The Neurophysiology of Ano-Rectal Sensation

A thesis submitted to the University of Manchester for the degree of Doctor of Medicine in the Faculty of Medicine, Dentistry and Nursing.

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Abstract

Despite recent interest in the physiology of gut sensation, human ano-rectal sensory neurophysiology is still poorly understood. The neurophysiological characteristics of ano-rectal primary afferents and the ascending pathways have been explored in animal studies. However, little is known about the central nervous system processing of ano-rectal sensation in man, due largely to the lack of non-invasive neurophysiological techniques to investigate the human brain function. Recently imaging tools have been developed that allow human brain function to be studied non-invasively and can now be used to study the neurophysiology of visceral sensation in man.

Cerebral evoked potentials (CEP) have been recorded following both electrical and mechanical rectal stimulation. Differences in CEP latencies between studies using these two stimulation modalities have led to the speculation that different afferent pathways are activated by each stimulation modality. However, no direct comparisons of these two stimulation modalities have been performed in the same subjects, so this hypothesis has not previously been tested. I have compared rectal CEP using electrical and mechanical rectal stimulation in the same subjects. My results demonstrate that both electrical and mechanical rectal stimulation activate similar afferent pathways, because the latency differences between the CEP can best be accounted for by the differences in the stimulus characteristics of these two stimuli.

There are important anatomical and physiological differences between the function and afferent innervation of the rectum and proximal gut, with the rectum having a greater sensory function than most of the proximal gut organs. Whether these differences are reflected in the central processing of sensations arising from these gut regions is unexplored. I have compared CEP following oesophageal, duodenal and rectal stimulation and shown that rectal CEP have a shorter latency than both oesophageal and duodenal CEP. Furthermore, the duodenal CEP amplitude was smaller than both oesophageal and rectal CEP amplitude. This demonstrates that the physiological and anatomical differences between the different gut regions are reflected by differences in the neurophysiological characteristics of their afferent pathways.

The brain areas involved in processing ano-rectal sensation remain unknown. Using functional MRI (fMRI) I have demonstrated a wide cortical network processing both rectal and anal canal sensation. This network includes areas involved in spatial discrimination, attention and affect.

In conclusion, I have demonstrated the feasibility of studying ano-rectal sensation with both CEP and fMRI. I have identified the cortical network that processes ano-rectal sensation and demonstrated differences between the central processing of rectal and proximal gut sensation

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning

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Abbreviations:

5-HT	Serotonin
ACC	Anterior Cingulate Cortex
CEP	Cerebral Evoked Potential
cm	Centimetres
BOLD	Blood Oxygen Level Dependent
EEG	Electroencephalogram
ERS	Electrical Rectal Stimulation
FGD	Functional Gastrointestinal Disease
fMRI	Functional Magnetic Resonance Imaging
GIT	Gastrointestinal Tract
Hz	Hertz
IBS	Irritable Bowel Syndrome
LL	Long Latency
mA	milli Amperes
MEG	Magnetoencephalography
MRI	Magnetic Resonance Imaging
MRS	Mechanical Rectal Stimulation
ms	Milliseconds
PET	Positron Emission Tomography
SI	Primary Somatosensory Cortex
SII	Secondary Somatosensory Cortex
SIII	Sensory Association Cortex
SLA	Short Latency Abrupt
SLS	Short Latency Sustained
SPECT	Single Photon Emission Computer Tomography
μV	Micro-volts

Acknowledgements:

I wish to acknowledge the support and encouragement of Dr. Qasim Aziz and Professor David Thompson, without whom the work described in this thesis would not have been possible. All of my work has been built on the foundations already laid by the pioneering work of Dr. Qasim Aziz in the use of functional brain imaging to study the neurophysiology of gastrointestinal tract sensation.

Mr. Anthony Hobson and Dr. Josephine Barlow from the department of Gastrointestinal Physiology, Hope Hospital provided valuable technical assistance in teaching me how to record cerebral evoked potentials. Dr. Paul Furlong from the University of Aston provided invaluable assistance in understanding and interpreting CEP. Mr. Tom Howell and Mr. Rob Hammer from the Department of Medical Physics at Hope Hospital designed and constructed the pump used in these studies.

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Finally I would like to acknowledge the help and support of my wife who has not only proof read this thesis, but also supported and encouraged me while I have been working on this research and while "writing up".

The Author:

I qualified in medicine from Sheffield University in 1991. Following pre-registration house jobs at Doncaster Royal Infirmary and Lodgemoor Hospital in Sheffield I spent three years in general medical SHO posts at Tameside General Hospital and Doncaster Royal Infirmary. During these jobs I gained experience in all the major medical specialities. I passed the MRCP (UK) in March 1995 and have since then been a collegiate member of the Royal College of Physicians of Edinburgh.

I was a registrar in Gastroenterology and General Internal Medicine at Tameside General Hospital for one year and at Wythenshawe Hospital for 18 months. Following these jobs I obtained a two year research training grant from the Digestive Disorders Foundation to perform the work described in this thesis. Since the completion of my Digestive Disorders Foundation fellowship I have worked as a Specialist Registrar in Gastroenterology and General Internal Medicine at the Royal Lancaster Infirmary and Hope Hospital, Salford.

Publications:

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The Role of Functional Brain Imaging in Gastroenterology in Health and Disease (editorial). Digestive and Liver Diseases, 32: 101-103, 2000

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Comparison of Cortical Potentials Evoked by Mechanical and Electrical Stimulation of the Rectum. Neurogastroenterology and Motility, 12(6): 547-554, 2000 (Chapter 3)

D I Hobday, Q Aziz, N. Thacker, I Hollander, D G Thompson, A Jackson

A Study of the Cortical Processing of Ano-Rectal Sensation Using Functional Magnetic Resonance Imaging. Brain, 124 (2): 101-108, 2001 (Chapter 6)

Preface

At the start of the 20th century it was widely argued that the viscera were insensitive to all stimuli, and that abdominal pain only resulted from the activation of somatic nerves in the parietal peritoneum. This was supported by studies showing that the non-inflamed stomach was insensitive to stimuli. In addition the colon was insensitive to normally painful stimuli, such as cutting and burning, allowing it to be operated upon without anaesthesia. Since this time the existence to true visceral pain has been established by studies demonstrating pain after stimulation of the gastrointestinal tract (GIT) which can be abolished by sympathectomy.

Until recently there was an assumption that the central processing of visceral sensation was the same as somatic sensation, which has been extensively studied. However, the differences in the perception of sensation from the GIT and somatic tissues would suggest that differences are likely to exist in the central processing of sensation from these structures. This hypothesis is supported by animal studies, which have shown important differences between the peripheral innervation of the GIT and skin. The skin is innervated by a rich supply of afferents with specialised receptors sensitive to a range of stimuli, while the gut is innervated by fewer afferents without specialised receptors. Animal studies have also shown convergence of somatic and visceral afferents onto single spinal neurones. However, there are differences in the functional importance of ascending spinal pathways for somatic and visceral sensation. Somatic sensation is conducted in both the spinothalamic tracts and dorsal columns while the dorsal columns are functionally more important for visceral sensation. The brain's role in sensory processing has been less intensively studied, but animal studies have shown convergence of somatic and visceral sensation in the somatosensory cortex.

Until recently it has been difficult to investigate the neurophysiology of GIT sensation in humans, because of the lack of non-invasive tools for studying human brain function. Therefore little is currently known about the neurophysiology of GIT sensation in man. Several recent technological advances have led to the development

of imaging tools (such as functional Magnetic Resonance Imaging [fMRI], Positron Emission Tomography [PET], Cerebral Evoked Potentials [CEP] and Magnetoencephalography [MEG]), which allow human brain function to be studied noninvasively. These functional brain imaging tools have now been used to investigate the central processing of gut sensation in man. These studies have, however, largely concentrated on investigating the central processing of oesophageal sensation. The rectum, unlike the most of the GIT (excluding the stomach), functions as a sensory organ. In addition, there are differences in afferent innervation between the rectum and the rest of the GIT. The rectum has afferent innervation from the sacral spinal cord only, while the rest of the gut has a dual afferent innervation from the vagus nerve and thoraco-lumbar spinal cord. Therefore, it is possible that the central processing of rectal sensation is also different from the rest of the gut. It follows that information gained from studies of oesophageal sensation may not be applicable to the processing of rectal sensation.

Cerebral evoked potentials (CEP) have been recorded following both electrical (ERS) and mechanical rectal stimulation (MRS). Comparisons between studies have shown differences in the CEP latencies using these two stimulation modalities, with MRS having a longer latency. This has led to the speculation that these two stimulation modalities are stimulating different ascending pathways. However, no direct comparisons of these two stimulation modalities in the same subjects have previously been published. Therefore, comparisons between these two stimulus modalities rely upon comparisons between different studies. The methodologies used for rectal stimulation and CEP recording in these studies differed, so the results cannot be directly compared. To identify the afferent pathways activated by these two stimulation modalities it is necessary to compare ERS and MRS in the same subjects using optimal parameters. This would also help to develop CEP as a useful tool for investigating the neurophysiology of rectal sensation.

The brains processing of rectal sensation is poorly understood, with the two previous studies that investigated this giving conflicting results. The first study only identified activation of the thalamus and post-central gyrus, while the second

identified only anterior cingulate cortex activation. These studies used different modalities of rectal stimulation (rapid phasic distention, and painful tonic distention respectively) and different imaging tools (PET and fMRI respectively). No published studies have investigated the brain areas processing anal sensation. Therefore, the brain areas that are involved in processing ano-rectal sensation are not fully known.

Aims of the thesis:

a) To study the neurophysiological characteristics of the rectal afferent pathways.

b) To compare the neurophysiological characteristics of afferent pathways from the rectum and other gut organs.

c) To identify the cortical areas involved in processing ano-rectal sensation

Chapter 1:

Anatomy and Neurophysiology of the Rectum and Anal Canal

This chapter reviews the anatomy and neurophysiology of ano-rectal sensation.

Chapter 2:

Basic Principles of Functional Brain Imaging and its Role in Studying Visceral Sensation

This chapter reviews the basic principles of both cerebral evoked potentials and functional magnetic resonance imaging, before reviewing previous studies of gut sensation using these techniques.

Chapter 3:

Characterisation of rectal afferent pathways using CEP

This chapter describes experiments comparing CEP recorded following electrical and mechanical rectal stimulation, in the same subjects. I demonstrate that the longer CEP latency with mechanical stimulation is likely to be due to the delay in

balloon inflation, and that both electrical and mechanical rectal stimulation activates similar afferent pathways.

Chapter 4:

Comparison of afferent pathways from the proximal and distal gut

This chapter describes experiments comparing CEP recorded following stimulation of the rectum and the proximal gastrointestinal tract (oesophagus and duodenum) in the same subjects. I demonstrate that rectal CEP have a shorter latency than both oesophageal and duodenal CEP, while duodenal CEP have a smaller amplitude than both oesophageal and rectal CEP. This suggests that there are differences in the neurophysiological characteristics of rectal afferent innervation in comparison to proximal gastrointestinal organs.

Chapter 5:

Studies of the Cortical Processing of Human Ano-Rectal Sensation using Functional Magnetic Resonance Imaging

This chapter describes studies comparing the cortical areas processing anal and rectal sensation using fMRI. I demonstrate a similar pattern of cortical activation with both anal canal and rectal stimulation activating cortical areas involved in spatial discrimination (primary and secondary somatosensory cortex), attention (anterior cingulate) and affect (anterior cingulate and pre-frontal cortex). There were however, important differences in the somatosensory representation of these two organs.

Chapter 6:

General discussion

This chapter discusses the results of the studies described in the previous chapters and provides suggestions for future research into the central processing of visceral sensation in health and disease.

Chapter 1

Anatomy and Neurophysiology of the Rectum and Anal Canal

Anatomy and Neurophysiology of the Rectum and Anal Canal

1.1 Ano-rectal anatomy

The rectum and anal canal form a continuous tubular structure situated within the pelvis and perineum. The anal canal is 3 cm in length and positioned below the pelvic floor in the perineum. The rectum is the superior continuation of the anal canal above the pelvic floor and measures 12 cm in length.

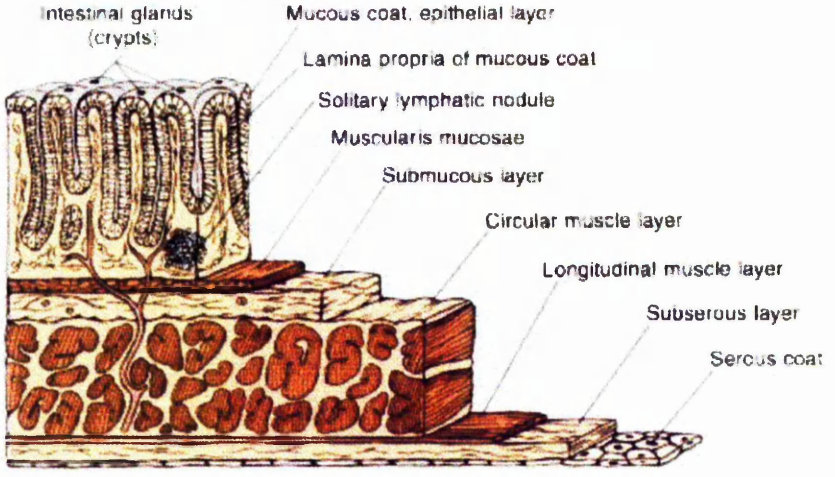
The embryological development of the ano-rectal region is from two different sources. The rectum and upper anal canal develop from the endoderm with the rest of the GIT, while the lower anal canal develops as a pit in the ectoderm. The boundary of these two embryological regions is marked by the dentate line, which marks a boundary in the anatomy and physiology of these two regions. During embryological development there is a membrane between these two regions of the anal canal, which breaks down leaving small valve like remnants (the anal valves). The area of anal canal below the anal valves is lined by skin and so has a rich afferent innervation from the pudendal nerve, which also supplies the skin of the perineum. The afferent innervation of the rectum and superior portion of the anal canal is from the inferior hypogastric plexus. These anatomical differences in afferent innervation between the rectum and anal canal result in differences in sensations arising from these two organs (discussed later). The blood supply of these two regions is also different, with the blood supply of the anal canal coming from the inferior rectal artery, which is a branch of the internal pudendal artery. Whereas the rectum's blood supply comes from the superior and middle rectal arteries, which are branches of the internal iliac artery.

The rectal wall consists of several layers (figure 1.1). The mucosal and submucosal layers are surrounded by two muscular layers, the circular and the longitudinal muscle layers. The superior third of the rectum is covered by

peritoneum anteriorly and laterally, while the lower two thirds is below the level of the peritoneum (figure 1.2). Although the word rectum originates from the Latin word "rectus" meaning straight, the human rectum follows the curve of the sacrum and has three lateral curves. These curves result in three folds in the rectal mucosa, called the rectal valves. The superior border of the rectum is defined by the start of a mesentery, from this point the GIT is called the sigmoid colon.

Figure 1.1:

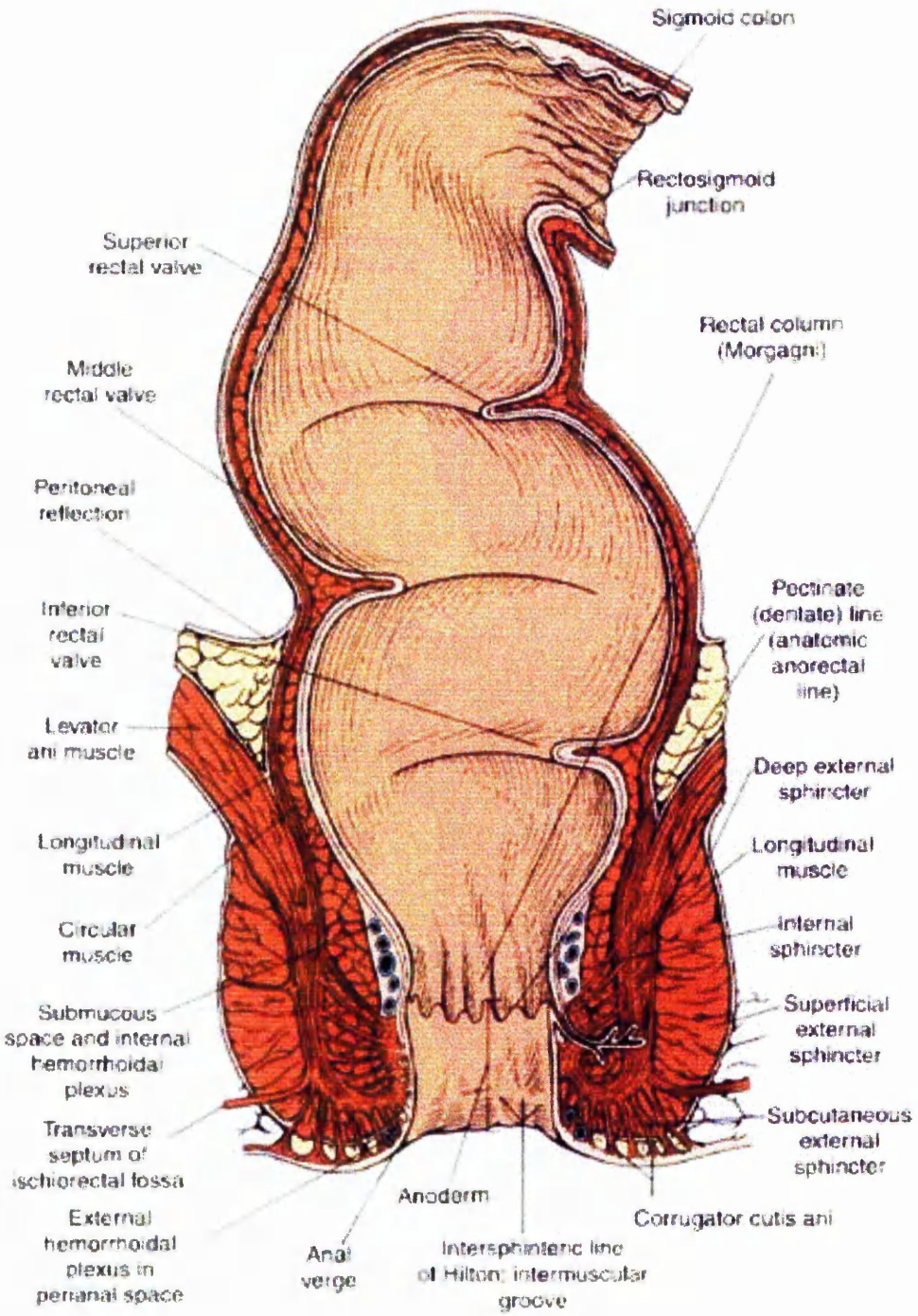
Layers of the colonic and rectal wall



The smooth muscle of the circular muscular layer extends down to the anal canal where it forms the internal anal sphincter. Anatomically the muscle of the internal anal sphincter is indistinguishable from the circular muscular layer of the rectum. Functionally however, this area of muscle works as a sphincter under reflex spinal control. The internal anal sphincter is surrounded by striated skeletal muscle of the external anal sphincter (figure 1.2). The external anal sphincter extends lower than the internal anal sphincter and is under voluntary control. The levator ani muscle, which forms the pelvic floor, also forms a loop around the ano-rectal junction. Contraction of this muscle results in an increased angulation of the ano-rectal junction, so helping to maintain rectal continence. The internal anal sphincter relaxes on rectal distention due to a spinal reflex. Therefore, continence is dependent upon rectal sensation and the voluntary contraction of the external anal sphincter and the levator ani muscle.

Figure 1.2:

The anatomy of the anal canal and rectum



1.2 Ano-rectal neurophysiology

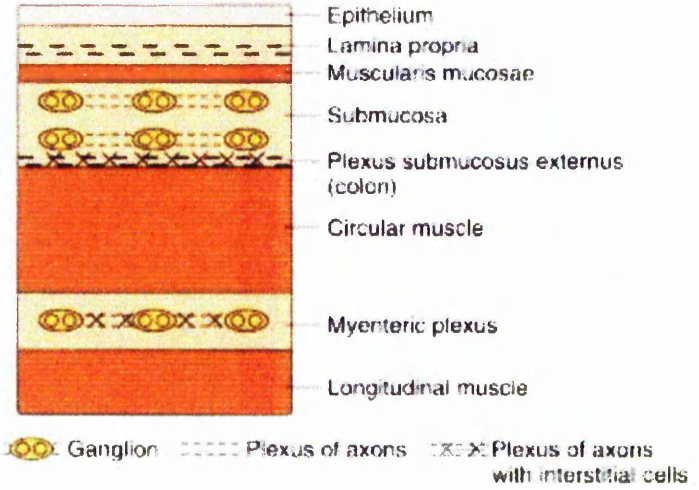
1.2.1 Rectal afferent innervation:

The whole of the gastrointestinal tract (GIT) has two inter-related neuronal networks: the intrinsic enteric nervous system and the extrinsic nervous system, consisting of the peripheral nerves and central nervous system.

1.2.1.1 The enteric nervous system:

The enteric nervous system stretches throughout the length of the GIT, from the oesophagus to the rectum. The neurones of the enteric nervous system are arranged into two groups of ganglia, the myenteric plexus positioned between the longitudinal and circular muscle layers of the bowel wall and the submucosal plexus positioned in the submucosal layer of the bowel wall (figure 1.3). The function of this intrinsic nervous system is to regulate GIT motor and secretory function. Although the enteric nervous system can accomplish this function independently from extrinsic innervation, it remains under central modulation via the autonomic nervous system (1). Neurones in the enteric nervous system do not have axonal projections outside the gut. Therefore sensations from the GIT are conveyed to the brain by afferent neurones of the peripheral nervous system.

