in patients than in control subjects [93]. This finding suggests that the cortical representations of painful stimuli are altered in IBS patients possible due to reorganization of the cortical areas involved in visceral pain processing. After experimental acid sensitization of the oesophagus in healthy volunteers, a posterior shift and latency reduction of the ACC dipole was seen

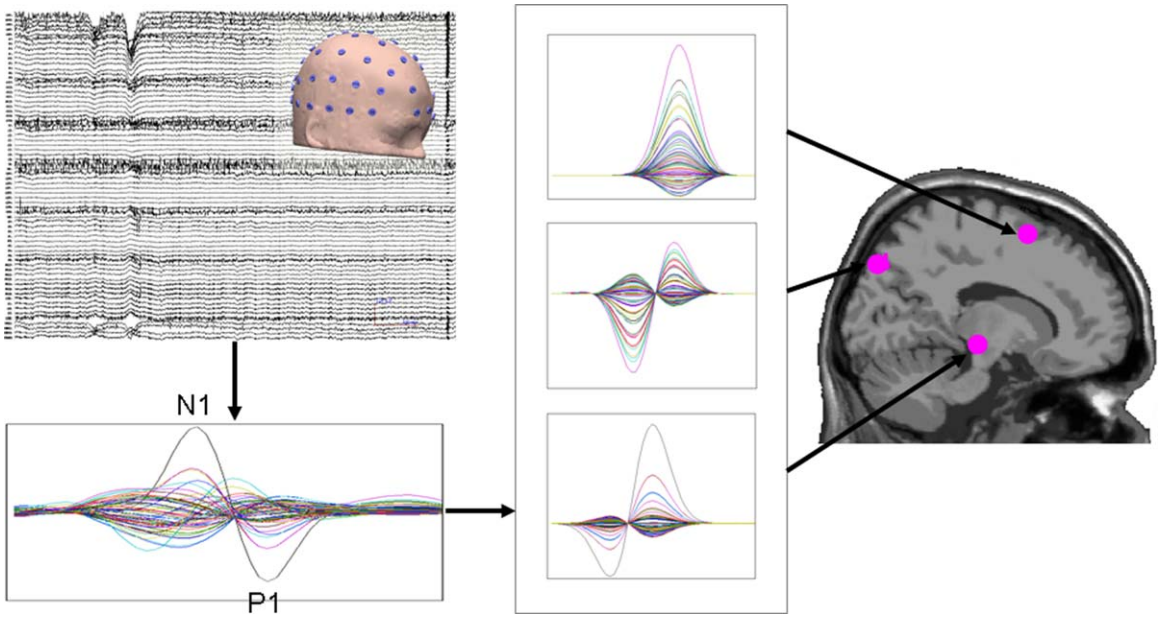

Fig. 4. Source localization of evoked potentials in combination with multichannel matching pursuit to estimate brain activation. Recording from multiple electrodes on the scalp gives continuous EEG signals as shown to the left of the figure on top. When the subject is stimulated a number of times, these signals can be averaged and evoked potentials can be identified as peaks, as seen on the bottom of the figure to the left. The averaged signal is then decomposed into a sum of atoms using multichannel matching pursuit as seen in the centre of the figure. Each of these atoms is well defined in time and frequency and when they are summed up, they represent the signal shown in the bottom left of the figure. Lastly, inverse modelling is done on each of the atoms in order to give a result similar to what is shown to the right of the figure. Now, not only are the active brain source locations known, but also their time and frequency distributions.

[94]. This finding may translate to the clinic where patients with non-erosive gastro-oesophageal reflux disease (NERD) often have unpleasant chest sensations and pain despite the fact that a normal oesophagus is evident at endoscopy [94]. Hence, the symptoms in these patients may be partly explained by alterations in the central processing of visceral pain. Along this line, a modification of bilateral insular dipoles localizations was seen in chronic pancreatitis patients following upper gut stimulations [83]. As the upper gut and pancreas share afferent neuronal pathways these findings likely represent a reorganization of the visceral cortical projections induced by recurrent pain attacks due to pancreatitis. This parallels findings from somatic pain studies where reorganization of the cortical pain matrix has been commonly reported [95].

In a study of diabetes patients with autonomic neuropathy and GI symptoms, the evoked brain potentials to painful oesophageal stimulation were analyzed using the fixed Multiple Signal Classification (MUSIC) algorithm in CURRY [96]. The patients had a posterior shift of the ACC dipole and additional sources close to the posterior insula and medial frontal gyrus, indicating that central neuronal reorganization may contribute to our understanding of the GI symptoms in these patients.

