Independent component analysis of the EEG: is this the way forward for understanding abnormalities of brain-gut signalling?

A R Hobson, A Hillebrand

A combination of electroencephalography and independent component analysis has the potential to contribute towards our understanding of brain-gut signalling

Our understanding of the sequence of physiological events that leads to the perception of a gastrointestinal sensation remains limited. What is it that determines whether acid in the oesophagus results in the sensation of heartburn? Why is it that some patients with extensive gastrointestinal inflammation are asymptomatic while others with functional gastrointestinal disorders report severe symptoms in the absence of any apparent biomedical abnormality?

These are questions that we are still some way from adequately addressing. For many years, researchers have attempted to design experiments with the aim of identifying robust biological markers of gastrointestinal pain hypersensitivity.^{1,2} Ultimately, the sensitivity and specificity of such approaches, which predominantly rely on subjective reporting of symptomatic episodes, have been generally poor.^{3,4} Moreover, even if such provocation tests produce a "positive" result, these data still reveal little about the underlying pathophysiology of the condition.

The primary reason that sensory testing alone is not the answer to solving these clinical conundrums is that this type of approach limits us to examining the gastrointestinal tract in isolation. However, there is a body of evidence which dates back more than a century that tells us that sensory information arising from the gastrointestinal tract comprises one component of a complex neurobehavioral system involved in homeostasis, pain, and emotional-motivational processes.^{5,6} Therefore, it is clear that new approaches are needed if progress is to be made in this field.

NEUROPHYSIOLOGY AND BRAIN-GUT SIGNALLING

In the past 10–15 years, gastrointestinal researchers have turned to their

neuroscience colleagues in order to develop the investigative tools needed to study brain-gut signalling in more detail.⁷ Neurophysiologists have used evoked potentials (EP) to study somatosensory, visual, auditory, and pain pathways for over half a century. This technique involves recording of the electroencephalogram (EEG) via electrodes placed on the scalp surface, with the recordings time and phase locked to the brief presentation of a sensory stimulus. The recorded signal is then averaged in response to repeated stimuli and the EP waveform develops.⁸

Frieling *et al* were the first to record EP responses to oesophageal stimulation⁹ and they, along with other investigators, suggested that the oesophageal evoked response was predominantly mediated via vagal afferent pathways. However, further studies have shown that the stimulus response characteristics of the visceral EP point to mediation via both spinal and vagal afferents.¹⁰ It has since been demonstrated that EPs can be recorded in response to stimulation throughout the gastrointestinal tract.¹¹

EP studies have shown that as stimulation intensity and sensory perception increases towards pain, there is an associated reduction in the latency and increase in amplitude of the EP components.¹⁰ This phenomenon is common across all evoked potential modalities and reflects recruitment of an increasing number of afferents. This relationship between amplitude, latency, and the intensity of sensory perception is an extremely important property, as visceral EP measures become objective neurophysiological correlates of gastrointestinal sensory processing. The ability to record objective neurophysiological measures that correspond directly to subjective pain ratings overcomes many of the limitations of previous studies of

visceral hypersensitivity discussed previously.

While EPs have provided important information regarding the transmission of gastrointestinal sensory information, this approach has not allowed detailed study of the brain regions that subsequently process this information within the brain. To overcome this, researchers have used functional brain imaging (FBI) techniques.

FUNCTIONAL BRAIN IMAGING AND BRAIN-GUT SIGNALLING

FBI techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) detect changes in cortical blood flow and metabolism associated with local changes in cortical activity. These techniques have been used by several investigators to study the cortical regions involved in gut-brain signalling.¹² These studies have revealed that gastrointestinal sensory information is processed by a broad network of cortical and subcortical structures with only subtle differences from activation patterns observed following stimulation of somatic structures.¹³