Figure 1.3:
Enteric nervous system:



In addition to the enteric nervous system there is a second class of afferent neurones with cell bodies within the bowel wall (2). Intestinofugal neurones are primary afferents with cell bodies within the bowel wall, but with processes synapsing in the pre-vertebral ganglia, with sympathetic neurones which project back to the proximal GIT. These afferents are more common in the rectum and colon than proximal GIT, where they form the afferent limb of entero-enteric reflexes. The role of this class of primary afferent is to regulate GIT motility and secretion, providing feedback from the distal bowel to the proximal GIT so helping to control the flow of chyme into the distal bowel.

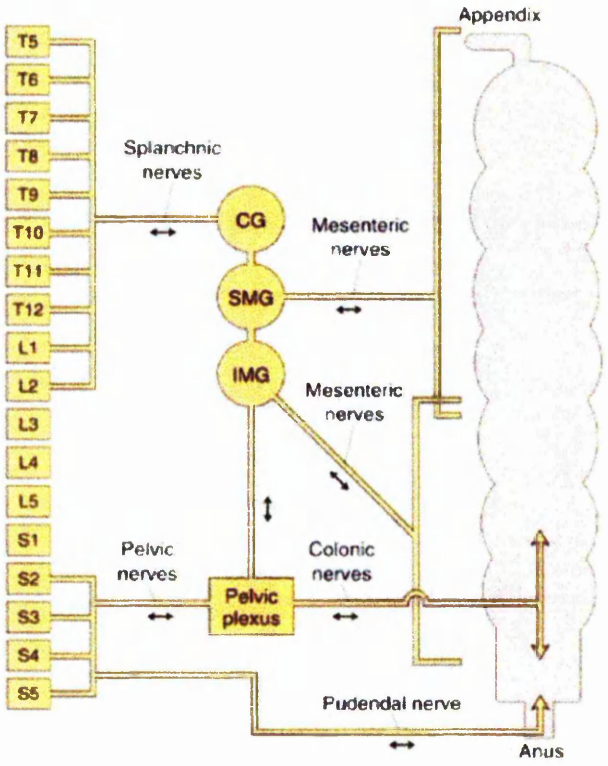
1.2.1.2 Primary rectal afferents:

The afferent innervation of the rectum is solely from the pelvic nerve, which originates from the inferior hypogastric plexus, situated lateral to the rectum. The inferior hypogastric plexus contains both parasympathetic nerves from the sacral roots and sympathetic nerves from the lumbar roots which reach the inferior hypogastric plexus via the pelvic part of the sympathetic chain and superior hypogastric plexus (figure 1.4). Afferents responding to rectal stimulation have been identified in the sacral roots, but not however, in the lumbar roots (3-5). Histological studies show that most rectal afferents have free nerve endings, without specialised receptors. The exception to this is the serosal surface of the bowel, where specialised receptors have been identified (6).

It has been speculated that enteroendocrine cells within the mucosa may act in part as receptors for the mucosal afferents (7). The luminal surface of these cells is covered with micro-villi, so these cells are well adapted for sensing the luminal contents. The cell cytoplasm contains secretory granules which contain serotonin (5-HT), which can be released into the mucosa and activate afferent neurones in a paracrine fashion. Work by Hillsley et al. (8) has suggested that this afferent nerve activation is mediated via the 5-HT_3 receptors.

Figure 1.4

Extrinsic innervation of the anal-rectal region



Diagrammatic representation of the neuronal innervation of the colon and anal canal. CG- coeliac ganglion, SMG- superior mesenteric ganglion, IMG inferior mesenteric ganglion.

Several electrophysiological studies have investigated the response of sacral spinal afferents to rectal stimulation in both intact animals and in vitro. These studies show that visceral afferents constitute less than 10% of the afferents entering the spinal cord in these segments (9). These studies have also divided rectal afferents into several functionally different groups according to their stimulus response characteristics (3) as in the more proximal GIT. Most studies trying to characterise these afferents have used distention as the only stimulus. Therefore, the resulting classification is based on the afferent response to different distention pressures.

Low threshold and wide dynamic range afferents:

Low threshold and wide dynamic range afferents respond to both rectal distention within the physiological range, and peristalsis (3, 4). Both of these afferent types encode a range of distention pressures by an increased frequency of axonal discharges. These afferents differ in the intensity range of stimuli they can encode. Low threshold afferents encode a limited range of distention pressures within the physiological range. Wide dynamic range afferents encode a larger range of stimuli including non-painful and painful distention pressures.

High threshold afferents:

High threshold afferents have been identified which are insensitive to rectal distention within the physiological range but do respond to distention within the noxious range (3). High threshold afferents are insensitive to peristalsis and have a low frequency of spontaneous activity, in contrast to the low threshold afferents that are sensitive to peristalsis and have a higher frequency of spontaneous activity (3, 10, 11).

Spinal mucosal afferents:

Recent studies have identified spinal afferents from both the oesophagus (12) and rectum (4) that are sensitive to light brushing of the mucosa. These afferents which are insensitive to stretching of the gut, can be activated by brushing the mucosa with a 10 milligram brush and are sensitive to a range of chemical stimuli applied to the mucosal surface of the rectum. These afferents have been called mucosal afferents, because of their response characteristics, but without histological proof of their anatomical position.

Silent afferents:

Several studies have identified a group of afferents that under physiological conditions are insensitive to high pressure rectal distention. These afferents, however, have been shown to be sensitive to rectal distention following the induction of rectal inflammation (13). While some mechanosensitive rectal afferents have been shown to be polymodal (14), the response of silent afferents to thermal and chemical stimuli has not yet been tested, so whether these afferents are truly silent is unknown.

The term "mechanically silent afferents" is therefore a more appropriate description of these afferents.

1.2.1.3 Spinal neurones:

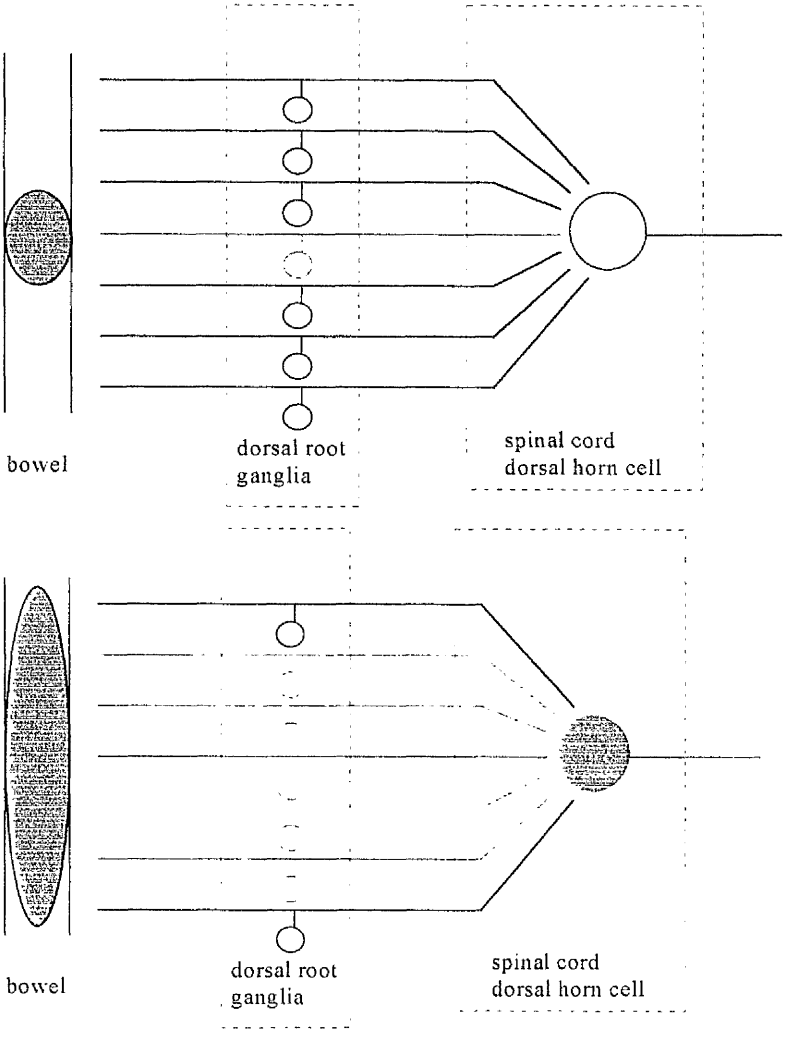
Rectal afferents enter the spinal cord in the dorsal roots to synapse with neurones within lamina I and V to VII of the dorsal horn grey matter (5, 15, 16). Before synapsing with these dorsal horn neurones the primary afferent axon can travel up or down several segments of the spinal cord. This results in afferents from one segment of GIT synapsing with spinal cord neurones in several segments, (17, 18). This arrangement is partly responsible for the poor spatial awareness of rectal sensation. An additional consequence of the convergence of afferents is that each dorsal horn neurone receives afferents from a long segment of GIT, and other visceral organs. Stimulation of a long segment of the GIT therefore activates more afferents than if the same stimulus was applied to a shorter segment. This results in a greater afferent discharge reaching the dorsal horn neurones as the length of the stimulated GIT increases (figure 1.5). The consequence of this is that a low-level stimulus that is not able to activate dorsal horn nocioceptive neurones when applied to a short segment of the GIT could do so if applied to a longer segment. This amplification of sensory information is called spatial summation (18).

Studies investigating the stimulus response characteristics of neurones within the lumbosacral spinal cord have led to a classification dependent upon the temporal characteristics of the neurones response to rectal distention (15, 16). The dorsal horn neurones in the L5 to S2 segments of the spinal cord can be divided into short latency abrupt (SLA), short latency sustained (SLS), long latency (LL) and inhibited (15). Most SLA and SLS neurones also respond to somatic stimulation. These two groups which also have ascending projections, respond to rectal distention with a short latency of less than one second for SLA and less than two seconds for SLS. On termination of the rectal distention the SLA cells activation stops abruptly while that of SLS continues for up to 120 seconds. Few LL cells respond to somatic stimuli, or have ascending projections. These neurones respond to rectal distention with a long

latency, having a mean of 8 seconds and reach maximal activation at or after the end of the rectal distention. These neurones also produce a sustained response, lasting a mean of 40 seconds after the termination of rectal distention. While responding to rectal distention with different latencies, SLA SLS and LL neurones respond to both physiological and noxious intensities of rectal stimulation.

Figure 1.5

Spatial summation:



Diagrammatic representation demonstrating the spatial convergence of primary afferents from a long segment of bowel onto a single dorsal horn cell. Demonstrating the effects of spatial summation where by a low intensity stimulus, which can not activate the dorsal horn cell if applied to a short segment of bowel, can do so if applied to a longer segment.

Inhibitory dorsal horn neurones have tonic activity, which is inhibited by noxious rectal distention, few of these neurones have ascending projections (15). These inhibitory spinal neurones can be sub-classified into inhibitory abrupt, inhibitory sustained and inhibitory excitatory according to the time course of their response to rectal distention (16). Both inhibitory abrupt and inhibitory sustained neurones are inhibited by rectal distention, with a short latency, but differ in the duration of their inhibition following termination of the stimulus. Inhibitory excitatory neurones show a mixed response to rectal distention with a period of inhibition being followed by a period of excitation (16).

1.2.1.4 Ascending spinal pathways:

Anatomically the ascending spinal pathways are divided into two groups, the dorsal columns and the antero-lateral systems. The dorsal columns consist of ascending tracts containing both postsynaptic fibres from spinal neurones and presynaptic fibres from primary afferents. The antero-lateral system contains several different ascending and descending tracts. The most important ascending pathway in the antero-lateral system is the spinothalamic tract, which consists of postsynaptic fibres from spinal neurones only.

Electrophysiological studies have demonstrated ascending pathways for rectal sensation in both the spinothalamic tracts and the dorsal columns (19). However, studies have shown that the medial portion of the dorsal columns are functionally more important than the spinothalamic tracts in mediating rectal sensation (20, 21). Animal studies have shown that lesions to the dorsal columns have a larger effect on the response of thalamic neurones to rectal stimulation than lesions to the spinothalamic tracts (20). In man restricted lesions in the medial dorsal columns have been shown to "virtually eliminate" pain due to pelvic cancer (22), suggesting that the dorsal columns are also important in mediating visceral sensation in man.

1.2.1.5 Descending spinal pathways:

The dorsal horn neurones are under tonic inhibition from descending spinal pathways. Cooling the rostral spinal cord results in a temporary spinalisation of the experimental animal, removing dorsal horn cells from the influence of these descending pathways. This leads to an increased response of dorsal horn cells to GIT distention (15, 23). This effect is reversible on re-warming the rostral spinal cord. This descending inhibitory spinal pathway provides one mechanism by which the higher cortical centres can modulate the processing of peripheral sensation.

The vagus nerve has recently been shown to have a role in modulating pain perception. Stimulation of the vagus nerve results in increased activation of the descending inhibitory spinal pathway and so increases the inhibition of dorsal horn cells (24). Serotonin and glutamate in the nucleus of the solitary tract (24) mediate this anti-nocioceptive affect of vagal stimulation. Several strands of evidence suggest that this anti-nocioceptive affect of vagal stimulation is of physiological importance. Firstly, physiological vagal stimulation using intravenous volume expansion in rats results in a reduction in pain behaviour (25). Secondarily, vagotomy results in an increase in pain behaviour to colorectal distention in rats (26). The role of the vagus nerve in modulating pain perception in man has not been explored.

1.2.2 Comparison of rectal and proximal gut innervation:

The proximal GIT, unlike the rectum, has a dual afferent innervation, receiving afferent neurones from both "sympathetic" spinal and "parasympathetic" vagal nerves (27). These two sets of afferents have different properties and are involved in differing but overlapping aspects of visceral sensation.

The vagus nerve:

The vagus nerve innervates the GIT from the oesophagus down to the level of the descending colon. While initially considered to be a pure efferent nerve, recent studies have demonstrated that up to 90% of the vagal nerve fibres are afferent (sensory) nerves (28). These afferent neurones are mainly unmyelinated C fibres and

thinly myelinated A-delta fibres. The receptive fields of both the A-delta and C fibre neurones are within the mucosa and superficial muscular layers of the gut wall (12, 28, 29). Studies of the stimulus response characteristics of these afferents show that these afferents are sensitive to low intensity stimuli, including peristalsis, physiological levels of bowel distention and chemical stimuli (12, 29). These afferents are considered to be involved in the reflex regulation of the GIT function and to mediate physiological non painful sensations but not pain (5).

Spinal afferents:

Spinal afferents arise from the thoracic and lumbar regions of the spinal cord and travel with the sympathetic nerves to the GIT (28). These neurones however, have cell bodies in the dorsal horn nucleus, in common with somatic and rectal afferents. As with the vagal afferents, these spinal afferents are a mixture of unmyelinated C fibres and thinly myelinated A-delta fibres. The receptive fields of spinal afferents are distributed throughout the gut wall from the mucosal to the serosal surface. Electrophysiological studies of these afferents have identified similar classes of afferents as in the rectum [low threshold, wide dynamic range, high threshold and mechanically silent afferents (3, 4, 11, 28)]. Spinal afferents are believed to mediate pain arising from the GIT (28, 30).

1.2.3 Anal afferent innervation:

1.2.3.1 Primary afferent innervation:

The anal canal, unlike the rectum, has dual afferent innervation. The area of the anal canal above the dentate line shares its embryological development from the endoderm with the rectum. This area of the anal canal is innervated by the pelvic nerve and has similar afferents to the rectum. The area of the anal canal below the dentate line develops from the ectoderm and is innervated by the pudendal nerve (figure 1.4). While the sacral roots of the two sets of anal afferents are the same, there are important differences in the innervation of these two areas of the anal canal.

In contrast to the rectum the somatic portion of the anal canal has a rich supply of afferent nerves with a mixture of free nerve endings, and specialised receptors. There is also a greater diversity of afferent fibres innervating the anal canal, with unmyelinated C fibres, myelinated A delta and beta fibres. This allows for the rapid conduction of sensation from the anal canal to the brain. The rich diversity of receptors allows for the discrimination between different sensory stimuli, with different receptors responding to different sensory modalities (such as light touch, pinprick and temperature). The higher density of afferent nerves results in a greater capacity of spatial discrimination.

It is common experience that sensations arising from the skin and the GIT feel different. This difference is evident when comparing the sensation of abdominal pain following GIT injury due to gastroenteritis with pain following injury to the arm. The neurophysiological differences between rectal and anal afferent innervation in part explain these differences in sensations. The differences in sensory characteristics suggests that the differences in peripheral innervation may also be mirrored by differences in the central processing of sensations from the GIT in comparison to somatic structures.

1.2.3.2 Spinal neurones:

The primary afferents form the somatic portion of the anal canal enter the spinal cord in the dorsal roots. The distribution of anal afferents in the spinal cord is wider than that of rectal afferents. The larger myelinated fibres enter the spinal cord medial to the unmyelinated and thinly myelinated fibres. These larger myelinated fibres enter the dorsal columns to form the ascending tract without synapsing. While the smaller diameter afferents enter the lateral aspect of the dorsal horn and synapse with interneurones. These interneurones then send projections to the anterior motor horn of the spinal cord, and to the ascending pathways in the spinothalamic tracts.

Electrophysiological recording from individual dorsal horn cells following peripheral somatic stimulation has lead to classifications of these cells on the basis of their response characteristics, which differs to that for rectal sensation (31). As with

primary afferents dorsal horn cells are divided into low threshold (class 1), wide dynamic range (class 2) and high threshold (class 3 or nocioceptive cells) according to their response to the intensity of peripheral stimulation.

An alternative classification of dorsal horn cells is based on their response to visceral and somatic stimuli. There is a high degree of viscero-somatic convergence at the level of the spinal cord, with most dorsal horn cells responding to both somatic and visceral stimulation (15). Somatic neurones predominantly have class 1 (low threshold response characteristics), while visceral-somatic neurones predominantly have class 2 or 3 response characteristics (i.e. respond to painful somatic stimulation) (15). Very few dorsal horn neurones that respond only to visceral stimulation have been described. This visceral-somatic convergence is believed to be the mechanism responsible for the referral of visceral sensation to somatic structures (18).

1.2.3.3 Spinal pathways:

Anal canal sensation is mediated by both the dorsal columns and spinothalamic tracts, as with rectum sensation. However, important differences exist. The two ascending pathways are involved in mediating different modalities of somatic sensation. The spinothalamic tracts mediate the sensory modalities of temperature, pinprick and pain from the contralateral side of the body, while the dorsal columns mediate light touch and spatial discrimination (two point discrimination). The portion of the dorsal columns mediating anal sensation is also more lateral than that mediating rectal sensation (20).

Studies of somatic sensation have identified two functionally separate ascending spinal pathways within the anterior lateral spinal columns, the lateral and medial pain pathways (32, 33). The "lateral pain pathway" consists of the neo-spinothalamic tracts that project to the somatosensory cortex via the ventral-posterior and posterior nuclei of the thalamus (32). This pathway is believed to be involved in the spatial discriminative aspects of sensation, with little involvement in mediating the subjective awareness of pain (32). The medial pain pathway consists of the paleo-spinothalamic tracts and the spinoreticular tract, which projects to the limbic

cortex including the anterior cingulate cortex via the medial intra-laminar nucleus of the thalamus. These pathways are believed to mediate the affective and motivational aspects of pain sensation (32).

As with rectal sensation somatic and anal canal sensation is under tonic inhibition by descending spinal pathways. The characteristics of these descending pathways and the effect of vagal stimulation are similar for both visceral and somatic sensation (15, 24, 25).

1.3 Cortical processing of sensation

While a limited awareness of sensation is possible at a subcortical level, conscious awareness is largely a function of the cerebral cortex. Information about cortical function has largely been obtained from studying the effects of destructive cortical lesions and stimulating the cortical surface in conscious neurosurgical patients. Though providing some information about cortical function these methods are limited. Producing sensations by stimulating the cortex suggests a role for that cortical area in sensory processing, however, the precise role played can not be identified. Likewise the interruption of sensory processing by damage to a cortical area does not identify its precise role in sensory processing. Therefore, these methods of investigating the sensory function of the cortex provide only limited information about the functional role of cortical areas.

At the start of the 20th century Brodmann divided the cerebral cortex into regions based on histological features (figure 1.6) (34). Perhaps not surprisingly this histological subdivision of the cortex has been shown to correlate well with functional organisation of the cortex (35), and is still used to describe functional cortical regions. While several cortical areas have be shown to be involved in specific functions, these areas work as an integral part of a larger cortical network. There are several cortical areas that from a network involved in sensory and pain processing (fig 1.7) (36).