One of the major limitations with inverse modelling has been the instability of algorithms to model several simultaneously active sources and sources in deep brain structures such as thalamus or brainstem. To bypass these problems, signal decomposition methods have been developed in order to separate the signal into a sum of waveforms, whereby signals corresponding to specific evoked brain activity can be separated from artefacts and noise. Once the signals are decomposed, inverse modelling can be applied on each of the retrieved waveforms. Some of the most common approaches for signal decomposition are blind source separation algorithms such as independent component analysis (ICA) and second order blind identification (SOBI) [97]. Drewes et al. have successfully used ICA to study sequential brain activation and cross talk between brain centres following electrical stimulation of the oesophagus in healthy controls [97]. Recently, multichannel matching pursuit (MMP) was introduced, which decomposes the EP data into a sum of waveforms (usually termed atoms), each of them being defined in time, frequency and space, see Fig. 4. We showed that inverse modelling on MMP atoms is superior to inverse modelling on instantaneous EP data, ICA and SOBI components with high accuracy to localize superficial, deep, and simultaneously active sources [97,98]. Recently, we used inverse modelling with MMP to study the effects of morphine on oesophageal EPs in healthy subjects, where the brain source shifted frontally in atoms in delta band due to morphine, whereas the source was stable in the placebo condition (unpublished data).

6. Magnetoencephalography

MEG is a non-invasive technique for mapping brain activity by recording magnetic fields produced by electrical currents in the brain. The electrical currents (as measured by the EEG) accompanying brain activity are extremely weak, and resulting magnetic fields are 1/8 of the magnetic field of the earth [99]. Nevertheless, these tiny fields can be measured in magnetic shielded rooms by superconducting detectors (supraconducting quantum interference devices), for technical details see [100]. Modern MEG uses a helmet-shaped dewar that typically contain arrays of detectors covering most of the head.

MEG has been extensively used for investigation of the SI and SII areas in health and disease [99]. These areas are rather superficial and their neuronal columns result in equivalent current dipoles with a marked tangential direction, the magnetic fields of which are optimal for MEG measurement. In particular, MEG has added to characterization of the somatotopic organization of SI and SII areas as well as the association between pain and cortical reorganization in somatic and neuropathic pain studies [95,99,101]. However, the use of MEG in the study of visceral pain has until today been very limited. For example in a study by Hobson et al. MEG was used to determine the sequential brain activation following oesophageal electrical stimulation in healthy volunteers [102]. They found the earliest cortical activities in the SI and SII cortices and posterior

insula, followed by later activities in the anterior part of insula and cingulate cortex.

Advantages: The main advantages follow from the fact that MEG signals are not highly distorted by conduction through the skull between sensors and scalp. Consequently, in contrast to EEG, MEG posses a high spatial resolution comparable to fMRI and PET. In addition, it has unique capabilities for real-time imaging of neuronal activity with millisecond response (comparable to EEG) [103].

Limitations: MEG is a very technically demanding technique and is only available in few specialist centres. Furthermore, it is limited by its incapability to resolve radial currents generated by deep brain sources, e.g. in the cingulate cortex. Radial dipoles generate magnetic fields which do not penetrate the head, since volume currents shield the magnetic fields. In order to investigate pain generators in the cingulate cortex and other deep brain structures, alternative methods such as multichannel EEG therefore have to be used [99].

7. Summary

This review gives an overview of the methods used in the assessment of neurophysiologic mechanisms behind visceral pain with focus on the advantages and limitations of the single techniques. Even though there has been a tremendous development in the methods for imaging the processing of pain, it should be kept in mind that all of these methods are not direct measures of brain activity and will never be able to assess the true neuronal activity and pathophysiological mechanisms. It should be realised that no single method has the potential to give the ultimate answer. However, as outlined in the review there seems to be some hope ahead since the development in hardware, computer power and post-processing is still ongoing allowing high quality data and new ways to interpret the data. Furthermore, the multimodal combinations of methods utilising the advantages of each single technique (see Table 1) will likely in the future bring us closer to true events in the brain, e.g. the fusion of hemodynamic and electrophysiological methods using for instance classification methods. The future aims are to identify still better and more specific biomarkers for abnormal processing of sensory input underlying the pain and suffering in patients groups, eventually to identify different sub-groups with different aetiology, and give directions for the development and testing of new treatment options with benefit for individuals and society.

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