The advantages and disadvantages of using FBI to study brain-gut signalling have previously been described in detail,¹⁴ and what follows is a very brief explanation of the most salient points. It was anticipated, as had been demonstrated with EP responses, that FBI techniques would provide objective indices of neural activity associated with gastrointestinal sensory processing. However, recent studies have shown that similar patterns of cortical activation can be observed in the presence or absence of peripheral stimuli, meaning that the activation measured by these techniques may not be directly related to the experimental stimulus.¹⁵ While this may provide us with interesting information regarding many aspects of brain-gut signalling, lack of specificity renders interpretation of these data difficult, not least when considering the translation of these findings into a clinical population. The major problem of metabolic imaging techniques is that they do not have sufficient temporal resolution to differentiate between activity directly related to the stimulus from that which is produced endogenously representing higher cognitive functions, such as executive function and self regulated attention.

We have addressed this issue somewhat using a technique called magnetoencephalography (MEG) which combines both high temporal and spatial resolution.¹⁶ However, MEG is currently not widely available, and therefore the question is whether there

Figure 1 Illustration of the use of independent component analysis (ICA) for analysis of electroencephalography/magnetoencephalography (EEG/MEG) data. Two analysis paths are shown here. The first analysis path shows how ICA can be used to decompose the recorded data which contains a mixture of signals from different sources. The spatiotemporal properties of the neuronal sources underlying each ICA component can subsequently be determined using a source reconstruction algorithm. In the second analysis path, the source reconstruction algorithm is directly applied to the raw dataset. Note that the interpretation, and source reconstruction, of the raw data is in general more complex than interpretation of the separate components. Also note that EEG/MEG provides information about the temporal dynamics of the neuronal sources, unlike imaging techniques such as functional magnetic resonance imaging or positron emission tomography.

is an approach which may combine the best features of all of the techniques discussed above, which is more widely available, and could therefore be adopted on a larger scale?

IS EEG IN COMBINATION WITH ICA THE WAY FORWARD

The paper by Drewes and colleagues¹⁷ in this issue of *Gut* describes the use of independent component analysis (ICA) to study EEG responses after painful oesophageal stimulation (see page 619). ICA is a relatively novel tool for extracting individual components from a mixture of signals and it has recently been applied to biomedical signals by various groups.^{18–}

²¹ Here we will briefly describe how this decomposition of the data is achieved, as well as some of the assumptions that are made. This knowledge will enable those readers not familiar with ICA to critically

evaluate the results of the study by Drewes and colleagues.¹⁷

EEG measures the signals at the scalp electrodes that arise from electrical neuronal activity in many regions of the brain, as well as from external noise sources (such as signals from the heart or electrical equipment). These signals can subsequently be analysed in signal space,¹⁰ for example by examining the effect of stimulus parameters on the latencies of the responses. Moreover, one can try to solve the so-called inverse problem—that is, try to reconstruct the spatiotemporal properties of the neuronal sources that gave rise to the EEG signal measured on the scalp.²² With both approaches, data interpretation is enhanced if the recorded mixture of signals is decomposed into meaningful physiological components (fig 1).

Initially, this was attempted by decomposing the signal into uncorrelated

components using principal component analysis but the interpretation of these orthogonal components is not straightforward.²³ ICA on the other hand not only requires the components to be uncorrelated but also to be statistically independent (the correlation and the higher order cross moments between components should be zero).

One way of achieving this statistical independence is to require the components to be as non-Gaussian as possible. Mixtures of signals (such as the measured EEG signal), by the central limit theorem, conform to a Gaussian distribution. If one assumes that Gaussian distributions only arise from mixing, and that mixtures in themselves are not interesting, then the only signals of interest must be non-Gaussian signals.²⁴

The most popular ICA algorithms (FastICA, infomax, JADE) only differ in the way in which the search for

non-Gaussian signals is achieved, although other methods for constructing statistically independent components exist that are not based on the assumption that the components have a non-Gaussian distribution.²⁰ Besides the non-Gaussianity assumption, one also assumes that signals from distinct neuronal sources are linearly mixed in the recorded signals (which is a reasonable assumption since the time it takes for the signals to travel from a neuronal source to the sensors is negligible for the frequencies in the EEG), and that the components are spatially stationary. On a practical level, one needs to ensure that enough data are supplied to the algorithm so that the statistical independence between the components can be established.²⁵