Figure 1.6

Brodmann areas

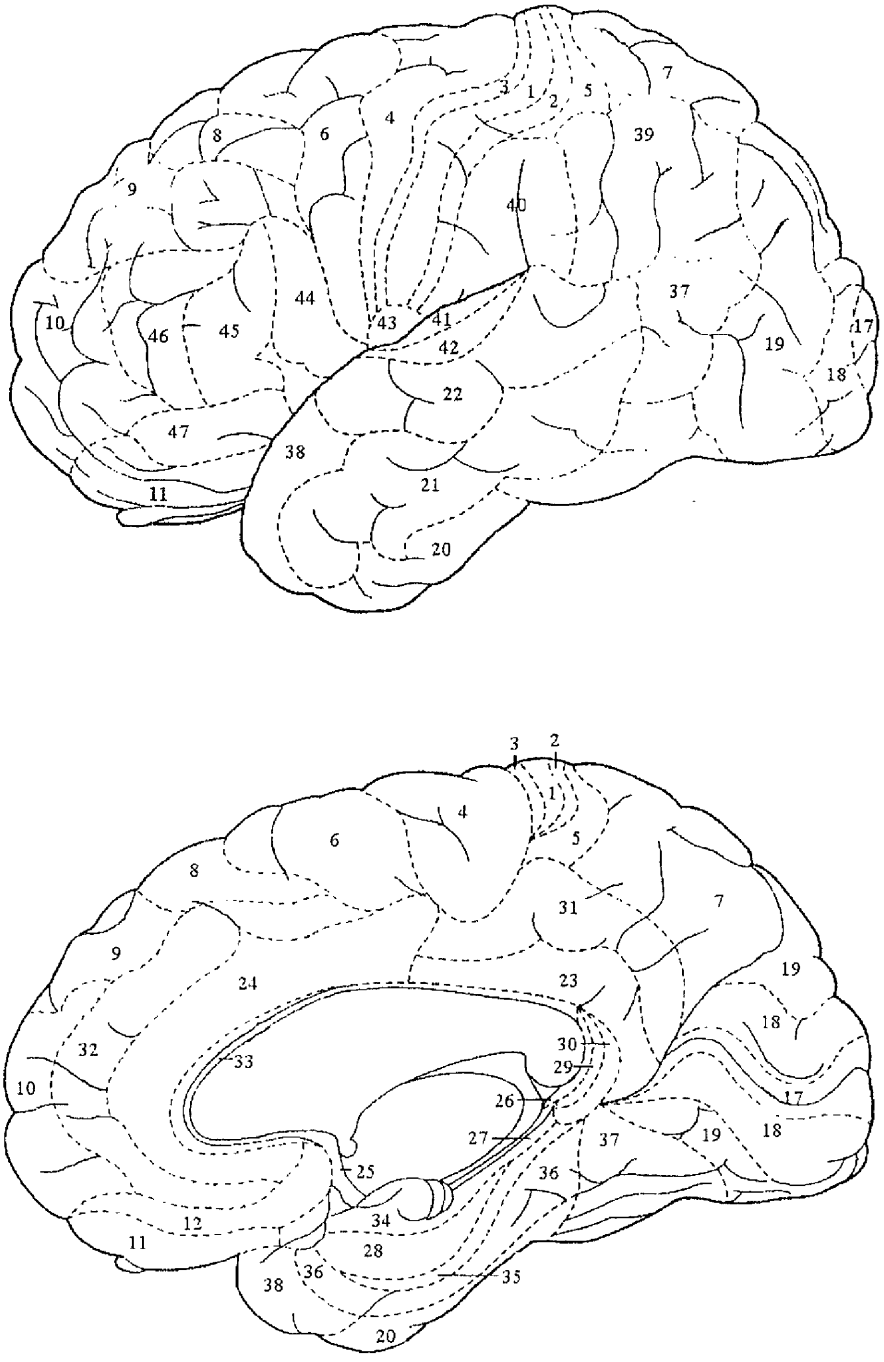
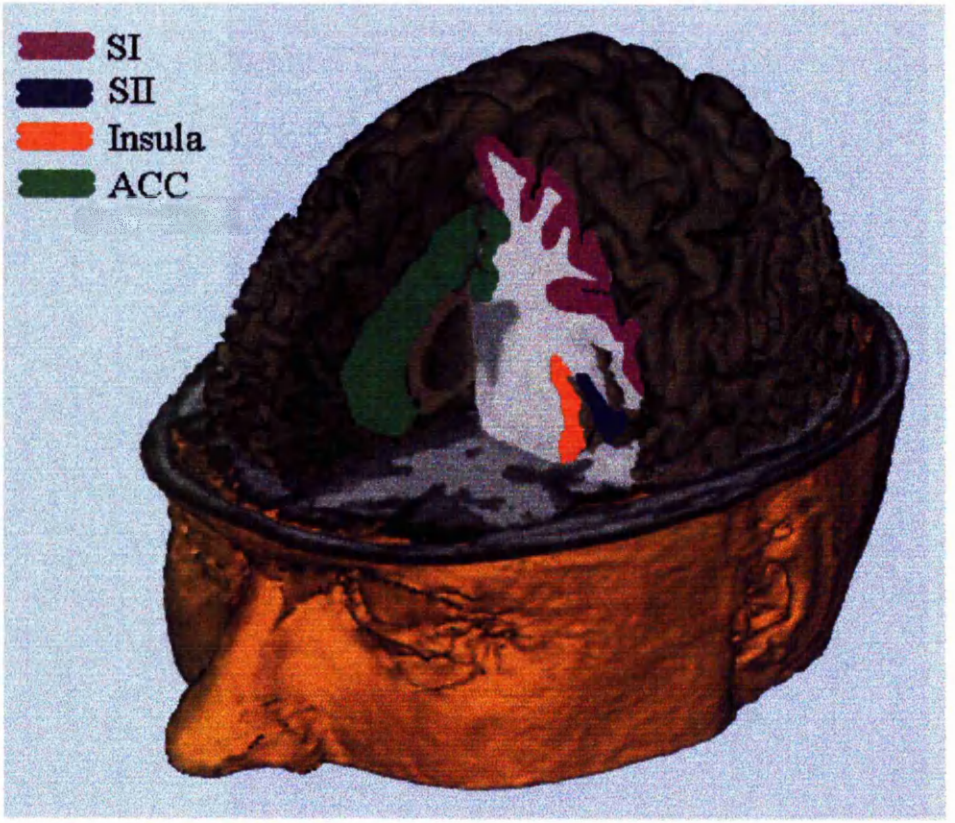


Figure 1.7

Location of cortical areas involved in sensory processing



1.3.1 Primary sensory-motor cortex

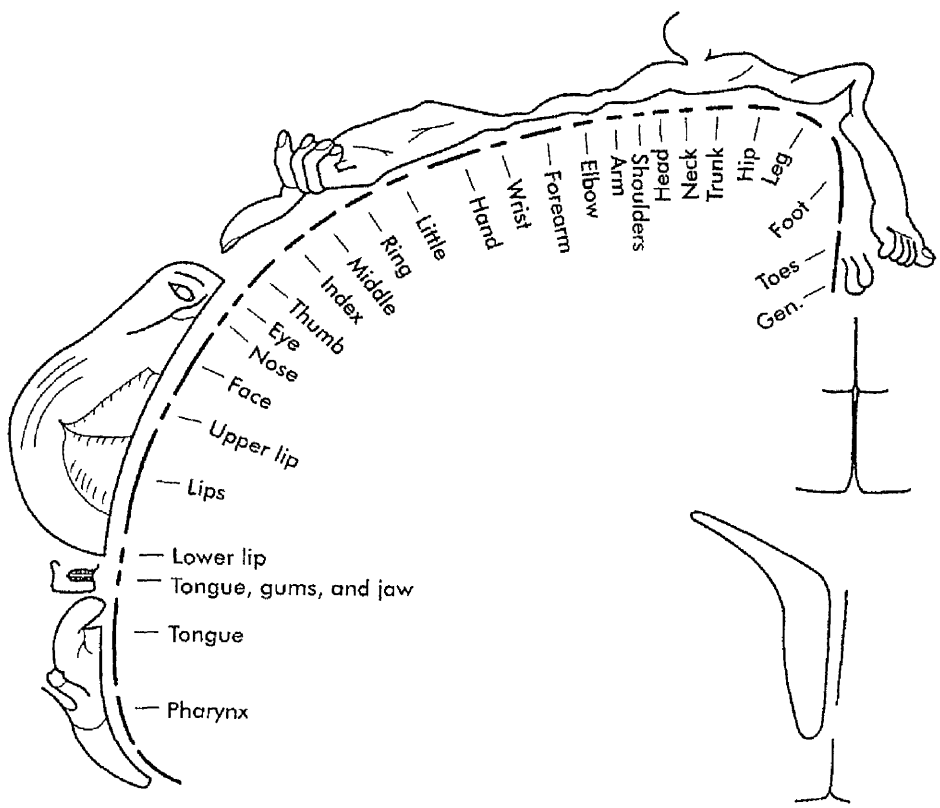
The primary sensory motor cortex is situated in the pre and post central gyri, and consists of Brodmann areas 1, 2, 3 and 4. The pre-central gyrus (Brodmann area 4) is predominantly involved with motor function, while the post-central gyrus (Brodmann areas 1, 2 and 3) is predominantly involved with sensation and often called the primary somatosensory cortex (SI). This distinction into motor and sensory cortex is not absolute, as sensory neurones have been identified within the pre-central gyrus and motor neurones within SI (37).

The afferent innervation of SI comes from the ventral-posterior nucleus of the thalamus (35). There is a high degree of spatial organisation in the SI sensory representation. Stimulation of the cortex along the post-central gyrus results in well-

localised contralateral sensation allowing a representation of the body to be reproduced along the gyrus, the homunculus (figure 1.8) (37). Studies on monkeys have identified neurones responding to GIT stimulation within the inferior part of SI (38). This study demonstrated a high level of convergence of visceral organs, with most cortical cells that responded to visceral stimulation responding to stimulation of several regions of the GIT (38). Studies on lower mammals have, however, shown marked variation between species in the representation of the GIT in SI (39). Functional brain imaging studies of human oesophageal stimulation have given conflicting results, with some identifying SI activation (40-43) while others do not (44-47). This discrepancy in results could be due to technical differences between studies and the limitations in spatial resolution of the techniques used. Magnetoencephalography (MEG) studies of somatic sensation have shown that SI is the first cortical area to be activated by sensory stimulation (48).

Figure 1.8:

The somatic homunculus



While functional imaging studies of somatic sensation have shown increased activation of SI with increasing stimulus intensity (49), several strands of evidence suggest that SI is not involved in processing pain. Direct stimulation of SI in man produces a range of sensations but fails to produce pain regardless of the intensity of stimulation (32, 37). Surgical destruction of SI while preventing the localisation of painful stimuli does not abolish the sensation of pain. Therefore, the role of SI is believed to relate to processing the spatial discriminative aspects of sensation.

1.3.2 Secondary somatosensory cortex

The secondary somatosensory cortex (SII) is a smaller cortical area than SI and is situated in the superior wall of the lateral sulcus, Brodmann area 43 (35). This area receives afferents from both the thalamus and SI (50). However, animal studies have shown surgical removal of SI prevents activation of SII (51), suggesting that the SI afferents are functionally more important than the thalamic ones. MEG studies of somatic sensation are in keeping with this, demonstrating that activation of SII occurs after SI (45, 48). While there is still a well organised spatial representation of the body in SII, this area unlike SI, has a bilateral representation of the body (52).

The functional role of SII is less well understood that other parts of the cortical network processing sensation. As activation of SII occurs after SI this area appears to be involved in the sequential higher processing of sensation after SI. Some neurones within SII respond to painful somatic stimulation (35, 52), suggesting a role in the pain processing. Functional imaging studies have also shown that SII activation is related to the intensity of a somatic stimulation (53), so supporting a role for SII in processing the intensity of sensations.

1.3.3 Sensory association cortex

The area of parietal cortex situated posterity to SI consisting of Brodmann areas 5 and 7 is called the sensory association area or the tertiary somatosensory

cortex (SIII). This area receives a rich afferent supply from SI, with reciprocal connections with the lateral thalamus (35). Cells within this area have been shown to respond to complex sensory information, including encoding the direction of movement of somatic stimuli (54). Destructive lesions of this area leave awareness of basic sensation intact but prevent the integration of sensory information, so that patients are unable to recognise objects by touch alone. This suggests that the sensory association cortex is involved in the higher processing and integration of sensory information.

1.3.4 Anterior Cingulate Cortex

The cingulate cortex is part of the limbic system, which forms most of the cortex in lower mammals, having been "pushed out on a limb" by the neo-cortex in man. The anterior cingulate cortex (ACC) is situated just above the corpus callosum on the medial surface of the brain Brodmann areas 24 and 32. This area receives afferents from the anterior nucleus of the thalamus and the insular cortex. Efferent connections from the anterior cingulate cortex go to the pre-motor cortex (55).

Although not forming part of the somatosensory cortex anatomically, functional imaging studies of somatic pain have often demonstrated ACC activation (49, 56, 57). Surgical lesions of the limbic cortex including the ACC have been performed to treat chronic pain and result in the relief of the negative emotional aspects of pain while leaving the sensory aspects intact (55). For these reasons the ACC has often been considered as a "pain centre". This is however, an over simplification of the function of the ACC.

Anticipation also activates the ACC in a similar but slightly anterior area to that activated by pain (58, 59). Attending to non painful cognitive tasks such as silent word generation also activates the ACC in a region anterior to that activated by pain (60). These studies demonstrate a role of the ACC in attention. Studies looking at cerebral blood flow have shown that the ACC has a reduced blood flow during depression (61), suggesting reduced activity in this area. Inducing transient emotions

result in changes in limbic cortex and ACC activity (62), so demonstrating the importance of these areas in emotions. Direct stimulation of the ACC produces feelings of unpleasantness and often a desire to leave. It has therefore been suggested that the role of the ACC is in generating affective response and in planing appropriate behavioural response to stimuli i.e. response selection (55).

The ACC also has efferent connections with the nucleus of the solitary tract, motor nucleus of the vagus nerve and sympathetic neurones in the thoracic spinal cord. Direct stimulation of the ACC results in changes in autonomic function including changes in blood pressure, heart rate, respiration and gastric motility (63). This shows that the ACC has an additional role in regulating autonomic function (55).

1.3.5 Insular Cortex

The insular cortex is situated deep within the lateral sulcus, and forms part of the limbic cortex. The insula is divided anatomically and functionally into two parts by the central sulcus.

The posterior insula forms a link between the somatic and limbic cortex (64). This area receives afferent sensory information direct form the vagus nerve (65, 66) and from SII (64). Efferent projections from the insular cortex go to the rest of the limbic cortex, including the ACC. The neurones within this part of the insula only respond to complex high intensity sensory stimulation having large, often bilateral, receptive fields with some covering most of the body (64, 67). As with lesions to the ACC, lesions of the insula result in the preservation of the sensory aspects of pain but relieve the distress and emotional aspects.

Animal studies have demonstrated changes in cardiovascular tone during insular stimulation (63, 68), suggesting that this area is also involved in regulating autonomic function. Stimulation of the anterior part of the insula in man results in changes in intestinal motor function and "visceral sensory phenomena" (69). These

sensations were largely described as "nausea", "something funny", "gurgling", "rolling" and "sick to my stomach" (69). These sensations are always accompanied by changes in intestinal motility (69). Therefore, it remains uncertain whether these sensations reflect a sensory function of the anterior insular, or are the result of intestinal motility, reflecting the role of the insular in regulating autonomic function.

1.4 Summary

There are differences in the subjective sensations arising form the GIT and somatic structures. This is in part due to differences in their peripheral afferent innervation and ascending pathways, but also suggest that their central representation is likely to differ. In addition there are important differences in the innervation and function of the rectum and the proximal GIT, with the rectum having a greater sensory function than most other gut organs. This suggests that the neurophysiology of rectal sensation could also be different to that of other GIT organs.

In order to understand the pathophysiology of visceral sensation in disease it is first necessary to understand the physiology of visceral sensation in health. However, little is currently known about the neurophysiology and central processing of visceral sensation in man. Most studies investigating the central processing of GIT sensation performed so far have investigated the oesophagus. The differences in function and innervation of the rectum in comparison to the oesophagus, however, means that information about the central processing of rectal sensation can not be inferred from these studies. Therefore, it remains important to develop tools for investigating the central processing of rectal sensation in man.

Chapter 2

Basic Principles of Functional Brain Imaging and its Role in Studying Visceral Sensation

Basic Principles of Functional Brain Imaging and its Role in Studying Visceral Sensation

2.1 Introduction

In order to understand both the physiology and pathophysiology of rectal sensation it is necessary to develop clinical research tools to allow the processing of rectal sensation to be studied non invasively in man. Several recent advances in computer technology have led to developments in neuroimaging, which now allow the function of the human brain to be studied non invasively. These tools can be utilised to study the central processing of GIT sensation. The ideal functional brain imaging tool should have several characteristics:

1) Good spatial resolution
2) Good temporal resolution
3) Non invasive and repeatable
4) Cheap technology, so widely available

None of the currently available functional imaging tools fulfil all of these requirements. However, by using a combination of different techniques the limitation of each when used alone can be overcome.

Positron Emission Tomography (PET), Single Photon Emission Computer Tomography (SPECT) (see reference (70) for a review) and functional Magnetic Resonance Imaging (fMRI) (see references (70-72) for a review) detect haemodynamic changes secondary to neuronal activity. These allow the cortical sources of neuronal activity to be localised with good spatial resolution (sub centimetre). However, the temporal resolution of these techniques remains poor (seconds for fMRI and minutes for PET and SPECT). An additional disadvantage of PET and SPECT is that they rely on administering radioactive isotopes to subjects. This places limits on the subjects who can be studied and the number of times each subject can be studied. This prevents a single subject from either being followed up with repeated studies or studied under several experimental conditions.

Cerebral evoked Potentials (CEP) (see reference (73) for a review) and magnetoencephalography (MEG) (see references (74, 75) for a review) rely on recording the electric and magnetic fields produced by neuronal activity. Both of these techniques have good temporal resolution (milliseconds) and so can be used to study the sequence of neuronal activity. While MEG also has good spatial resolution (sub centimetre) this technique is limited in that it is insensitive to subcortical neuronal activity. CEP can detect subcortical activity, but this technique lacks the spatial resolution of the other functional imaging tools, as the electrical currents are distorted by being conducted round the scalp.

2.2 The Role of Cerebral Evoked Potentials

2.2.1 The cellular basis of electromagnetic fields

Depolarisation and repolarisation of neuronal cells is due to the flow of charged ions across the cell's surface membrane (74, 75). This flow of ions is driven by the difference in the ion concentration across this membrane, with ions flowing down their concentration gradient into the cell. While this reduces the difference in ion concentration across the cell membrane, it also generates a small electrical charge across it. This electrical charge drives an electrical current in a loop consisting of trans-membrane, intra-cellular and extra-cellular components. The extra-cellular component of this current is often called the volume current (75), and for cortical neurones flows through the whole volume of the head. As any electrical current will generate a magnetic field, the electrical currents generated by neuronal activity will also be accompanied by a magnetic field.

Basic principles of physics states that the strength of a magnetic field will reduce with distance at a rate proportional to the square of the distance. As a result the small magnetic fields generated by single neurones can not be detected outside the brain. However, the cerebral cortex is organised with columns of pyramidal cells (which are believed to be the primary source generating the recordable magnetic fields) lined up parallel to each other, and perpendicular to the cortical surface. This

results in the weak magnetic field generated by each cell being added together into a larger field. It has been estimated that the smallest area of cortex needed to generate a magnetic field which can be detected outside of the head is 2 mm^2 (74). In order for this summation of magnetic fields to occur, all of the cells must be active simultaneously. The short duration of axonal impulses probably exclude them from contributing to this summation, so the recorded potentials are believed to originate from postsynaptic depolarisation of cortical pyramidal cells (75). The orientation of the cortical pyramidal cells is essential for the summation of magnetic fields. In spherical groups of cells the magnetic fields generated will cancel each other out and so no magnetic field will be detectable outside of the spherical group. This results in the magnetic fields of sub-cortical nuclei being undetectable outside of the head.

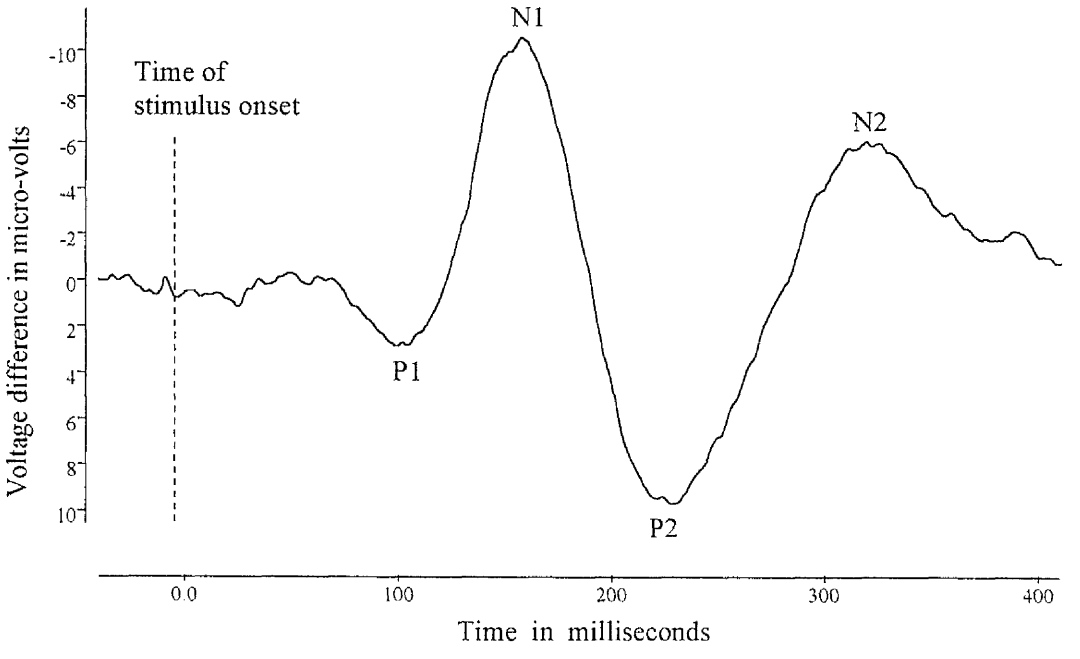
2.2.2 Recording of cerebral evoked potentials

The volume currents generated by cortical neural activity can be recorded easily from the surface of the scalp. This requires the use of two surface electrodes positioned on the scalp and a differential amplifier to amplify the voltage difference between the two electrodes. Recording this continuously results in the standard electroencephalogram (EEG), which is used clinically to monitor epileptic activity and in assessing the level of consciousness. Changes in the pattern of the EEG during painful stimuli have been reported. However, changes in the EEG gives little information about the cerebral processing of sensory information.

In order to study the brain's processing of specific sensory stimuli it is necessary to "extract" the stimulus specific cerebral evoked potential (CEP) from the background noise of the EEG. This can be achieved by averaging the EEG recorded following a sequence of identical stimuli (76). The stimulus specific CEP occurs at a fixed time after each stimulus while other brain activity does not. Therefore, by giving a series of stimuli and recording the EEG for a fixed time interval after each stimulus, the stimulus specific CEP will occur at the same place within each segment of recorded EEG. Averaging these segments of EEG reduces the background noise, so that the stimulus specific CEP is "extracted".

By convention CEP are represented in a graph format, with voltage difference in microvolts (between the active recording electrode and reference electrode) on the vertical axis, over time in milliseconds on the horizontal axis. This results in a single line containing several positive and negative peaks. Positive peaks are labelled P, and numbered in sequence (i.e. P1, P2, P3 ...), negative peaks are labelled N and are also numbered in sequence (i.e. N1, N2 ...) (see figure 2.1 for an example). Neurophysiological convention has positive voltage differences (the active electrode is more positive than the reference electrode) represented as a downward deflection in the graph.

Figure 2.1:
Example of a CEP recording



Example of a CEP recording, showing a series of positive and negative peaks with the time scale measured from the time of the stimulus.

By recording CEP from several locations on the scalp it is possible to generate topographical maps of the brain's electrical activity in response to a stimulus. While it would be useful to calculate the location of the neuronal sources generating the CEP the volume currents are distorted by the differences in electrical conductivity of the scalp layers. This results in the volume currents being conducted around the scalp (instead of through it) and so in distortion of the topographic maps recorded on the scalp's surface (73). In order to overcome this problem in source localisation, complicated computer models of the head have been developed. While the more advanced of these models attempt to model the conductivity of each layer of the head, these rely on several untestable assumptions about the conductivity of the human cranium. Therefore this limits the spatial resolution of CEP for identifying the anatomical sources generating neuronal electrical activity.

2.2.3 Magnetoencephalography

The basic principles of electromagnetism state that any changing magnetic field will induce an electrical current in a wire within the magnetic field. This principle can now be harnessed allowing the magnetic field generated by cortical neurones to be recorded; this technique is called magnetoencephalography (MEG) (77). The simplest way to achieve this is to place a single loop of superconducting wire over the scalp, attached to a series of special amplifiers (74). This simple arrangement is called a magnetometer (or zero order gradiometer). The disadvantage with the magnetometer is that it will detect any changing magnetic field, including any environmental noise, giving rise to a poor signal to noise ratio. The magnetometer can be made insensitive to distant magnetic fields by having two loops of wire positioned parallel to each other, but wound in opposite directions (first order gradiometer).

The magnetic fields generated by neuronal activity, unlike the electrical fields, are not distorted by passage through the scalp. This means that localising the source of neuronal activity is more accurate with MEG than with CEP (78, 79). Both magnetometers and gradiometers are only sensitive to the component of the magnetic

field perpendicular to their plane. The pyramidal cells in the walls of the sulci are arranged so that the resulting magnetic field is perpendicular to the scalp and so ideally orientated for detection. The pyramidal cells at the top of the gyrus, however, are positioned so that their magnetic field is parallel to the scalp's surface, and therefore are not detected by MEG (74). This apparent disadvantage of MEG is reduced, as only a small proportion of the cortex is exactly parallel to the scalp.

2.2.4 Advantages and limitations of CEP

There are two major advantages of CEP over other functional imaging tools. Firstly it has a high temporal resolution (milliseconds) which allows investigation of the sequence of neuronal activation. Functional imaging tools that rely on detecting haemodynamic changes (fMRI, SPECT and PET) are unable to reach this level of temporal resolution. The second advantage of CEP over other functional imaging tools including MEG is its cheapness and wide availability. Investigating patients (in a research or clinical setting) will require an imaging tool which is cheap, safe and widely available. The recording equipment for CEP is cheap and simple in comparison to that needed for MEG, PET or fMRI, only consisting of a modern computer and an amplifier. Most hospitals will already have equipment and expertise in recording and interpreting CEP making this technique widely available.

The main disadvantage of CEP is its poor spatial resolution, which prevents the sources of neuronal activity from being accurately identified from CEP alone. The consequence of this poor spatial resolution could be reduced if the anatomical sources of each CEP component were already known. This could be achieved by the combined use of CEP with MEG in preliminary studies while CEP was still being developed as a useful clinical tool.

2.2.5 Studies of visceral sensation

2.2.5.1 Studies using CEP:

Cerebral evoked potentials have an established role in investigating the central processing and the integrity of pathways involved with vision, auditory and somatic sensation. The recent interest in GIT sensation has prompted several research groups to consider the possibility of using CEP to investigate visceral sensation. CEP have now been recorded following both mechanical and electrical stimulation of the oesophagus (80-84) and rectum (41, 85-89).

The optimal parameters for recording CEP from the oesophagus (83, 84) and rectum (88) following both mechanical and electrical stimulation have been determined, and are similar for these stimuli:

Stimulation frequency:

The optimal frequency of stimuli for recording oesophageal CEP has been shown to be 0.2 Hz for both electrical (84) and mechanical (83) stimulation. With higher stimulation frequencies the CEP amplitude reduces. Following rectal stimulation, CEP with two different morphologies and latencies, to the first component, have been described. Stimulation frequencies in the range of 0.6 to 3.1 Hz do not affect the quality of the short latency rectal CEP (88). In contrast the long latency rectal CEP following rectal stimulation is affected by stimulation frequency, with larger amplitude CEP being obtained at lower stimulation frequencies (88, 90).

Number of stimuli:

The number of stimuli delivered and so EEG segments averaged has an important influence on the CEP recorded. As more stimuli are delivered and averaged the background noise will be reduced leading to an improvement in the signal to noise ratio of the recorded CEP. However, studies of oesophageal CEP following both mechanical and electrical stimulation has shown that the CEP amplitudes decrease with repetition of the stimuli (83, 84). The amplitude of the long latency rectal CEP also reduces with stimulus repetition, in contrast the short latency

rectal CEP does not (88). This results in the signal to noise ratio of the recorded CEP reducing as the number of stimuli averaged increases. In order to overcome this problem is it possible to average several short CEP runs of stimuli recorded from the same subjects with a short rest between each run (83, 84). This results in a reduction in the signal noise by averaging a larger number of EEG segments, without reducing the CEP amplitude. The optimal number of stimuli in each run and the number of runs recorded is a compromise between the time taken to record the CEP and the CEP quality. In practise recording between two and four runs of fifty stimuli results in reproducible robust CEP (83, 84, 91).

The reason for the reduction in CEP amplitude with stimulus repetition is not known. There are several possibilities that could account for this. CEP recorded following somatic stimulation from both the insular cortex and SII are known to reduce in amplitude with stimulus repetition (35). This probably reflects a heightened processing of novel stimuli by these cortical areas, so that with repetition of the stimuli its novelty and insular processing reduces. Alternatively if the recorded CEP were the consequence of secondary, higher processing of the stimuli, then novelty of and attention to the stimuli would affect the amplitude of the CEP. Therefore, with stimulus repetition if the attention to the stimulus reduced so would the CEP amplitude.

Filter settings:

While recording CEP it is customary to filter the EEG signal. This results in a reduction in the noise in the signal and also fluctuations in the baseline, so improving the CEP quality. The choice of filters used is important, as they could also filter out part of the CEP signal if chosen inappropriately. Studies investigating the optimal filter settings for recording oesophageal CEP have shown these should be set at 100 for the low pass filter and 1 Hz for the high pass filter (83, 84). Studies of rectal CEP have shown a different response to filter setting between the short and long latency CEP. The short latency CEP is relatively unaffected by the choice of filter setting, while the amplitude of the long latency CEP is reduced by low pass filter settings above 250 Hz (88, 89). To facilitate the comparison of CEP recorded from different GIT regions the technique used for recording CEP, including the filter settings should

be standardised. Filter settings of 100 Hz for the low pass filter and 1 Hz for the high pass filter would be consistent with the investigation of the optimal setting for both the oesophagus and rectum.

Source localisation with CEP:

Information from CEP studies about the neuronal sources generating the CEP is limited. Differences have been reported in the topographical distribution of CEP responses between the distal and proximal oesophagus (80). This would suggest that different cortical areas are activated by the proximal and distal oesophagus and that several cortical areas are contributing to the CEP. The only attempt to locate the cortical sources following oesophageal stimulation using CEP concluded that a midline and two lateral sources were active (92). These were suggested to be the anterior cingulate and insular cortex, however, the spatial resolution of CEP prevented confirmation of this. There have been no studies looking at either the topographical distribution of CEP or source localisation following ano-rectal stimulation.

2.2.5.2 Studies using MEG:

The better spatial resolution of MEG allows the cortical generators to be localised with greater accuracy using this technique. Studies localising the cortical activation following oesophageal stimulation using MEG have given conflicting results. The first study reported did not use a whole head MEG system, so required four separate recording over different regions of the head in order to obtain results (93). This study reported that SI was activated followed by the insula, with topographical differences in the region of SI activated by distal and proximal oesophageal stimulation. Subsequent studies have either identified activation of SI followed by SII and the insula (43), or to SII activation alone (45) or followed by frontal activation (46). Stimulation of the anal canal has been shown to activate a region of SI between the hand and foot areas of SI (94). MEG has, however, not been used to study rectal stimulation.

2.3 The role of functional magnetic resonance imaging

Cerebral neuronal activity is accompanied by a series of haemodynamic changes. Activation, or inhibition of neuronal cells results in an increase in glucose utilisation, and uptake from the blood (70). This increased metabolic activity is accompanied by vasodilatation of local blood vessels, which results in a local increase in cerebral blood volume, and blood flow. This increases the delivery of glucose and oxygen to the metabolically active region of the brain. This increased oxygen delivery is greater than the increased oxygen utilisation (95), so the oxygen concentration of venous blood in metabolically active brain regions increases. Therefore there is an apparent uncoupling of cerebral blood flow and metabolic requirements. However, it has been speculated that as the cerebral blood flow increases the percentage oxygen extraction is reduced due to the reduced transit time of the blood through capillaries. Therefore in order to supply an increased metabolic requirement, the cerebral blood flow must increase more than expected and lead to the observed increase in venous oxygen concentration.

Both PET and SPECT rely upon using labelled radioisotopes to measure cerebral blood volume or glucose uptake. Measurements are made during a resting period without any stimuli and during the test state with sensory stimulation. Changes in either blood volume or glucose uptake can be calculated by subtracting the scan results obtained in the two states, after they have been aligned to each other.

2.3.1 Magnetic resonance imaging

Magnetic resonance imaging (MRI), which was previously called nuclear magnetic resonance imaging, relies upon several basic principles of nuclear physics:

a) As all nuclei are charged their constant motion generates a small magnetic field. Therefore, nuclei with an odd number of protons and neutrons, such as hydrogen, behave as small bar magnets.

b) If these nuclei are placed in a strong external magnetic field they try to line up parallel to this external field, the angular momentum of the nuclei will, however, prevent this. Therefore, the nuclei will spin around the axis of the external magnetic field, this is termed procession.

The magnetic field of each nucleus will therefore have a component parallel to the external magnetic field (longitudinal magnetism) and a component perpendicular to the external magnetic field (transverse magnetism). The transverse magnetism of the nuclei will be cancelled out by surrounding nuclei, unless they can be entrapped to spin in phase with each other.

c) The frequency at which the nuclei will spin is called the Larmor frequency and is directly proportional to the strength of the external field, and dependent on the type of nuclei. The Larmor frequency for hydrogen in a 1.5 Tesla field is 63.9 MHz.

b) A radio frequency pulse at the Larmor frequency will impart energy to the nuclei. This will result in some of the spins of the nuclei flipping over to be anti-parallel to the external magnetic field. In addition the nuclei will be entrapped to spin in phase with each other, so generating a net transverse magnetism.

e) Following an excitatory radio frequency pulse, nuclei will start to lose phase with each other, resulting in a reduction in transverse magnetism. This will fall in an exponential manner with a time constant T2 (transverse relaxation time), which is dependent upon the inconsistencies in the magnetic field experienced by the nuclei, (which is a combination of the external magnetic field and internal magnetic fields). With time the nuclei will also start to processes in parallel to the external magnetic field again. Therefore the longitudinal magnetism will also increase in an exponential manner with a time constant T1 (longitudinal relaxation time), which is dependent upon the rate of transfer of energy between nuclei.

All MRI scanners consist of three basic parts.

i) A large magnet for generating the strong static external magnetic field needed for MRI scanning.

ii) A radio frequency coil, which is used to transmit excitatory radio frequency pulses, and to record the transverse magnetic field, generated in the subject being imaged. As a receiving coil close to the area being imaged can obtain better images a range of special receiving coils have been developed.

iii) A services of gradient coils, which are used to generate smaller magnetic fields which have a linear gradient across the MRI scanner. These gradient coils are used to alter the lambda frequency across the subject being imaged. This allows for slice selection, with a thin two-dimensional slice of the subject being imaged.

Several imaging sequences have been developed for use in MRI scanners, by changing the sequence of radio pulses and use of the gradient coils, images can be obtained which are predominantly influenced by the longitudinal relaxation time (T1 weighted) or the transverse relaxation time (T2 and T2* weighted).

2.3.2 BOLD fMRI

Oxygenated and deoxygenated haemoglobin have different paramagnetic properties (96, 97). Therefore a change in the level of oxygenation of blood will effect its transverse relaxation and so the intensity of T2 and T2* weighted MRI images. An increase in the level of oxygenation results in an increase in the T2 weighted MRI signal intensity. This phenomenon was first utilised by Ogowa et al. who produced MRI images of the cerebral vasculature in rats by subtracting MRI scanning obtained when the rats were hypoxic and when breathing oxygen (98).

This technique has also been adapted for use in man to localise areas of neuronal activity and is the theoretical basis for Blood Oxygen Level Dependent (BOLD) functional MRI (fMRI) (96, 97). As described above neuronal activation is associated with an increase in oxygenated blood in metabolically active areas of cortex resulting in an increase in T2 weighted MRI images.

Neuronal activation is also accompanied by an increase in cortical blood flow (95). The nuclei in blood flowing into the brain after the initial radio pulse will not be synchronised with the other nuclei in that slice. Therefore flowing blood will effectively reduce the transverse relaxation time and so increase the intensity of T2 and T2* weighted MRI images. The BOLD effect observed in fMRI experiments is a combination of this flow effect and the true BOLD effect, which are additive.

2.3.2.1 fMRI Image analysis:

The percentage signal change obtained with BOLD fMRI is dependent of the strength of the MRI scanner, being larger with higher strength magnets (99). With 1.5 Tesla magnets the maximum signal change that can be obtained is with flashing visual stimuli, which produce a signal change of less than 4% (100). Sensory stimuli will normally give a smaller percentage change in signal. This signal change is similar to the level of noise in the MRI signal. Therefore, it is not possible to identify BOLD activation by simply comparing two MRI scans obtained during a stimulus and at rest. During a normal fMRI experiment a series of MRI scans will be obtained during alternating periods with and without stimulation (97, 100). There is then a series of image processing steps necessary to analyse the fMRI experiment.

Image co-registration:

Head movement during fMRI scanning will result in tissue with different transverse relaxation times moving into or out of the volume covered by an individual image element (voxel). This will result in a change in the average intensity of that voxel due to movement. This movement related signal change will be greatest in areas of the MRI scan were there is a larger difference in intensities of adjacent voxels (such as at the cortex / bone boundary, which is where most of the true activation will also occur). Because of the small size of the signal change that fMRI is trying to detect this movement related signal change can be as large as the true fMRI signal change. Therefore movement artefacts can either hide a true activation or cause false activation if related in time to the periods of stimulation (101, 102).

In order to overcome this problem of movement artefacts it is necessary to minimise the effect of movement during the fMRI scan. Restraining the head in the MRI scanner can reduce, but not adequately prevent head movement. It is also possible to detect head movement in the scanner by using navigator echoes to detect the centre of the head. This allows for correction, within the scanner, of the position of each image slice so reducing the relative movement of the head in relation to the MRI slices (103). However, both of these methods are not adequate to remove all movement artefacts. Therefore it is also necessary to correct for the movement during the image processing (101, 104). This is the first step in the sequence of analysis of fMRI data. The amount of movement and rotation of each MRI image from a reference image in the fMRI sequence is calculated. This is achieved using computer programmes to either match the edges to each image, or minimise the difference in image intensity between each image and the reference image.

Reslicing:

Having calculated the movement and rotation necessary to align each image to the first, each image has to be resliced. This results in each voxel of each image correspond to the same portion of brain.

Temporal smoothing:

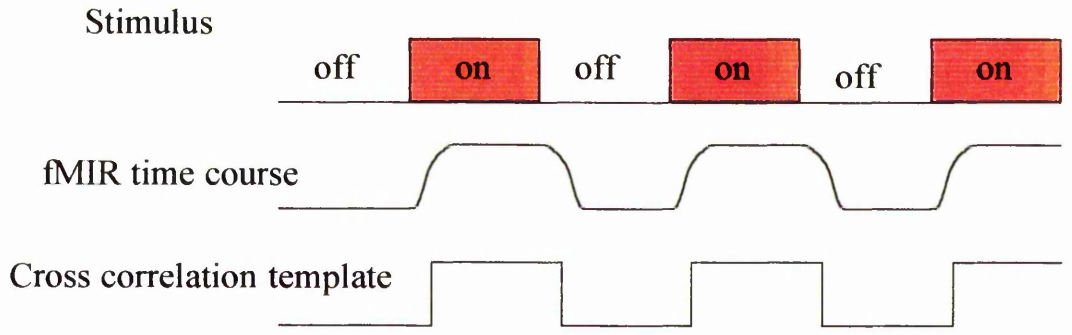
It is common practice to either smooth or normalise fMRI data across time, to correct for temporal fluctuations in MRI image intensity over time, due to either scanner or, physiological fluctuations, such as changes in blood and cerebral spinal fluid flow with heart rate and respiration. This results in a reduction in noise, in the time domain, without affecting the true fMRI signal.

Time course analysis:

Having corrected for head movements, there are several ways to identify the fMRI signal change. The simplest method would be to average the MRI images from the two stimulus states and then subtract these two averages. However, better results are obtained by comparing the intensity time course of each voxel with the time course of the stimuli using crosscorrelation (71, 105) (figure 2.3). There is a time

delay between the onset of a stimulus and the resulting neuronal haemodynamic change. Therefore, the maximal fMRI signal can be up to seven seconds after the stimulus onset (72). During the crosscorrelation this time delay can be accounted for be shifting the time course of the activation template to coincide with the expected time course of the haemodynamic change (figure 2.2).

Figure 2.2:
fMRI cross correlation time course



The principle of fMRI crosscorrelation, showing the on-off pattern of the stimulus, the brains haemodynamic response as measured by the fMRI and the template used for crosscorrelation, which has been offset from the stimulus to account for the delay in the brains haemodynamic response.

Cluster analysis:

In the typical fMRI study each MRI volume can contain up to 102,000 voxels (25 slices with a 64x64 matrix). Using conventional levels of significance to analyse this data will result in multiple false positive results. The use of conventional Bonferroni corrections for multiple comparisons will lead to the risk of missing true positives, due to the number of comparisons being corrected for. Therefore, alternative methods to correct for multiple comparisons have been developed. The false positive voxels will be randomly distributed across the MRI volume, while true positive voxels will occur in groups, or clusters. By discarding single positive voxels, or small groups of positive voxels, from the results it is possible to increase the

significance level of the results (106). The significance level of each cluster of active voxels can be calculated from size of the cluster and the significance level of the individual voxels. This type of cluster analysis improves the chances of detecting large areas of activity by reducing the probability of a type 2 statistical error, but at the risk of missing small areas of true activity smaller than the cluster size used.

2.3.3 Advantages and limitations of fMRI:

The main advantage of fMRI is its good spatial resolution, being able to localise cortical areas of activation with sub-centimetre accuracy. When compared to the gold standard of intra-operative cortical mapping, fMRI has been shown to give good results (107-109). Previously studies of this type have been performed using positron emission tomography (PET), which uses radio-labelled isotopes of water or glucose to measure cerebral blood flow, or glucose utilisation. In comparison with PET, fMRI has been shown to have a better spatial resolution, so is able to differentiate between two close but separate areas of cortical activation (110-112). This higher spatial resolution has been utilised to map small functional sub-regions of the human visual cortex (113). As fMRI can be performed using standard clinical MRI scanner, fMRI is cheaper and more widely available than PET. An additional advantage of fMRI is the avoidance of the radiation exposure inherent in PET. This allow subjects to be studied repeatedly, so allowing the processing of sensory stimuli to be investigated with different experimental paradigms.

The main limitation of fMRI is its poor temporal resolution. As fMRI detects haemodynamic changes in cerebral blood flow which take seconds to occur (72), its temporal resolution will always be longer than the time course of neuronal activation. Therefore fMRI can not be used to identify the sequence of cortical activation following a stimulus.

2.3.4 Studies of rectal sensation using PET

Both PET and SPECT have been used to study the cortical processing of both rectal (41, 42, 44), oesophageal (40) and stomach (114), but not the anal sensation. The studies of rectal sensation have given conflicting results. The first study used PET with non painful rapid phasic rectal distention, this identified activation only within the thalamus and pre and post central gyrus (SI / primary motor strip) (41). This study does not indicate the site of the somatosensory cortex activation. The second study also used PET to identify the brain activation with painful tonic rectal distention, this only identified activation of the anterior cingulate gyrus, which occurred with both real and sham rectal distention in healthy volunteers (44). IBS patients in this study showed a different pattern of cortical activation, only activating the pre-frontal cortex. This difference in pattern of cortical activation was used by the authors to argue for a psychological aetiology of IBS. A recent study used SPECT with tonic painful rectal distention and demonstrated activation of both the anterior cingulate and pre-frontal cortex in healthy volunteers (42).