In the context of recorded EEG/MEG data, ICA can separate the channel data into *signal components* with independent time courses. This can be very useful in itself.²⁶ However, these statistically independent components are simply components in the signal space, and one still has to solve the inverse problem if one is interested in the properties of neuronal activity (such as its spatial location) that gave rise to these components. In those cases, ICA does not provide a short cut to avoid the inverse problem and after the recorded data has been preprocessed with ICA the inverse problem still needs to be solved (fig 1).

This means that volume conduction must be modelled accurately and a source reconstruction method must be selected that uses assumptions that are appropriate for the specific dataset.²⁷

Drewes and colleagues¹⁷ chose a single equivalent current dipole fitting approach to reconstruct neuronal activity but different source reconstruction approaches might be more appropriate for other studies. For example, a multiple dipole fit approach could be used when an ICA component reflects the activity of two or more correlated sources, an imaging technique such as the weighted minimum norm could be chosen when an ICA component reflects the activity from an extended source, or a beamforming approach might be more appropriate when responses that are not phase locked to the stimulus are localised.^{22 27 28}

In fact, Drewes and colleagues¹⁷ report a relatively high residual variance for some of their reconstructed sources, which could be an indication that some ICA components were incorrectly modelled by a single equivalent dipole source, and an extended, or multiple, source model might have been more appropriate. A high residual variance is particularly suspicious when modelling ICA components as each component reflects activity from a statistically independent source and modelling of these

components should be relatively straightforward.^{29 30}

Drewes and colleagues¹⁷ have highlighted the *potential* contribution that the combination of EEG and ICA can make to the understanding of brain-gut signalling, as they were able to reveal the order in which the nodes in the network processing painful oesophageal stimulation were activated, as well as the brain rhythms that were involved. Imaging methods such as fMRI or PET have not got this capability. However, one should interpret their results with caution, as the source analysis applied to some of the ICA components might not have been optimal.

In summary, ICA can be successfully applied to EEG/MEG data in order to decompose the recorded data into statistically independent components and the interpretation of these components is in general easier than interpretation of the raw data (assuming that the assumptions behind ICA, and its particular implementation, were appropriate). However, deducing information about the neuronal activity underlying the ICA components still requires the inverse problem to be solved. Although modelling of an ICA component can often be relatively straightforward,^{29 30} incorrect modelling would still lead to erroneous interpretations of the measured data.

CONCLUDING REMARKS

The question posed by this commentary was whether ICA was the way forward for understanding abnormalities of brain-gut signalling. The answer is that ICA is another useful tool in the armoury of the gastrointestinal neuroscientist. It provides a different snapshot of brain-gut signalling, adding a further piece to the panoramic picture of gastrointestinal sensory processing. As always, this technique will be at its best when used in conjunction with other neuroscience tools and it is important to remember the clinical problems which are the driving forces behind this research. Finally, progress in this area will only be made if the data obtained from the type of study published by Drewes and colleagues¹⁷ are of equivalent quality to that published in other areas of neuroscience and short cuts should be avoided.

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Enteric glial

Glial regulation of neuronal plasticity in the gut: implications for clinicians

A Rühl

Enteric glia regulate gastrointestinal physiology by controlling neurochemical phenotypes in the enteric nervous system

The enteric nervous system (ENS) is a complex network in the gut wall, extending throughout the gastrointestinal tract and coordinating vital gastrointestinal functions, such as motility, perception, mucosal permeability and secretion, blood flow, as well as immune and inflammatory processes.¹ The ENS is connected to the central nervous system (CNS) by sympathetic and parasympathetic nerves which relay information to and from the brain via pre- and paravertebral ganglia, spinal cord, and medulla; yet, it can perfectly function independently of the CNS.