2.3.5 Studies of rectal sensation using fMRI

The pioneering work using fMRI to image human cortical activation was performed using visual stimuli (99, 100, 115). Since then fMRI has been used to detect activation following a number of different stimuli (107, 109, 116). fMRI has also been compared to the gold standard of direct cortical mapping during neuro surgery with good results (108). There are a limited number of recent studies that have investigated visceral sensation using fMRI. Oesophageal stimulation has been shown to activate the somatosensory cortex, insular and anterior cingulate cortex with painful stimulation (117, 118).

Two recent papers, published after my studies were completed, have investigated the cortical processing of painful rectal stimulation with fMRI (51, 119). The first of these studies (119) investigated the cortical processing of painful rectal sensation in eight subjects, after adjusting the level of significance and cluster size in

each subject they reported the results in six. This paper reported anterior cingulate, pre-frontal, insular and primary somatosensory (SI) cortex activation; the level of SI activated is not identified in the paper. Adjusting the analysis to obtain results on an individual basis instead of using a pre-defined significance level weakens the methodology of this paper. The second study (120) used a region of interest analysis, only looking at the anterior cingulate, pre-frontal, insular cortex and thalamus. This study demonstrated activation in all four areas analysed. No published studies have investigated the cortical representation of the anal canal with fMRI.

All but one of the PET and fMRI studies of rectal sensation have only investigated painful rectal stimulation. Therefore, the pattern of activation with non painful rectal sensation and the role of stimulus intensity on cortical activation is unknown. The conflicting results from the earlier PET studies also required the processing of painful rectal sensation to be further investigated.

2.4 Summary

Several recent technological developments have led to the development of imaging tools for studying brain function non invasively. These have been used to study the processing of GIT sensation. Most of these studies have investigated oesophageal sensation, therefore there are still unanswered questions about the processing of ano-rectal sensation. Two methods of rectal stimulation have been used for recording rectal CEP, the afferent pathways activated by each is unknown, with speculation that each might activate a different pathway. The network of cortical areas processing ano-rectal sensation is not fully understood, in particular the processing of non painful rectal sensation and anal canal sensation is not known. Whether the anatomical and physiological differences between the afferent innervation of the rectal and proximal GIT are reflected in neurophysiological differences in the afferent pathways and central processing is unexplored.

Chapter 3

Characterisation of rectal afferent pathways using CEP

Characterisation of rectal afferent pathways using CEP

3.1 Introduction

CEP have been recorded following both electrical (ERS) (85, 88, 90, 100, 121) and mechanical rectal stimulation (MRS) (41, 87, 89, 122, 123). However, the methodology used in these different studies has varied, giving rise to a wide variation in CEP characteristics. The methodological differences have included using different frequency and intensity of rectal stimulation, using different positions of both the active and reference electrodes and using different filter settings (see table 3.1 for details). This has resulted in CEP with different morphology and latencies, which can not be meaningfully compared to each other due to the methodological differences used in recording them.

In order to determine the utility of CEP as a research tool for clinical studies, the reliability and robustness of the available methodologies must be assessed; and an understanding of the afferent pathways activated by each stimulus modality developed. While there are some similarities in the morphology of CEP following ERS and MRS, a direct comparison of these two stimulus modalities in the same subjects has not been published. Work comparing CEP following mechanical and electrical somatic stimulation has shown that mechanical CEP have a longer latency but their amplitude is similar to electrical CEP (124), whether the same applies to visceral evoked CEP, however, is not known.

Table 3.1: Summary of all the previous studies demonstrating CEP following rectal stimulation

year	ref.	Stimulus	Stimulus position from the anal verge	active electrode position	reference electrode position	stimulus frequency in Hz	stimulus intensity	filter settings	CEP latencies in milliseconds	CEP amplitudes in μV
1987	85	electrical	4 to 8 cm	Cz Fz Cz	R ear R ear Fz	2	non painful	2 – 100 Hz	Short: P1 36 N1 45 Long: N1 90-92 P1 120	not stated
1988	87	balloon	5 to 8 cm	Cz	R ear	0.6 to 0.8	20 ml	3 – 10,000 Hz	N1 109 ±- 0 P1 122.8 ± 0.4	not stated
1989	121	electrical	20 cm	Cz	Fz	3	1.5 to 7.5 mA	20 – 2,000 Hz	P1 72.7 ± 8.8 N1 96.5 ± 11.7	0.9 ± 0.4
1991	90	electrical	10 cm	Cz	Fz	3 and 1	1.5 and 2.5 times sensory threshold	2 – 200 Hz and 1 – 250 Hz	short: P1 34.2 ± 2 N1 44.4 ± 1.7 long: P1 58.7 ± 5.2 N1 66.0 ± 3.9	short: P1 – N1 2.7 ± 1.2 long: P1 – N2 3.4 ± 2.4
1992	125	electrical	10 – 15 cm	Cz	Fz	3	maximum non painful	2 – 200 Hz	P1 29 – 73	not stated

Table 3.1 continued: Summary of all the previous studies demonstrating CEP following rectal stimulation

year	ref.	Stimulus	Stimulus position from the anal verge	active electrode position	reference electrode position	stimulus frequency in Hz	stimulus intensity	filter settings	CEP latencies in milliseconds	CEP amplitudes in μV
1992	88	electrical	10 cm	Cz	Fz	1	highest tolerated without pain	1 –250 1 – 1500 5 – 1500 10 – 250 10 – 1500 30 – 1500	short: P1 34.7 ± 2 N1 45 ± 2 long: P1 61 ± 5 N1 70 ± 8	not stated
1993	89	balloon	10 cm	Cz	Fz	0.167	10 to 30 ml	1 – 250 1 – 1500 10 – 1500 10 – 250 30 – 1500	short: P1 128 ± 26 N1 157 ± 18 Long: N1 210 ± 15 P1 316 ± 24	short: P1 – N1 3.4 ± 2.0 Long: N1 – P1 4.8 ± 1.8
1995	126	electrical	10 cm	Cz	linked ears	0.1 to 10	1.5 times sensory threshold	0.1 – 200	N1 50 – 90 P1 60 – 125	< 5
1996	41	balloon	15 cm	Cz	not stated	0.7	5 – 20 ml	not stated	N1 120 ± 16 P1 281 ± 15	22.0 ± 2.3
1999	127	balloon		Cz	Fz	0.1	Definite sensation Call to stool	3-300	119 ± 9 121 ± 8	9.4 ± 1.9 8.6 ± 1.6

3.2 Aim of the study

The aim of this study was to compare the characteristics of CEP recorded following MRS and ERS in the same subjects.

3.3 Methods

Ethical approval was obtained and 14 right handed healthy subjects (3 female) with a mean age of 29 years (range 21 to 44) were studied after obtaining written informed consent. None of the subjects had gastrointestinal or neurological symptoms, and none were taking any regular medication.

3.3.1 Rectal stimulation

Electrical stimulation:

Electrical stimulation was performed using a pair of bipolar platinum ring electrodes placed 2 cm apart on an insulated catheter, with an external diameter of 3 mm. The catheter was constructed from nylon tubing covered with stainless steel braid and sheathed in silicone rubber (Gaeltec, Dunvegan, Isle of Skye, IV55 8GU). The electrical stimulus was a square wave of 200 microseconds duration, which was produced by a constant current high voltage stimulator (model DS7, Digitimer Ltd., Welwyn Garden City, Herts, UK).

Mechanical stimulation:

Mechanical stimulation was produced by rapid phasic distention of a 2 cm latex balloon positioned 2 cm from the tip of a polyvinyl catheter with an external diameter of 4 mm. A mechanical pump (Medical Physics Department, Hope Hospital, Manchester, UK) was used to inflate the balloon. Following triggering of the pump there was a constant delay of 65 milliseconds before the initiation of balloon inflation, then a constant inflation time of an additional 165

milliseconds. The volume of balloon inflation could be adjusted by altering the inflation pressure (pressure range 0 to 25 psi). Increasing the pressure in the system resulted in an increased rate of airflow, so that a greater volume was delivered to the balloon during the inflation cycle. The balloon was completely deflated after each inflation. In vitro, this pump was capable of delivering a maximum balloon volume of 30ml. The pump was triggered using a laboratory interface (CED 1401plus, Cambridge Electronic Design Ltd, Cambridge, CB4 4FE, UK). The same pump was used to trigger the pump and CEP recording.

3.3.2 CEP recording

CEP were recorded using silver silver-chloride surface electrodes, with the active electrode positioned at the vertex (Cz) in accordance with the international 10-20 system of electrode placement, and the reference electrode on the right ear lobe. The electrodes were applied using electrode paste and the impedance was kept below 5 K ohms. The CEP data were acquired using a CED 1902 programmable signal conditioner (Cambridge Electronics Design, Cambridge, CB4 4FE, UK) and a IBM compatible desktop computer, running SIGAVG software (version 6.04 Cambridge Electronics Design, Cambridge, CB4 4FE, UK). The CEP data were sampled at a frequency of 2000 Hz, with an epoch duration of 2000 milliseconds, of which the first 200 milliseconds was pre-stimulation time. The amplifier gain was set at 100 000, with on-line artifact rejection. The bandpass filters were set at 1 and 100 Hz. The stimulus frequency for both ERS and MRS was 0.2 Hz. These parameters were chosen to be identical to those we have previously shown to be optimal for recording visceral CEP from the oesophagus (83, 84); and are consistent with the results of previous studies investigating the optimal parameters for recording rectal CEP following both MRS (89) and ERS (88).

3.3.3 Protocol

The electrical and mechanical catheters were tied together and inserted into the rectum so that the centre of the balloon and ring electrodes were positioned in the mid rectum, 10 cm above the anal verge. The sensory and pain thresholds for both MRS and ERS were determined by increasing the balloon inflation pressure in steps of 1 psi or the intensity of electrical stimulation in steps of 1 mA. All subsequent stimuli were applied at an intensity of 75% of the difference between the sensory and pain thresholds. This has been shown to be the optimal stimulus intensity for recording oesophageal CEP (84), and my preliminary studies confirmed this as the optimal intensity for recording rectal CEP.

The CEP were recorded in a quiet semi darkened room with the subject in a semi recumbent position. ERS and MRS studies were performed consecutively on the same day. The sequence of ERS and MRS was randomised between subjects. Four runs of fifty stimuli were averaged for both ERS and MRS, with a five minute rest period between each run. During ERS the balloon was inflated with 5 ml of air in all subjects to improve contact between the ring electrodes and rectal mucosa. My preliminary studies showed that this resulted in the electrode impedance and stimulus intensity being more consistent, due to more reliable electrical contact between the electrodes and the rectal mucosa. In order to mask the pump noise, the subjects wore headphones connected to a white noise generator (65 dB output, Medical Physics Department, Hope Hospital, Manchester, UK). CEP were also recorded during sham stimulation by disconnecting the pump from the rectal catheter and reconnecting the pump to a second balloon catheter position next to the subject. CEP were then recorded as described above while the subject listened to white noise.

3.3.4 Definition of terms

Latency:

Latency was defined as the time in milliseconds from the triggering of the stimulus to the peak of each CEP component.

Interpeak latency:

Interpeak latency was defined as the time interval in milliseconds between consecutive CEP peaks.

Amplitude:

Amplitude was defined as the voltage difference in microvolts between consecutive CEP peaks.

3.3.5 Data analysis

The four CEP runs from each subject were averaged for both MRS and ERS. The CEP characteristics to both stimulus modalities were assessed by a researcher blinded to the stimulus used. The positive peaks of the CEP were labelled P1 P2, while the negative peaks were labelled N1 N2. The latency, interpeak latency and amplitude of each component of the CEP obtained for both MRS and ERS from each subject were averaged to obtain group mean data for the whole group.

3.3.6 Statistical comparison:

The latencies to each peak, the amplitude and interpeak latencies of the CEP evoked by MRS and ERS were compared by the Mann-Whitney U test, using Arcus Quickstat software version 1.0 (Addison Wesley Longman Ltd). A Bonferroni calculation was performed to correct for multiple comparisons, the results are reported both before and after correction. A p value of < 0.05 was accepted as a statistically significant difference.

3.4 Results

3.4.1 Electrical stimulation:

Reproducible polyphasic CEP were recorded in all subjects. The mean stimulus intensity used was 42 mA (range 23 to 70 mA), this was felt as a sharp but non painful pulse situated deep in the pelvis. The CEP obtained consisted of two morphologies. The common morphology, recorded in 13 subjects, consisted of a P1 N1 P2 N2 wave form (figure 3.1) with a median latency to the P1 component of 82 milliseconds (range 54 to 119 ms). The amplitudes and latencies for the other peaks are given in table 3.2. In one male subject aged 30, the CEP morphology was different consisting of a N1 P1 N2 P2 wave form with a latency to the N1 component of 66ms (figure 3.2). Because of these discrepant findings CEP were recorded from this subject following ERS on a second occasion, which demonstrated the same morphology as on the first occasion.

3.4.2 Mechanical stimulation:

CEP could only be recorded from 11 subjects on the first occasion. The average stimulus intensity used was 15 psi (range 8 to 25 psi), this was felt as a poorly localised non painful pulse. The study was repeated on a second day in the 3 subjects in whom CEP could not be recorded initially, and in two of these subjects CEP were successfully recorded. As with ERS, CEP with two morphologies were obtained. In 12 subjects the morphology consisted of a P1 N1 P2 N2 wave form (figure 3.1) with a median latency to the P1 component of 203 milliseconds (range 135 to 214 ms), the amplitudes and latencies for the other peaks are given in table 2. In the one male subject who had the uncommon CEP morphology following ERS the CEP following MRS also showed the uncommon N1 P1 N2 P2 morphology (figure 3.2).

Figure 3.1

Common morphology rectal CEP, from the same subject

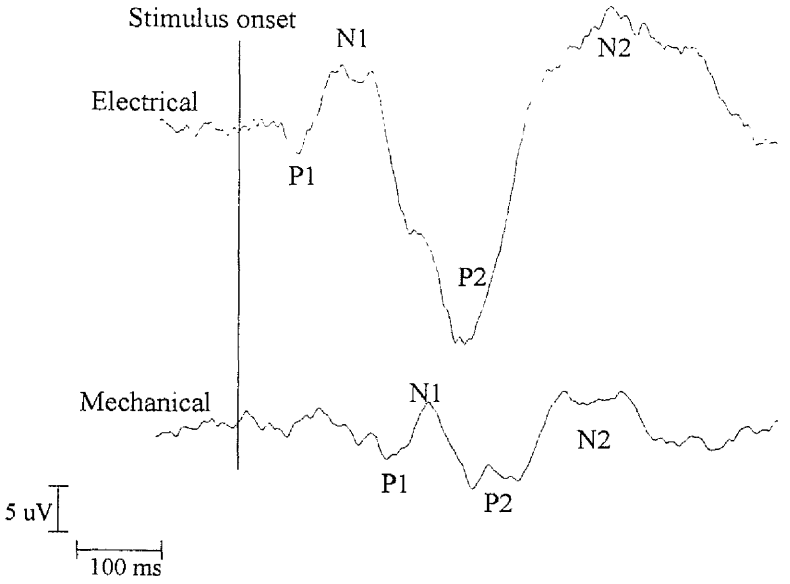
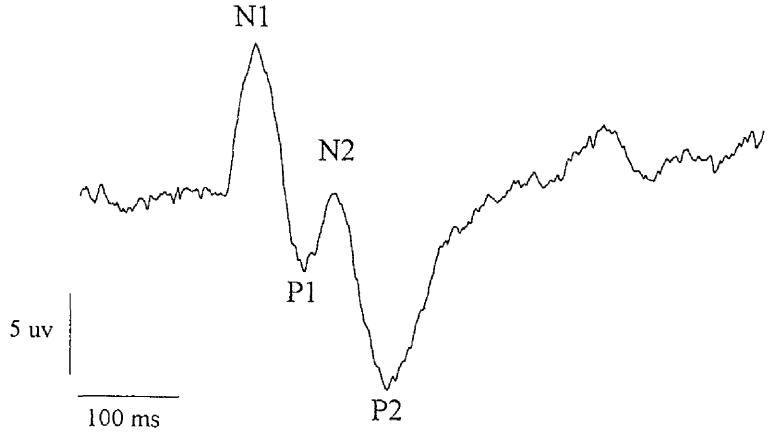


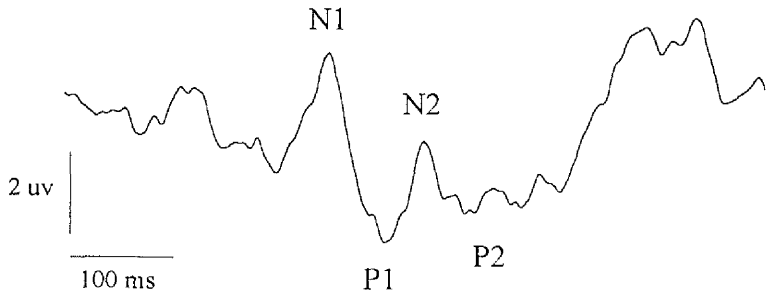
Figure 3.2:

Uncommon morphology rectal CEP, from the same subject

Electrical:



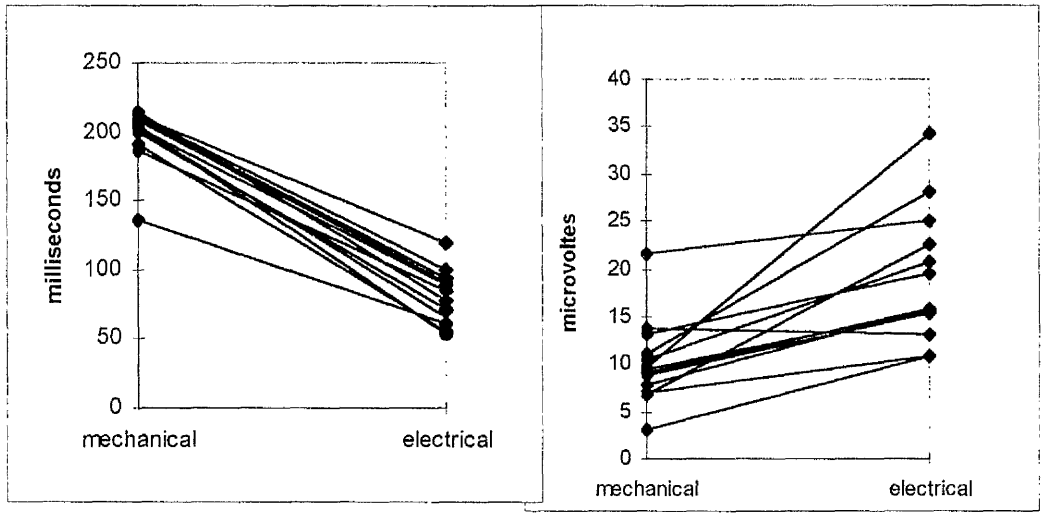
Mechanical:



3.4.3 Comparison of CEP to electrical and mechanical stimulation:

The 12 subjects in whom the common morphology CEP (P1, N1, P2, N2) was recorded were included in the comparison. MRS evoked CEP with a longer latency ($P<0.0013$, corrected for multiple comparisons) for all components and smaller amplitude ($P< 0.0039$, corrected for multiple comparisons) for the N1-P2 amplitude), see figure 3.3 and table 3.2 for details. There was however, no difference in the interpeak latencies between CEP recorded following ERS and MRS ($P = 0.29$ to 0.9 for different interpeak latencies; see table 3.2 for details).

Figure 3.3:
Comparison of the P1 latency and P2 to N2 amplitude of CEP following ERS and MRS:



Latency to P1 Amplitude of P2 to N2

Table 3.2

Latencies and amplitudes for the common morphology rectal CEP

	Median ERS (inter quartile range)	Median MRS (inter quartile range)	Median difference between MRS and ERS (95% confidence interval)	P value	corrected P value
P1 latency	81 (64-93)	203 (193-212)	-91 (-137 to -106)	< 0.0001	< 0.0013
N1 latency	128 (107-150)	254 (246-266)	-95 (-142 to -105)	< 0.0001	<0.0013
P2 latency	231 (210-242)	351 (326-366)	-88 (-136 to -94)	< 0.0001	<0.0013
N2 latency	402(388-418)	522 (512-563)	-95 (-161 to -102)	< 0.0001	<0.0013
P1 - N1 latency	45 (43-53)	53 (42-71)	-4.25 (-16 to 6)	= 0.29	= 1
N1 - P2 latency	100 (80-130)	95 (77-107)	6 (-17 to 31)	= 0.62	= 1
P2 - N2 latency	168 (148-195)	189 (153-202)	-11.75 (-49 to 15)	= 0.33	= 1
P1 - P2 latency	145 (140-179)	152 (130-172)	1 (-20 to 30)	= 0.94	= 1
P1 - N2 latency	329 (297-340)	327 (313-376)	-8.25 (-54 to 20)	= 0.68	= 1
N1 - N2 latency	276 (242-295)	280 (259-309)	-5 (-41 to 23)	= 0.68	= 1
P1 - N1 amplitude	6.67 (5.6-9.2)	4.47 (2.4-6.0)	1.66 (0.17 to 4.52)	= 0.031	= 0.4
N1 - P2 amplitude	13.4 (10.3-22.7)	6.68 (4.2-6.7)	5.93 (4.22 to 14.87)	= 0.0003	= 0.0039
P2 - N2 amplitude	15.8 (12.5-23.2)	9.3 (7.4-12.1)	7.78 (4.05 to 12.89)	= 0.0002	= 0.0026

Comparison of the median latencies and interpeak latencies in milliseconds, and amplitude in microvolts of CEP following electrical (ERS) and mechanical rectal stimulation (MRS).

3.5 Discussion

This study has directly compared for the first time the characteristics of CEP recorded following both electrical and mechanical rectal stimulation in the same subjects. My results have demonstrated that CEP with a similar morphology can be recorded following ERS and MRS, but that important differences exist between the two methods.

The rectum is innervated by unmyelinated C fibres and thinly myelinated A-delta fibres in the pelvic nerves (3, 5). The latencies of CEP in my study are shorter than would be expected from C fibre stimulation, which produces CEP with a latency of around 1000 milliseconds (128). This suggests that both electrical and mechanical rectal CEP are mediated via A delta fibre activation. Furthermore previous studies of somatic sensation have shown that it is difficult to recording C fibre mediated CEP because of latency jitter (128), therefore activation of these fibres in unlikely to have contributed to the CEP recorded in my study.

The similarity in morphology and interpeak latencies of CEP following ERS and MRS suggest that both stimuli activate the same cortical neuronal network, hence the pathways mediating CEP following both stimulation modalities are likely to be similar. However, this study does not prove this, as vertex CEP with a similar morphology can be generated from a number of discrete cortical locations (129). Magnetoencephalography (MEG) has a greater spatial resolution than CEP, as magnetic fields are not distorted by the scalp (75). Therefore the use of magnetoencephalography (MEG) is now required to test the hypothesis that ERS and MRS are stimulating the same cortical neuronal network.

This study has also identified some important differences between CEP recorded following ERS and MRS. In comparison to MRS, ERS produces CEP with a larger amplitude. There are two likely explanations for this greater

amplitude with ERS. First, ERS will stimulate all afferents within 200 microseconds, resulting in excellent synchronisation between the stimulus and afferent nerve discharge. In contrast, during MRS the balloon inflates over 165 milliseconds resulting in relatively poor synchronisation between the stimulus onset, afferent nerve discharge and the onset of CEP averaging. This will result in CEP of lower amplitude being recorded with MRS in comparison to ERS. Second, MRS will only stimulate rectal stretch receptors while ERS will stimulate all the rectal nerves including the mechanically silent afferents (13), resulting in a larger signal reaching the central nervous system. Gut injury is known to sensitise these mechanically silent afferents, which then become sensitive to rectal distention (13). It may be possible therefore, to monitor injury related gut sensitisation using MRS evoked CEP, as the CEP amplitude will be increased. This hypothesis, however, remains to be tested.

The results of this study showed that the CEP latencies following MRS were longer than those following ERS. While no previous studies have compared CEP following MRS and ERS in the same subjects, previous studies have also shown longer CEP latencies following MRS than with ERS (41, 85, 89, 90). The most obvious explanation for the longer CEP latency with MRS is the delay between the triggering of balloon inflation, and the maximal inflation of the rectal balloon (the 65 millisecond delay before the initiation of balloon inflation plus the 165 millisecond inflation time of the pump and balloon). However, the time delay between triggering CEP averaging and the maximal balloon inflation (230 milliseconds) is longer than the latency difference between ERS and MRS (122 milliseconds). This could be explained by the rectal afferents being activated before the balloon is fully inflated. It has previously been suggested that the longer latencies following MRS are due to activation of slower C fibre pathways, however, the data from this study is more consistent with both ERS and MRS stimulating A-delta pathways.

The inability to consistently record CEP in all subjects following MRS demonstrates that ERS is a more reliable stimulus for recording CEP. As all subjects could feel each balloon inflation, the likely explanation for this failure

to record MRS evoked CEP in some subjects is that the smaller amplitude of the CEP following MRS resulted in them being indistinguishable from the background noise in the signal. The use of a shorter balloon inflation time for MRS might result in better synchronisation of afferent fibre activation with stimulus onset and therefore, larger amplitude, more reliable CEP might be obtained. This speculation, however, remains to be tested.

Previous studies have also demonstrated CEP with two morphologies following ERS (88, 90) and MRS (89). However, unlike the results from my study these two CEP morphologies had different latencies (34.7 ± 2 milliseconds and 61 ± 5 milliseconds for ERS (88), and 128 ± 26 milliseconds and 210 ± 15 milliseconds for MRS (89)). As CEP are recorded as the voltage difference between two electrodes (the active and reference electrodes) the position of both scalp electrodes will affect the CEP morphology. These previous studies used different scalp electrode positions to my study (see table 3.1), the results are therefore not directly comparable. The authors in these previous papers speculated that the shorter latency CEP following ERS was due to stimulation of somatic afferents outside the rectum (88-90). I stimulated the rectum using a similar ring electrode to these previous studies, however, the use of a balloon to improve mucosal contact in my study is an important difference. The electrical current used to stimulate the rectum in the previous studies was not stated, but my experience suggests that the improved mucosal contact achieved by inflating the balloon allows a smaller current to be used. This would reduce the chance of stimulating somatic nerves outside the rectum, which may explain the lack of short latency CEP in my study. However, this interpretation is speculative and remains to be tested. The longer latency CEP demonstrated in previous studies using the same scalp electrode positions as in my study does have similarities with the common morphology CEP in my study (41). This indicates the need for using standardised stimulation and recording parameters to record reproducible rectal CEP.

Several previous studies have compared somatic CEP following electrical and mechanical stimulation of the skin using either tapping (124), a needle (130), or a puff of air (131) as the mechanical stimulus. The somatic CEP morphology following electrical and mechanical stimulation are similar (124, 130, 131). Furthermore in comparison to electrical stimulation, mechanical somatic stimulation produces CEP with a longer latency (124, 131, 132). This latency difference is normally explained as being due to the time taken for receptor activation with mechanical stimulation. These studies used a mechanical stimulus with a shorter duration (i.e. 3 milliseconds (131)) and showed a smaller latency difference between electrical and mechanical CEP (1 to 6 milliseconds (130, 131)) than in my study. Therefore, while receptor activation could account for some of the longer latency following MRS in my study, the delay in balloon inflation is likely to account for most of the latency difference. The CEP amplitude following electrical and mechanical somatic stimulation has varied between studies, with mechanically evoked CEP being reported as having a smaller (131), similar (124) or larger (133) amplitude than electrically evoked CEP. However, as no attempt was made in these studies to control for the intensity of electrical and mechanical stimulation it is difficult to interpret the reported amplitude differences. As in my current study, several studies have reported that somatic mechanically evoked CEP are less reliable that electrically evoked CEP (130, 134)

3.6 Conclusion

I have directly compared the reliability of both electrical and mechanical rectal stimulation for recording CEP for the first time; and demonstrated that electrical rectal stimulation results in more reliable, larger amplitude CEP than mechanical stimulation. The similarities in morphology and interpeak latencies suggest that MRS and ERS may be activating a similar cortical neuronal network.

The increased reliability and robustness of ERS over MRS makes this the stimulus technique of choice for most future studies. Whether gut inflammation will alter the amplitude of CEP following MRS by sensitising silent afferents needs to be explored. If this is the case then MRS could have a role in investigating peripheral sensitisation of the gut.

Chapter 4

Comparison of Afferent Pathways from the Proximal and Distal Gut

Comparison of Afferent pathways from the Proximal and Distal Gut

4.1 Introduction

There are important anatomical and physiological differences in the afferent innervation of the rectum in comparison to the rest of the gastrointestinal (GIT). Proximal GIT organs, such as the oesophagus and duodenum, develop from the foregut and are innervated jointly by vagal and spinal afferents from the cervical and thoracic spinal cord segments (5). In contrast, distal GIT organs such as the rectum, develop from the hindgut, and are innervated solely by spinal afferents from the sacral spinal cord (5).

Unlike most of the proximal GIT, which functions as a transport and absorptive organ, the main physiological role of the rectum is as a sensory and storage organ. This sensory function of the rectum is important in maintaining faecal continence by alerting the brain to the need to contract the external anal sphincter. While the stomach also shares these sensory and storage functions with the rectum, there is less conscious control of gastric function than of rectum function. Therefore, the rectum is unique in the GIT in needing fast afferent connections to the brain to fulfil its physiological role.

Despite the differences in the peripheral afferent innervation and physiological function of the rectum in comparison to the rest of the gut, electrophysiological studies in animals have shown convergence of afferent pathways from multiple visceral organs and the skin onto single cells in both the cortex (38) and thalamus (135). However, differences in the speed, magnitude or pattern of afferent pathway activation could exist, maintaining the physiological differences between the organs. CEP have been used to study both oesophageal (80, 84, 136-138) and rectal afferent pathways (41, 85, 87, 88).

There are, however, no published studies that compare CEP from different gut regions in the same subjects.

I hypothesised that differences in the afferent innervation of the rectum, in comparison to the rest of the GIT, would be reflected by differences in the rectal CEP characteristics in comparison to CEP evoked from other GIT organs.

4.2 Aim of study

To compare the morphology, latency and amplitude of CEP recorded following stimulation of the oesophagus, duodenum and rectum.

4.3 Methods

4.3.1 Subjects:

Ethical approval was obtained and six right handed healthy male subjects with a mean age of 28 years (range 23 to 34) were studied after obtaining written informed consent. None of the subjects had gastrointestinal or neurological symptoms, and none were taking any regular medication.

4.3.2 Gut Stimulation

Gut stimulation was performed using a catheter assembly containing platinum bipolar ring electrodes connected to a constant current, high voltage stimulator (model DS7, Digitimer Ltd., Welwyn Garden City, Herts, UK). A square wave stimulus of 200 microseconds duration was used in each gut region. Previous work has demonstrated that the optimal stimulus intensity for recording oesophageal and rectal CEP is 75% of the difference between the sensory and

pain thresholds (84, 139). Therefore, we used this stimulus intensity in each gut region.

Oesophageal Stimulation:

Oesophageal stimulation was performed using a catheter assembly with three pairs of bipolar platinum ring electrodes (2mm electrodes with an inter-electrode distance of 1cm) sited 5, 12.5 and 20cm from the tip of the catheter. Solid state pressure transducers were sited between each electrode pair to enable the catheter to be positioned using manometric assessment. The catheter was constructed from nylon tubing covered with stainless steel braid insulated with silicone rubber, and had an external diameter of 3mm (Gaeltec, Dunvegan, Isle of Skye, Scotland, IV55 8GU). The catheter was passed either nasally or orally depending upon subjects choice. The distal manometric sensor was positioned in the stomach and then slowly withdrawn until the proximal margin of the lower oesophageal sphincter was identified. The catheter was then withdrawn so that the distal stimulating electrodes were 5cm above the lower oesophageal sphincter. Oesophageal stimulation was then performed using the distal pair of ring electrodes.

Duodenal stimulation:

Duodenal stimulation was performed using the catheter assembly described above. The catheter was passed either nasally or orally depending upon the subject's choice until the distal manometric sensor was in the stomach 50-55cm from the incisors. The subjects were then asked to lie on their right side in a semi recumbent position. The catheter was then slowly advanced while the motility pattern was recorded. When characteristic duodenal activity was seen in all three manometric channels the catheter was secured and duodenal stimulation was performed using the middle pair of electrodes.

Rectal stimulation:

Rectal stimulation was performed using a different catheter assembly with a single pair of bipolar platinum ring electrodes (2mm electrodes with an inter-electrode distance of 2cm) sited 2cm from the tip of the catheter. The

catheter was constructed from nylon tubing covered with stainless steel braid insulated with silicone rubber, and had an external diameter of 3mm (Gaeltec, Dunvegan, Isle of Skye, Scotland, IV55 8GU). This catheter was tied to a second catheter, with an external diameter of 3mm, containing a 2cm latex balloon. During stimulation the balloon was inflated by 5-10ml of air. My experience suggests that this improves electrical mucosal contact without affecting the CEP characteristics (139). Additionally, another study has also shown that the volume of balloon inflation at the site of electrical stimulation of the gut does not affect CEP latency (140).

4.3.3 CEP recording

CEP were recorded using silver silver-chloride surface electrodes, with the active electrode positioned at the vertex (Cz) in accordance with the international 10-20 system of electrode placement, and the reference electrode positioned on the right ear lobe. The electrodes were applied using electrode paste and the impedance was kept below 5 K ohms. The CEP data were acquired using a CED 1902 programmable signal conditioner (Cambridge Electronics Design, Cambridge, CB4 4FE, UK) and a IBM compatible desktop computer, running SIGAVG software (version 6.04 Cambridge Electronics Design, Cambridge, CB4 4FE, UK). The CEP data were sampled at a frequency of 2000 Hz, with an epoch duration of 1000 milliseconds, of which the first 200 milliseconds was pre-stimulation time. The amplifier gain was set at 100,000, with on-line artefact rejection. The bandpass filters were set at 1 and 100 Hz. These parameters are similar to those previously shown to be optimal for recording oesophageal and rectal CEP (84, 88).

4.3.4 Protocol

Each gut region was studied on a separate day in a randomised order for each subject. On each study day, after the catheter was passed the sensory and

pain thresholds were determined by increasing the stimulation intensity in steps of 1 mA. All subsequent stimuli were applied at an intensity of 75% of the difference between the sensory and pain thresholds (84). For each gut region, four runs of 50 stimuli at 0.2 Hz were recorded, with a ten minute rest period between each run. The CEP to all 200 stimuli from each gut region were then averaged. This stimulation paradigm was chosen as faster stimulation rates, or longer stimulation runs results in poorer quality, smaller amplitude CEP (84, 88).

4.3.5 Definition of terms

Sensory threshold:

Sensory threshold was defined as the stimulus intensity in milli-amps (mA) when the subjects first became aware of any sensation.

Pain threshold:

Pain threshold was defined as the stimulus intensity in milli-amps (mA) when the subjects first reported pain.

Latency:

Latency was defined as the time in milliseconds (ms) from the onset of the stimulus to the peak of each CEP component.

Amplitude:

Amplitude was defined as the voltage difference in micro-volts (μV) between consecutive CEP peaks.

4.3.6 Data analysis

In each subject, the four runs of CEP for each stimulation site were averaged. The CEP characteristics from each site were assessed by a investigator blinded to the origin of the data. The positive peaks of the CEP were labelled P1 P2, while the negative peaks were labelled N1 N2. The latency and amplitude of each CEP component from each subject was averaged to obtain group mean data for each gut site.

4.3.7 Statistical comparison:

Using Arcus Quickstat software version 1.0 (Addison Wesley Longman Ltd.), the latencies to each peak and the amplitudes of CEP component evoked from each gut site were compared by the paired two tailed student T test, if the data was normally distributed. The Mann-Whitney U test was used if the data was not normally distributed. A p value of < 0.05 was accepted as a statistically significant difference.

4.4 Results

4.4.1 Stimulus Intensity:

The mean values of the stimulation intensities used for the three gut regions are given in table 4.1, these values represent the 75% difference between sensory and pain thresholds. While there was a trend towards the use of a higher stimulus intensity in the duodenum in comparison to the oesophagus this did not reach significance ($P= 0.09$). The stimulus intensity required in the rectum was significantly lower than that required in either the oesophagus or the duodenum ($P< 0.001$)

4.4.2 Stimulus Perception:

Oesophageal stimulation was described as a sharp retrosternal, non-painful pulse in all subjects. Duodenal stimulation was described as a sharp pulse in the peri-umbilical region, followed by a more diffuse dull sensation which outlasted the sharp pulse, also in the peri-umbilical region. Rectal stimulation was described as a sharp pulse felt deep within the pelvis.

4.4.3 CEP Morphology:

In all subjects oesophageal and duodenal CEP had similar triphasic morphologies, with three main components which were labelled P1, N1 and P2 (figure 4.1). Rectal CEP in four subjects were also triphasic and similar to those from the oesophagus and duodenum (figure 4.1). However, in two subjects a distinctly different morphology was seen, consisting of four main components. This was similar for both subjects and reproducible on a repeat study (figure 4.2).

Figure 4.1:
Comparison of oesophageal, duodenal and rectal CEP

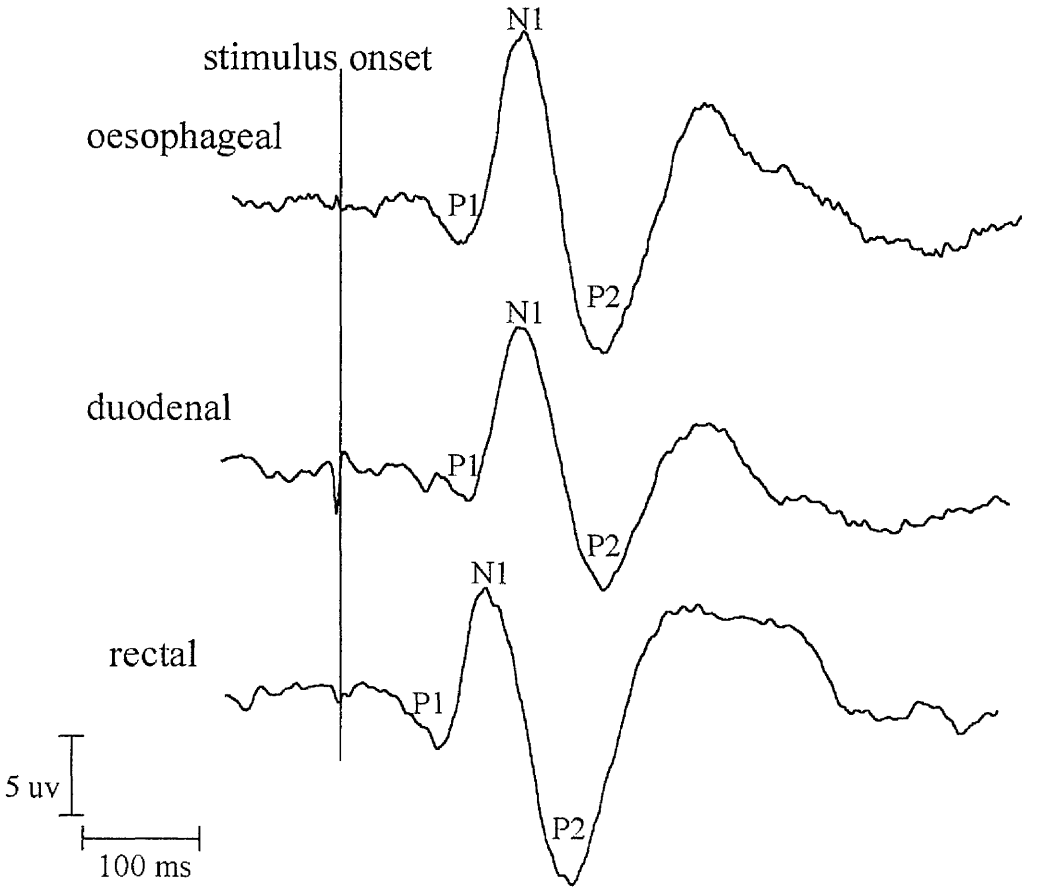
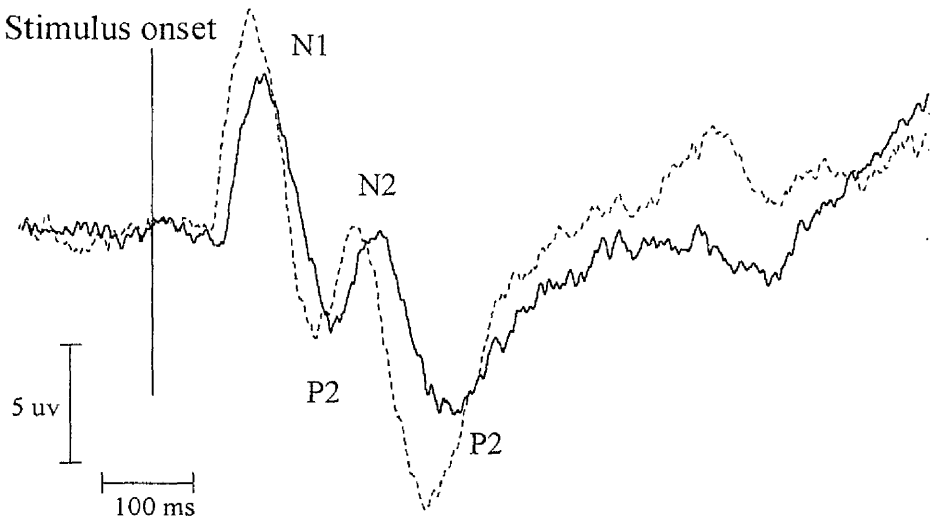


Figure 4.2:

Uncommon CEP morphology following rectal stimulation



The uncommon CEP morphology elicited following rectal stimulation in two subjects, one shown as a solid line, the second as a dashed line. The solid vertical line indicates the time of the stimulus onset.

4.4.4 CEP Latency:

The mean latencies of the CEP following stimulation of the three gut regions are shown in table 4.1. In comparison to the oesophageal CEP, duodenal CEP had a significantly longer latency to the first (P1) component (P =0.009). There was no difference in latencies to the later components of oesophageal and duodenal CEP. For the rectal CEP, the P1 and N1 latencies were significantly shorter than the corresponding latencies for both the oesophagus (P=0.02) and duodenum (P=0.005).

4.4.5 CEP Amplitude:

The mean CEP amplitudes following stimulation of the three gut regions are shown in table 4.1. The amplitude of rectal and oesophageal CEP were similar. The P1-N1 amplitude of the duodenal CEP was significantly smaller

than both the oesophageal (P= 0.01) and rectal (P= 0.04) CEP amplitude. However, the amplitude of the N1-P2 component was similar in all regions.

Table 4.1:

Summary of CEP characteristics from the three gut organs:

	Oesophageal Mean (± SEM)	Duodenal Mean (± SEM)	Rectal Mean (± SEM)
Stimulus intensity in mA	70.8 (± 12.5)	87 (± 20.5)	49.6 (± 15.7)
P1 latencies in ms	102.8 (± 3.8)	115.7 (± 4.9)	70.9 (± 10.1)
N1 latencies in ms	155.9 (± 6.3)	162.1 (± 5.6)	121.4 (12.7)
P2 latencies in ms	234.6 (± 15.7)	255.1 (± 17.2)	206.9 (± 22.6)
P1 -N1 amplitude in μV	7.5 (± 1.3)	5.9 (± 1.1)	7.7 (± 1.4)
N1-P2 amplitude in μV	15.2 (± 1.9)	12.7 (± 0.9)	15.0 (±3.4)

4.5 Discussion

The result of this study suggests that the anatomical and physiological differences between different gut organs are reflected by differences in their CEP characteristics. I also report for the first time CEP evoked by duodenal stimulation.