The ENS contains as many neurones as the spinal cord.¹ Enteric neurones have been neurochemically and immunohistochemically classified based on characteristic combinations of neurotransmitters, their synthesising enzymes, and neuronal markers, which ultimately constitute a “neurochemical code” (for review see Kunze and Furness²). Neurochemical coding has provided an important tool to identify functionally distinct neuronal subpopulations, and it is generally assumed that the neurochemical phenotype of differentiated postmitotic neurones is finally determined.^{3–4}

The most abundant cells in the ENS are glia.⁵ Enteric glia lie adjacent to neuronal cell bodies in the enteric ganglia, but probably as many of them accompany the extraganglionic nerve strands throughout all layers of the intestinal wall, from serosa to mucosa.^{5–7} Enteric glia envelop neuronal cell bodies and axon bundles without producing myelin. Historically, enteric

glia used to be seen as little more than packing material, holding the ENS together (“glia” derives from the Greek word for “glue”). Presently, however, evidence is accumulating to support a much more active role for glia in enteric neurotransmission and information processing (for review see Rühl⁸).

In this issue of *Gut*, Aubé and colleagues⁹ report exciting findings which further extend the potential roles of enteric glia in gastrointestinal physiology (see page 630). Employing a mouse model which has been previously used to ablate enteric glia in newborns—resulting in rapidly fatal intestinal inflammation¹⁰—the authors targeted enteric glia in the mature ENS. They injected activated influenza virus haemagglutinin (HA) specific CD8+ T cells into adult mice specifically expressing HA on enteric glia as well as on astrocytes and spinal glia.^{10–12} Surprisingly, this treatment did not induce any overt loss of enteric glia, nor was there any neuronal cell loss in the ENS of treated animals. However, when the authors analysed the neurochemical coding in the jejunum of treated animals, they found a marked reduction in the vasoactive intestinal peptide (VIP) and substance P (SP) immunoreactive neuronal populations in the submucosal plexus (SMP), whereas in the myenteric plexus (MP) the proportions of choline acetyltransferase (ChAT) and nitric oxide synthase (NOS) immunoreactive neurones were significantly increased and decreased, respectively.

The neurochemistry of the murine ENS is not yet well characterised, and

the authors did not try to further decipher the neurochemical code in this species. However, there is some evidence that nitric oxide (NO) and VIP contribute to nerve mediated relaxation in the mouse.¹³ While currently there is no evidence that VIP neurones are secretomotor neurones in the murine SMP, the primary transmitter of excitatory motor neurones in the mouse is acetylcholine.¹⁴ In addition, it has been postulated that SP containing nerves in the mouse gut may also be excitatory (table 1).¹³

The strength of this paper clearly lies in the detected correlation between the numerical changes in myenteric neuronal phenotypes and the functional alterations revealed in *in vitro* and *in vivo* analyses of gastrointestinal motor functions. An example of how neural regulation of gastrointestinal functions depends on the presence of specific neuronal phenotypes is provided by the fact that neurally mediated jejunal relaxation *in vitro* was dramatically impaired in the relatively NOS deficient transgenic mice, which in addition convincingly endorses the contention that in mouse—like in guinea pig—NOS immunoreactive neurones are inhibitory. Because the role of nitric oxide (NO) in normal gastric emptying and gastrointestinal transit is well established, loss of NOS immunoreactive neurones would also explain the observed delay in gastrointestinal transit.^{15–16} While it is currently impossible to account for the seemingly contradictory alterations observed in the SMP, together the reported data strongly indicate that enteric glia regulate gastrointestinal physiology by controlling neurochemical phenotypes in the ENS.

In spite of these exciting implications, some words of caution are necessary: On close examination, this study does not provide convincing evidence that enteric glia are indeed functionally impaired in transgenic mice. On the other hand, quite extensive inflammatory alterations have been previously described in the brain and spinal cord of these animals.¹¹ Thus gastrointestinal motility changes observed by the authors could directly result from CNS dysfunction via extrinsic neuronal modulation. Alternatively, the motility