The oesophagus and duodenum have a dual afferent innervation by vagal and spinal afferents. The vagus nerve is predominantly sensory (28) and consists mainly of unmyelinated C fibres and thinly myelinated A-delta fibres (12, 28, 29). These afferents are sensitive to low intensity stimuli within the physiological range such as peristalsis and physiological levels of gut distention (12, 29). Spinal afferents arise from the cervical, thoracic and lumbar segments of the spinal cord and travel with the sympathetic nerves to the gut (28). Like vagal afferents, spinal afferents are also a mixture of unmyelinated C fibres and

thinly myelinated A-delta fibres and encode a range of sensations, ranging from the innocuous to the noxious (4, 11, 28).

In contrast to the proximal gut, the rectum receives afferent innervation from only the pelvic nerve, which originates from the inferior hypogastric plexus and contains both parasympathetic nerves from the sacral roots and sympathetic nerves from the lumbar roots. As with the proximal gut, rectal afferents are also a mixture of unmyelinated C fibres and thinly myelinated A-delta fibres (4, 5, 11).

In addition to the anatomical differences between the innervation of the rectum and proximal gut, the rectum also has a different physiological role. It functions as a sensory organ, which is important in maintaining faecal continence. Upon rectal filling the internal anal sphincter relaxes reflexively, allowing rectal emptying. Maintenance of faecal continence is then dependent upon voluntary contraction of the external anal sphincter, which is regulated by conscious cortical involvement. For the cortex to maintain efficient sphincter control a regular and rapid sensory feedback is required from the rectum, so that the urgency of the defecatory need and the adequacy of sphincter control can be assessed. Therefore, in comparison to the other gut organs the rectum requires rapid afferent communication with the cortex to maintain its physiological role.

The main difference observed in this study between CEP responses evoked from the different gut regions is in their latency. Despite the greater length of afferent pathways from the rectum to the brain the P1 latency of rectal CEP was shorter than the corresponding latencies of both oesophageal and duodenal CEP. One reason for this could be that rectal stimulation activated afferent pathways with faster conduction velocities than oesophageal and duodenal stimulation. Previous investigators have speculated that electrical stimulation in the rectum can activate fast conducting A-beta fibres in the pudendal nerve (88), leading to a shorter CEP latency than would occur if CEP were mediated via A-delta fibres in the pelvic nerve. They reported CEP latencies of 40 (±2) ms following direct pudendal nerve stimulation, and a

similar CEP latency of 34.7 (±2) ms following rectal stimulation (88). The CEP latency following rectal stimulation in my study was 70.9ms, suggesting that activation of A-beta pudendal nerve fibres is unlikely.

While it could be argued that the presence of the rectal balloon affected the rectal CEP latencies, my previous studies shows that the presence of the rectal balloon does not affect CEP latencies (139). Additionally, another study has also shown that the volume of balloon inflation at the site of electrical visceral stimulation does not affect CEP latencies (140). Therefore, the shorter latency of rectal CEP can not be attributed to the presence of the rectal balloon.

A delta fibres have a conduction velocity that ranges from 7 to 11 meters per second. It could therefore be speculated that rectal sensation is mediated by A delta afferents that have a faster conduction velocity than those mediating oesophageal and duodenal sensation. While there are no direct comparisons of the conduction velocities of afferents from different gut organs in humans, the available animal studies suggest that rectal (3) and oesophageal (29) A delta afferents have a similar conduction velocity. It is possible therefore that the differences observed in this study represent a species difference related to greater cortical control of defecation in man. This speculation would require further investigation.

Another reason for the differences in CEP latencies from the different gut organs in this study could be due to differences in the cortical representation of these organs. However, this speculation is not supported by animal studies that have demonstrated marked convergence of oesophageal and rectal afferent pathways in the thalamus (135) and cortex (38). Nevertheless, differences in CEP recorded at the vertex could still result if the early cortical activation following oesophageal and duodenal stimulation did not result in changes in the vertex electrical potential, due to differences in orientation, or volume the cortical neurones representing these different gut regions.

A study of oesophageal CEP and magnetoencephalography (MEG) responses to electrical stimulation supports this possibility (43). In this study, vertex CEP were recorded following electrical distal oesophageal stimulation, with a similar latency as in my study (98.9 ± 8 ms). MEG in the same subjects demonstrated primary somatosensory cortex (SI) activation at 70 ms in two subjects, supporting the theory that the early oesophageal CEP components could have been missed in both studies. If the rectum had a larger SI representation than the oesophagus then the early rectal CEP components would be more likely to be detected at the vertex. The results of my study therefore, highlight the possibility of differences in the volume and / or orientation of the cortex representing the rectum and oesophagus. This would be an analogous with the somatic homunculus in which the face and thumb have a proportionally larger cortical representation due to the importance of their sensory and motor function.

The spatial resolution of CEP is limited because the scalp distorts electrical currents. However, the scalp does not distort the magnetic component of the electro-magnetic field generated by cortical neurones. Recording these magnetic fields using MEG, which has a greater spatial resolution in comparison to CEP (74) allows the volume and orientation of cortex generating the response to a stimulus to be calculated. Therefore the use of MEG to compare oesophageal and rectal evoked potentials could help to identify possible differences in the volume and orientation of cortical representing these two organs.

Duodenal CEP had a longer latency than both oesophageal and rectal CEP. The most likely explanation for this latency difference between duodenal and oesophageal CEP is that despite the considerable overlap in their spinal innervation the peak distribution of duodenal afferents is inferior to that of oesophageal afferents (5). Therefore the afferent volley from the duodenum has a longer distance to conduct to the cortex, which translates to a longer latency of the P1 component.

This study did not show any differences in the latencies of the later components of the CEP recorded following stimulation of the oesophagus, duodenum, or rectum. The later CEP components reflect secondary cortical processing of sensory information and are often more variable than the early components, as seen in my study. The small sample size in this study is therefore likely to have prevented us from identifying any differences in the latencies of these later CEP components.

This study has also shown that the early components of the duodenal CEP have a smaller amplitude in comparison to rectal and oesophageal CEP. There are three factors that affect CEP amplitude. First, increasing the stimulus intensity will increase CEP amplitude (84). However, in this study the stimulus intensity was fixed at 75% of the difference between the sensory and pain thresholds, which resulted in the use of a higher current in the duodenum than the oesophagus or rectum. This suggests that this difference in the amplitude of CEP from the different gut organs is not due to differences in stimulus intensity.

Second, a reduced density of peripheral afferents will result in a smaller afferent volley reaching the central nervous system, and so smaller amplitude CEP will result. The results of animal studies show that the composition of the vagal nerve changes along the gut, with a reduction in density of myelinated A-delta fibres (141, 142). However, little is known about the relative density of spinal afferents innervating the different gut organs in man (28). It is possible that the smaller amplitude of duodenal CEP is a reflection of a lower density of vagal and spinal innervation of the duodenum in comparison to the oesophagus and rectum.

Third, CEP amplitude is also related in part to the area of cortex activated by the stimulus. Therefore, the smaller amplitude duodenal CEP could be due to a smaller cortical representation of the duodenum in comparison to the rectum and oesophagus.

The CEP morphology from the three gut organs was similar. However, the morphology of vertex CEP following stimulation in several sensory modalities is known to be similar (129). Therefore, the similarity in vertex CEP morphology from different gut organs does not imply that the same cortical areas are being activated.

4.6 Conclusion

I have shown that it is possible to study afferent pathways from multiple gut organs using CEP. My results demonstrate that important differences exist in the characteristics of the afferent pathways and / or cortical representation of the different gut organs. Future studies that combine MEG and CEP will allow a greater understanding of the physiology of gut sensation and will allow CEP to be developed as an objective tool for assessing gut afferent pathways in health and disease.

Chapter 5

Studies of the Cortical Processing of Human Ano-Rectal Sensation using functional Magnetic Resonance Imaging

Studies of the Cortical Processing of Human Ano-Rectal Sensation using functional Magnetic Resonance Imaging

5.1 Introduction

Studies of the neurophysiology of the ano-rectal region have concentrated largely on investigating the motor control of defecation. While a spinal reflex controls defecation, maintaining continence when the rectum is full requires the voluntary contraction of the external anal sphincter. Therefore, while disorders of the pelvic floor musculature or spinal cord can lead to incontinence, maintenance of continence also requires voluntary cortical control, which is dependent upon the sensory feedback from the ano-rectum. However, little is currently known about the processing of ano-rectal sensation in man.

As discussed in chapter 1 there are important differences in the anatomy and physiology of the rectum and anal canal. Uniquely in the gastrointestinal tract the physiological role of the rectum is primarily as a sensory and storage organ. The rectum has a visceral afferent innervation from the pelvic nerve, while the anal canal is predominantly innervated by somatic afferents from the pudendal nerve. Sensation from these two areas is perceived differently, with rectal sensation feeling deep and is poorly localised, while anal canal sensation is perceived superficially and is well localised. These differences in the innervation and sensation suggest that the cortical areas processing sensation from these two sites might be different. The cortical representation of the anal canal has, however, not been studied in humans.

In recent years studies of the cortical processing of somatic sensation using functional imaging techniques have identified a network of cortical areas which are activated in response to pain (49, 56, 57). These cortical areas include

those responsible for spatial discrimination, such as the primary (SI), and secondary (SII) somatosensory cortex, and those involved in attention and emotion, such as the anterior cingulate cortex (ACC), and pre-frontal cortex. The differences in peripheral innervation and sensations from the rectum and skin suggest that there might also be differences in the cortical processing of these two structures, although there is little available evidence to support this view.

Rothstein et al. published the first study to investigate the cortical processing of rectal sensation using positron emission tomography (PET), and demonstrated activation of the pre and post central gyrus only (41). This study stimulated the rectum by painful rapid phasic distention. Silverman et al. also used PET to study cortical activation in response to painful tonic rectal distention in healthy volunteers and IBS patients (44). They showed a different pattern of cortical activation in IBS patients and healthy volunteers. In response to painful rectal distention the ACC only was activated in healthy volunteers, while in IBS patients only the pre-frontal cortex was activated. No cortical activation was identified in somatosensory cortex in either group. More recently Bouras et al. have used single photon emission computer tomography to investigate the response of the ACC and pre frontal cortex to painful rectal distention in healthy volunteers (42). This study showed consisted activation of the ACC in all subjects and activation of the pre-frontal cortex in half the subjects.

The inconsistencies in these studies demonstrate that the cortical network for processing rectal pain is poorly understood. In addition there are no published studies identifying the cortical representation of non-painful rectal sensation. Therefore the effect of increasing rectal stimulus intensity on the cortical processing is unknown.

5.2 Aims of the study

The aims of my study were a) to identify the cortical areas involved in processing non painful and painful rectal sensation, b) to compare the cortical areas that process rectal (visceral) and anal (somatic) sensation.

5.3 Methods

5.3.1 Subjects

Eight healthy right handed male volunteers with a mean age of 31 years (range 21 to 39) were recruited. None of the subjects had any gastrointestinal or neurological symptoms and none were taking any regular medication. All subjects were studied after giving informed consent and with ethical committee approval.

5.3.2 Ano rectal stimulation

Both rectal and anal stimulation was performed using a 2 cm long latex balloon attached 1 cm from the distal end of a polyvinyl catheter with an external diameter of 5 mm. The balloon was inflated at a frequency of 1 Hz using a mechanical pump, especially constructed to be compatible with the MRI environment (Medical Physics Department, Hope Hospital, Manchester, UK). The inflation pressure generated by the pump could be adjusted from 0 to 25 psi. The pump had a constant inflation time of 165ms, so increasing the inflation pressure resulted in a larger volume of balloon inflation. For rectal stimulation the catheter was positioned in the rectum with the middle of the balloon positioned 10 cm above the anal verge. For anal stimulation the lower end of the balloon was positioned just above the anal verge.

5.3.3 MRI scanning

Scanning was performed using a 1.5 Tesla Phillips ACS-NT MRI scanner in the Division of Imaging Science and Biomedical Engineering at Manchester University. The functional scans consisted of a series of 40 T2* weighted single shot gradient echo, echo planar image sets each containing 24 contiguous slices [TR (repetition time) 3000ms, TE (echo time) 50ms, voxel size 3.5 x 3.5 x 3.5 mm]. During each scanning sequence, 30 second periods of stimulation were alternated with 30 second rest periods without stimulation. Preliminary studies had shown a marked attenuation of sensation, and fMRI signal change if the scans were extended beyond two minutes. Two functional scans of two minutes duration were performed in each subject, with a five minute rest period between each scan as this was adequate for recovery of the fMRI signal. A T1 weighted inversion recovery image set [TR (repetition time) 6850ms, TE (echo time) 18ms, TI (inversion time) 300ms] was also obtained to provide anatomical information for each subject.

5.3.4 Experimental Protocols

Protocol 1 Painful v Non Painful Rectal Stimulation:

Seven right handed healthy male volunteers with a mean age of 31 (range 24 to 39) were studied. In each subject, the rectal catheter was inserted and the subject positioned in the MRI scanner. As repetitive painful recto-sigmoid distention has been reported to cause rectal sensitisation and hyperalgesia (143), non painful stimulation was always performed first. The pressure of balloon inflation needed to produce a definite but non painful rectal sensation was determined by increasing the pressure of rectal distention in steps of 1 psi. A functional scan was then performed using this intensity of stimulation. After five minutes rest the stimulus intensity was rechecked and a second functional scan performed. After another five minutes rest the balloon inflation pressure

needed to produce a painful stimulus was determined, and two functional scans performed at this stimulus intensity, separated by a five minute rest period. As the stimulus intensity was determined before each functional scan all subjects knew the intended stimulus intensity for each scan.

Protocol 2 Anal Canal Stimulation:

Anal canal stimulation was performed on a separate occasion to protocol 1. Six of the original subjects and one additional subject (aged 36) were studied. The catheter was inserted and the balloon positioned within the anal canal. A stimulus level producing a definite but non painful sensation was determined as in protocol 1. Two functional scans separated by five minutes were then obtained at this stimulation intensity in each subject.

5.3.5 Image Analysis

The images were transferred to a Sun Workstation for analysis using the TinaTool software package (144). Each of the 40 image sets was aligned to the 20^{th} image set by automated rigid body realignment. The algorithm employed maximises the correlation between the two images in three orthogonal planes, and has been shown to correct adequately for motion in fMRI experiments (102). The images were then resliced using a 3D sinc algorithm based on a 5 x 5 x 5 re-normalised Kernel (145). An individual activation map was calculated from each functional image set using crosscorrelation to a square wave template employing a crosscorrelation measure equivalent to that employed by other groups (146).

An averaged brain aligned to Talairach space was constructed by manually aligning high resolution T2 weighted MRI brain scans (TR 2300ms, TE 80ms, voxel size 0.45 x 0.45 x 3 mm) from five normal subjects into Talairach space, using the TinaTool software package. The anterior and posterior commissure were identified by an experienced neuroradiologist, the

MRI scans were then rotated so that both commissures were in the same horizontal plane. The scans were then scaled independently in the three orthogonal dimensions so that the brain size matched that of the Talairach brain.

The 20^{th} image, from each functional scan, was then aligned to Talairach space using this averaged T2 weighted brain as a template for the realignment. The transformation was performed using rigid body realignment and linear scaling in the three orthogonal dimensions. As the individual correlation maps were aligned to the corresponding fMRI scans, this transformation matrix was used to transform the corresponding activation map into Talairach space. The activation maps from all subjects were averaged to form a group mean activation map. To account for intersubject variation in cortical anatomy the group activation map was smoothed in the x-y plane using a Gaussian filter with a kernel width of one voxel (3.5 mm).

The statistical effects of all the processing were quantified by Monte Carlo simulation. The filtered group mean activation map was then thresholded at a significance value of 0.001 (uncorrected for multiple comparisons) to identify voxels showing significant group correlation with the stimulus. Only clusters of active voxels containing over three contiguous voxels were analysed so increasing the P value of the reported activations (106). The Talairach co-ordinates for these areas were then calculated, and the cortical areas identified from the Talairach atlas (147). These group mean activations were displayed on a 3D surface rendered MRI brain scan using previously described software (148).

5.3.6 Comparison of rectal and anal sensory processing:

In order to determine whether there was a real difference in the cortical areas activated by rectal and anal canal stimulation, a region of interest was drawn around the areas of activation within the somatosensory cortex and ACC. The number of active voxels from each individual fMRI scan within each region of interest was counted. The number of active voxels in each region was

statistically compared. Since there was a positive skew to the data the Man Whitney U test was used for this comparison.

5.4 Results

5.4.1 Protocol 1 Rectal Stimulation

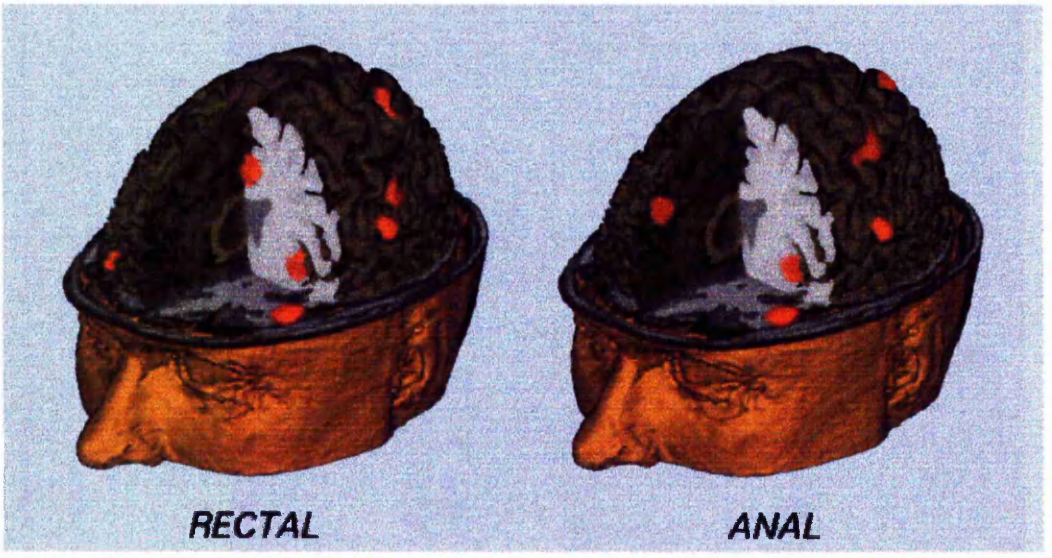
All the studies were completed without complication, the mean balloon inflation pressure was 11 psi (range of 5 to 19) for non painful stimulation, and 21 psi (range of 11 to 25) for painful stimulation. The stimuli were perceived as a deep seated, poorly localised pelvic pulsation. None of the subjects rated the intensity of the non painful stimuli as causing any pain or discomfort. All the subjects rated the intensity of the painful stimuli as consistently painful and could clearly distinguish between the stimulation and rest periods.

Non painful rectal stimulation:

Non painful rectal stimulation (figure 5.1) produced significant activations bilaterally in the secondary somatosensory (SII), sensory association cortex, anterior cingulate (ACC) (figure 5.2) and insular cortex. There was an area of activation bilaterally bordering the inferior primary somatosensory cortex (SI) and Brodmann area 40, but with its centre in the inferior posterior SI (figure 5.3). There was also bilateral activation extending from the peri-orbital cortex to cover part of the anterior temporal lobe (auditory association cortex). In addition there was bilateral activation of the pre-frontal cortex, but in different regions of each hemisphere. The Talairach co-ordinates, Brodmann areas and the number of voxels in each activation cluster are given in table 5.1.

Figure 5.1:

Cortical areas activated with non-painful rectal and anal canal stimulation



Diagrammatic representation of the group mean activations for non painful rectal (on the left) and anal (on the right) stimulation displayed on a 3D rendered MRI brain scan with the left frontal lobe removed to show the insular and anterior cingulate activations. This shows the similarities in activations in SII, insular and peri-orbital cortex, with the difference in position of the SI activation.

Figure 5.2:

Anterior cingulate activation with non-painful (left) and painful (right) rectal stimulation

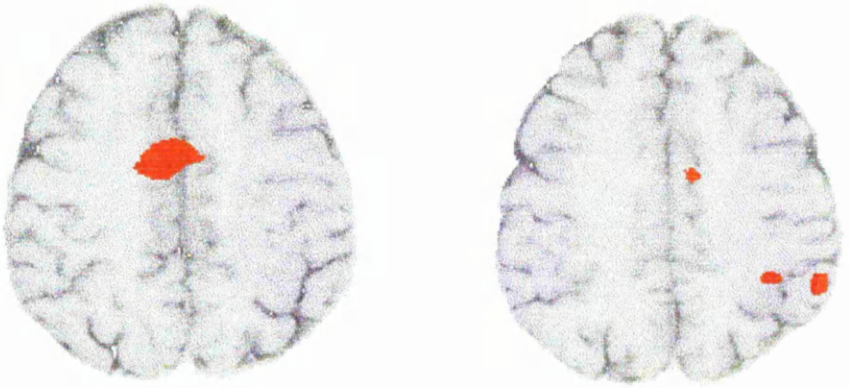


Table 5.1:

Brodmann areas, Talairach co-ordinates of the centre of the cortical areas activated, and the number of voxels activated by non-painful rectal stimulation.

	Brodmann area	Talairach co-ordinates			No of voxels in cluster
		X	Y	Z	
Left primary somatosensory cortex	1 / 2	+ 54	- 20	+ 23	20
Right primary somatosensory cortex	1 / 2	- 54	- 20	+ 23	15
Left secondary somatosensory cortex	43	+ 58	- 10	+ 13	25
Right secondary somatosensory cortex	43	- 58	- 10	+ 17	12
Left sensory association cortex	40	+ 45	- 40	+ 50	22
Right sensory association cortex	40	- 45	- 40	+ 50	18
Bilateral anterior cingulate cortex	24 / 32	0	+ 7	+ 36	18
Left insula		+ 50	+ 5	+ 3	27
Right insula		- 50	+ 5	+ 3	17
Left pre-frontal cortex	10	+ 21	+ 67	+ 7	7
Right pre-frontal cortex	46	- 41	+ 45	+ 7	12
Left peri-orbital	47 / 22	+ 45	+ 15	- 3	17
Right peri-orbital	47 / 22	- 45	+ 15	- 3	24

Painful rectal stimulation:

Painful rectal stimulation produced significant activations in similar areas as non painful stimulation, with two exceptions (see table 5.2). There was a marked reduction in the ACC activation (figure 5.2) to below the threshold set for clusters, with only one voxel showing activation. There were also additional areas of activation in the pre motor cortex, Brodmann area 6.

Table 5.2

Brodmann areas and Talairach co-ordinates of the centre of the cortical areas activated by painful rectal stimulation

	Brodmann area	Talairach co-ordinates			No of voxels in cluster
		X	Y	Z	
Left primary somatosensory cortex	1 / 2	+ 58	- 20	+ 23	14
Right primary somatosensory cortex	1 / 2	-54	- 17	+ 23	21
Left secondary somatosensory cortex	43	+ 58	- 17	+ 17	13
Right secondary somatosensory cortex	43	- 58	- 10	+ 17	3
Left sensory association cortex	40	+ 45	- 40	+ 46	8
Right sensory association cortex	40	- 45	- 40	+ 50	13
Anterior cingulate cortex	24 / 32	+ 5	+ 4	+ 40	1
Left insula		+ 54	+ 4	+ 3	16
Right insula		- 54	+ 4	+ 3	10
Right pre-frontal cortex	10	- 11	+ 67	- 3	12
Right pre-frontal cortex	46	- 38	+ 41	+ 7	10
Left peri-orbital cortex	47 / 22	+ 51	+ 15	- 3	4
Right peri-orbital cortex	47	- 41	+ 25	- 7	10
Left lateral pre motor cortex	6 / 44	+ 54	+ 11	+ 17	8
Medial pre motor cortex	6	0	- 3	+ 53	4

Comparison of painful and non painful rectal stimulation:

In comparison to painful rectal stimulation, non painful stimulation resulted in a larger number of activated voxels in the ACC in the group mean activation map. However, a statistical comparison of the number of activated voxels in individual scans showed no significant different (P = 0.57).

5.4.2 Protocol 2 Anal Canal Stimulation

All the scans were completed without complications, the average pressure of balloon inflation was 6 psi (range of 3 to 15). The stimulus was perceived as a well-localised non-painful peri-anal sensation, which was clearly distinguishable from the rectal stimulation.

The areas activated were the left SI, bilateral SII, sensory association, insular, pre-frontal, peri-orbital and right pre motor cortices (figure 5.1). Table 5.3 shows the Talairach co-ordinates and Brodmann numbers and the number of voxels in each activation cluster. While the group mean SI activation for the whole group was left sided, there was a cluster of 10 voxels in the right SI cortex which just failed to reach the significance threshold (Talairach co-ordinates -51, -14, +43).

5.4.3 Comparison of rectal and anal stimulation

In comparison to rectal stimulation, anal canal stimulation resulted in a more superior SI activation (figure 5.3), at a level above that previously identified as representing the hand (57), and there was no activation of the ACC. Comparing the number of activated voxels between rectal and anal scans showed that the inferior SI had statistically greater activation with rectal stimulation and the superior SI with anal stimulation (P= 0.029 and P= 0.021 receptively). The ACC showed a strong trend for greater activation in response to non painful rectal stimulation compared to non painful anal stimulation (P= 0.053) see table 5.4 for details.

Table 5.3:

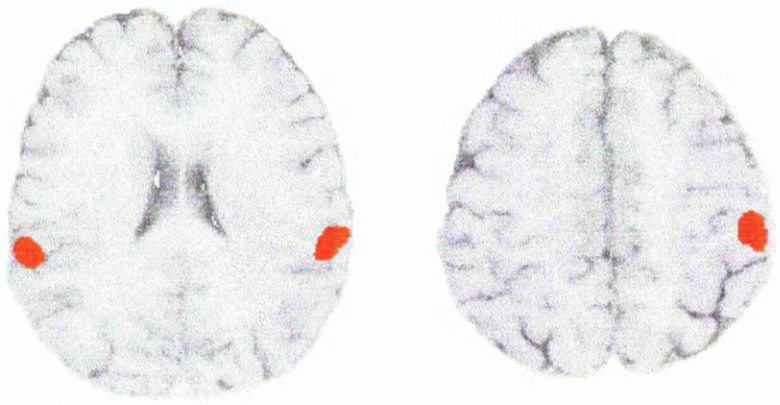
Summary of the cortical activations with anal canal stimulation

	Brodmann area	Talairach co-ordinates			No of voxels in cluster
		X	Y	Z	
Left primary somatosensory cortex	1 / 2	+ 51	- 14	+ 43	14
Left secondary somatosensory cortex	43	+ 48	- 14	+ 13	25
Right secondary somatosensory cortex	43	- 48	- 14	+ 13	13
Left sensory association cortex	7	+ 15	- 54	+ 60	7
Right sensory association cortex	7	- 15	- 54	+ 60	5
Left sensory association cortex	40	+ 51	- 31	+ 20	25
Left insula		+ 40	+ 4	0	60
Right insula		- 40	+ 4	0	39
Right pre-frontal cortex	46	- 41	+ 41	+ 10	11
Left pre-frontal cortex	9	+ 54	+ 15	+ 33	25
Right pre-frontal cortex	9	- 54	+ 15	+ 33	6
Bilateral medial pre-frontal cortex	9	0	+ 48	+ 36	23
Left peri-orbital cortex	47	+ 44	+ 22	- 3	25
Right peri-orbital cortex	47	- 44	+ 22	- 3	35
Right pre-motor cortex	6	- 41	+ 4	+ 43	19

Brodmann areas and Talairach co-ordinates of the centre of the cortical areas activated by non-painful anal canal stimulation.

Figure 5.3:

Primary somatosensory activation with rectal and anal stimulation



The group mean activation in the primary somatosensory cortex superimposed onto an anatomical MRI scan aligned to Talairach space, demonstrating the different levels of the two activations. The rectal activation is displayed on the left at a vertical Talairach level of + 23 mm, the anal canal activation is displayed on the right at a vertical Talairach level of +43 mm.

Table 5.4:

Comparison of the number of voxels activated by non painful rectal and non painful anal stimulation

	Non painful rectal stimulation	anal stimulation	P value
anterior cingulate	4.2 (± 0.99)	1.8 (± 0.4)	= 0.053
Inferior SI (Z= +23)	3.6 (± 0.7)	1.5 (± 0.3)	= 0.029
Superior SI (Z= +43)	1.6 (± 0.5)	3.5 (±0.8)	= 0.021

5.5 Discussion

The results of this study have identified a wide pattern of cortical areas processing ano-rectal sensation, including areas involved in spatial discrimination (SI, SII), and areas involved in affective and cognitive aspects of sensation (ACC, insula and pre-frontal cortex).

Despite evidence of convergence in the spinal cord (5), thalamus (135) and cortex (38), animal studies have identified functional differences in the ascending spinal pathways serving visceral and somatic sensation. The dorsal columns have been shown to be functionally more important for visceral than somatic pain (20), and restricted lesions in the medial dorsal columns in man have been reported to reduce pelvic pain (22). This study has extended knowledge of this difference between visceral and somatic sensation by identifying differences in the brain regions processing these two sensory modalities.

My study demonstrated activation on the border between the inferior part of SI and Brodmann area 40 following rectal stimulation. Although the centre of this activation is within SI, due to the inherent problems with the Talairach atlas a contribution from Brodmann area 40 cannot be excluded. The activation in the inferior part of the post-central gyrus following rectal stimulation in this study occurred in an area similar to that activated by oesophageal stimulation (40, 118) and swallowing (149). In contrast, the SI activation following anal canal stimulation occurred at a level superior to the area processing hand sensation (57). This suggests that the differences in perception of visceral and somatic sensation are reflected by differences in their cortical representation with visceral sensation being represented in the inferior part of SI, and somatic sensation more superiorly. This is consistent with the results of single cell recordings from the cortex of monkeys, which have demonstrated viscero-somatic convergence within the primary somatosensory cortex, but with the viscera only being represented within the inferior part of SI (38).

Previous PET studies of the cortical representation of oesophageal sensation have demonstrated asymmetry of SI activation (40). This study also demonstrated asymmetry in that the SI activation was lateralised to the left hemisphere for anal canal stimulation. However, the subthreshold cluster also present in the right SI cortex suggests activation of this hemisphere in some subjects. In contrast, rectal sensation was represented bilaterally. However, it must be accepted that the use of group data for analysis would have reduced the chance of finding evidence for asymmetry, so I can not exclude the possibility of lateralisation in individual subjects. Asymmetrical control of ano-rectal motor function has also been demonstrated recently (150). The significance of this lateralised cortical representation of midline structures is unknown, but might account for the observation that the somatic referral site of visceral pain is often asymmetrical.

The SII cortex was activated by both rectal and anal stimulation. The SII receives afferents from SI (51) and also directly from the thalamus (50). There is evidence to suggest that for somatic sensation the functionally more important afferents are those from SI (51) and that SII is involved in the serial secondary processing of sensory information after primary processing has occurred in SI (48). Recent magnetoencephalography (MEG) studies following oesophageal stimulation showed only SII activation (45, 46), suggesting that for visceral sensation SII may be functionally more important than SI. However, other studies of oesophageal stimulation using PET and MEG have identified activation in both SI and SII (40, 43, 93). Furthermore, the only previously published study of rectal sensation identifying somatosensory cortex activation, identified only SI activation (41). Binkofki et al. used fMRI to investigate the cortical representation of oesophageal sensation using two frequencies of rapid phasic oesophageal distention and two levels of tonic oesophageal distention (118). This study demonstrated only SII activation with the low intensity tonic oesophageal distention, but both SI and SII activation with the other stimuli. This suggests that the differences in SI activation following visceral stimulation could in part relate to differences in stimulus intensities used. It appears therefore that in most studies of human visceral sensation both SI and SII are

activated. The functional importance of these two areas of activation, however, remain to be determined.

Activation of the anterior insular cortex has been observed in previous studies of both somatic (56, 57) and visceral oesophageal pain (40). The insular cortex forms a link between the somatosensory cortex and the limbic system with efferent connections to both the cingulate and pre-frontal cortices and afferent connections from the thalamus and somatosensory cortex (64) in addition to direct afferents from the vagal nerve (65, 66). Lesions of the insula result in loss of the affective response but preservation of the spatial discriminative aspects of pain. Direct electrical stimulation of the insula at surgery (69) results in visceral motor as well as sensory responses that include abdominal pain and nausea. It is unknown, however, whether these visceral sensations are a direct result of insular stimulation, or secondary to changes in visceral motor function. The insular activation in this current study could therefore be due to either processing of the affective aspects of rectal sensation, or as a result of visceral sensory-motor responses due to rectal distention.

The anterior cingulate cortex is often identified in human functional imaging studies of visceral (40, 44, 151) and somatic (49, 57, 152) pain processing, and has been considered as "the pain centre". Direct electrical stimulation of the ACC results in changes in autonomic tone (55, 63), which is mediated by efferent connections with vagal nuclei and sympathetic columns in the thoracic spinal cord. It is likely that the ACC activation with painful stimuli is related to the generation of autonomic responses to the stimuli.

In the current study non-painful rectal stimulation produced strong ACC activation, while painful rectal stimulation resulted in a weak sub-threshold activation. While studies of somatic sensation have only demonstrated ACC activation with pain (56), studies of oesophageal visceral sensation have demonstrated ACC activation during non painful distention (151). This limbic representation of non-painful visceral sensation could explain the greater

autonomic reflexes and affective responses seen in response to visceral, in comparison to somatic, sensation (5).

The weak sub-threshold ACC activation with painful rectal stimulation in the current study requires an explanation. Both PET and fMRI rely on detecting haemodynamic changes between the stimulation and rest periods, and therefore cortical areas that are activated though out a study will not be identified. As the stimulus intensity was checked before each scan in this study, subjects knew the stimulus intensity they would experience during each scan. It is possible therefore that subjects were anticipating the painful stimuli during the rest periods in the scan. Anticipation of both somatic (59) and rectal (44) pain has been shown to activate the ACC. Therefore, if subjects were anticipating the painful stimuli this could activate the ACC during the rest periods, resulting in no net change in ACC activity occurring during the scan. While this is the most likely explanation for the lack of ACC activation being detected with painful rectal stimulation this hypothesis remains speculative. An alternative explanation is that as the order in which the stimuli were delivered was not randomised the ACC activation could have attenuated over time. This explanation is less likely however, as there was no attenuation of response from the other cortical areas.

The ACC forms part of the limbic system and has been shown in PET studies to be activated by sad emotions (62), and to be less active during depression (61). This suggests a role for the ACC in generating an affective response to a stimulus. In addition, the ACC has connections with the motor cortex and it has been suggested that it plays an important role in selecting appropriate behavioural responses to a stimulus (55).

The pre frontal cortex is involved with cognition and memory, and receives inputs from the sensory association cortex. Animal studies have demonstrated pre-frontal cortex activation with painful visceral stimulation (153), and an increase in pain thresholds and reduction in pain behaviour after local anaesthetic injections into the pre-frontal cortex (154). These studies

demonstrate a role of this cortical area in processing of pain.. Silverman et al. (44) hypothesised that altered cortical processing of rectal sensation occurs in IBS based on their observation of activation in the pre frontal cortex in IBS patients but not healthy volunteers. However, my study and that of Bouras et al (42) have demonstrated that this pattern of cortical activity is a normal component of the processing of rectal sensation in health. Bouras et al. used a region of interest analysis over the pre-frontal cortex, therefore the precise location of the activation within this area can not be determined in their study. As in my study the pre frontal activation observed in the study by Silverman et al. was also in Brodmann area 10 (44). However, no Talairach co-ordinates for this activation were given in the study by Silverman et al. therefore, a more detailed comparison of the location of the activations in the pre-frontal cortex with that observed in this study is not possible.

Both anal and rectal stimulation resulted in bilateral activation of the periorbital cortex. The periorbital cortex has connections with the limbic cortex, as well as receiving direct afferents from the spinal cord (155). This area is involved in maintaining homeostasis and in regulating autonomic function. The activation of the periorbital cortex seen in this study is therefore likely to reflect changes in autonomic function in response to ano rectal stimulation; however, this speculation remains to be investigated.

This study has identified more areas of cortical activation with non-painful rectal stimulation than the previous studies of rectal pain. This probably reflects methodological differences in particular the use of repeated phasic distention which causes repeated stimulation of rectal stretch receptors, and so increased activity of ascending spinal pathways to the cerebral cortex. My preliminary work confirmed that this also gives a larger fMRI signal change than tonic distention used in other studies (42, 44).

5.6 Conclusion

This study has demonstrated a wide pattern of cortical areas processing anal and rectal sensation, including areas involved with spatial discrimination, attention, and affect. In addition, differences between the areas of somatosensory cortex responsible for processing the visceral and somatic components of ano-rectal sensation have been identified. The role of the anterior cingulate and pre-frontal cortices in normal rectal sensation, and how this is modulated by attention and affect needs to be further investigated in order to develop a full understanding of the pathophysiology of diseases of ano-rectal function.

Chapter 6

General Discussion

General Discussion

6.1 Present studies

Animal studies investigating the cortical representation of gut sensation have demonstrated activation of primary somatosensory (SI) cortex following gut stimulation (38). Marked variation in the SI activation has been demonstrated, dependent on the animals studied and the anaesthetics used (39). The insular cortex has also been shown to receive afferent connections from the vagus nerve (65, 66), implying a role for the insula in processing proximal gut sensations. Whether the insula also receives afferents from the distal gut, which is not innervated by the vagus nerve, is, however, not known. Human studies using functional brain imaging tools have demonstrated activation of a wide network of cortical areas following oesophageal stimulation (43, 45-47, 93, 117, 118, 151, 156). These include areas responsible for spatial discrimination (somatosensory cortex SI and SII) and the affective aspects of sensation and pain (anterior cingulate cortex). However, there is less detailed information available about the brain areas processing human rectal sensation.

There are important physiological and anatomical differences between the rectum and the proximal gut. The physiological role of the rectum is primarily as a sensory and storage organ, with the proximal gastrointestinal tract (GIT) acting predominantly as a conduit for transport and absorption. While the stomach also has a sensory role, the control of gastric function is not under voluntary control like the rectum. Therefore, the rectum (together with the anal canal) has a unique position in the GIT of requiring fast sensory feedback to the cortex, which is important in maintaining faecal continence.

Animal studies have identified important anatomical and neurophysiological differences in the peripheral afferent innervation of the rectum in comparison to the proximal GIT. While the proximal GIT has a dual afferent innervation from vagal and spinal afferents, the rectum's afferent

innervation is from only the sacral spinal cord (3-5). Animal studies, however, also suggest that there is marked convergence of afferents from multiple viscera in the spinal cord (17, 18), thalamus (135, 157) and somatosensory cortex (38). However, differences in the central processing of sensation from different human GIT organs have not previously been investigated.

Each of the functional imaging techniques available for studying human GIT sensation has advantages and limitations. The high temporal resolution of CEP allows the afferent pathways to be characterised, but its poor spatial resolution prevents accurate localisation of the cortical sources generating the CEP. The high spatial resolution of fMRI allows for the localisation of cortical activation, but its poor temporal resolution prevents study of the sequence of cortical activation. While MEG combines the high temporal resolution of CEP and spatial resolution of fMRI, its limited availability prevents wide spread use of this technique. To gain the most information about the neurophysiology of human rectal sensation studies will need to utilise a combination of these techniques.

In order to investigate possible neurophysiological differences in the afferent pathways from the rectum and proximal GIT it is necessary to use imaging tools with a high temporal resolution and a stimulus with the same temporal characteristics in each gut region. The volume (and so time) required to inflate an intra luminal balloon would be dependent on the physical characteristics of the organ being investigated and would therefore be different for the different organs of the GIT. Therefore a mechanical stimulus can not be used to compare the neurophysiological characteristics of the afferents from different GIT organs. Electrical stimulation of the GIT fulfils this requirement for identical temporal characteristics of the stimulus in each GIT organ. However, before using electrical stimulation it is necessary to confirm that this is simulating only the GIT afferents, and not also activating somatic afferents outside of the GIT.

Previous investigators have recorded CEP following both electrical and mechanical oesophageal stimulation, and reported longer CEP latencies with mechanical stimulation (91). This lead to the speculation that these stimulation modalities could be stimulating different afferent pathways. However, comparison of CEP latencies following stimulation of the proximal and distal oesophagus have shown that both electrical and mechanical oesophageal CEP are mediated via activation of A-delta fibres with similar conduction velocities (91). This suggests that both electrical and mechanical oesophageal stimulation activate similar afferent pathways and therefore, the latency difference between these two techniques is due to the delay in balloon inflation with mechanical stimulation.

Previous investigators have also reported CEP following both mechanical and electrical rectal stimulation (85, 89). Comparisons between studies have suggested that mechanical rectal stimulation results in CEP with a longer latency than electrical rectal stimulation (41, 85, 87, 126). This has lead to the speculation that electrical stimulation could be activating faster conducting afferents in the pudendal nerve (88). However, methodological differences between these studies prevent comparison between them. Furthermore, no direct comparisons of electrical and mechanical rectal stimulation have been performed in the same subjects. Therefore, it is still unknown whether these two stimulation modalities are activating the same afferent pathway. Before progressing to the comparison of afferent pathways from different GIT organs it is first necessary to perform a direct comparison of CEP evoked after electrical and mechanical rectal stimulation in the same subjects.

My studies described in chapter 3 demonstrate that the longer CEP latency following mechanical rectal stimulation is likely to result from the delay in balloon inflation, as is the case with oesophageal CEP (91). My studies also demonstrate that both electrical and mechanical rectal CEP are mediated by A-delta fibres, and that electrical stimulation results in larger amplitude more reliable CEP. There are important methodological differences between my studies and previous studies. I used a rectal balloon to improve the electrical

contact between the rectal mucosa and the electrical catheter. Preliminary studies show that this results in more reliable CEP and allows the use of a lower stimulation current. While the authors of the previous studies have not stated the current used for rectal stimulation it is possible that they used a larger current that did stimulate somatic afferents outside the rectum.

Having validated the methodology for recording rectal CEP, I then compared CEP following stimulation of the rectum and proximal gut (described in chapter 4). The main finding of these studies was the shorter latency of rectal CEP in comparison to both oesophageal and duodenal CEP. This demonstrates that despite the convergence of oesophagus and rectum afferents onto the same thalamic neurones (135, 157) important differences in the central processing of rectal and proximal gut sensation exist. This could be due to either faster afferent conduction of rectal afferents or a greater cortical representation of the rectum in comparison to the proximal gut. This is consistent with the unique physiological role of the rectum as a gut sensory organ, and the need for the cortex to maintain control of the external anal sphincter with rapid sensory feedback from the rectum.

My demonstration of a shorter CEP latency following rectal stimulation in comparison to oesophageal and duodenal stimulation highlights the possibility of differences in the cortical representation of the rectum and the proximal GIT. However, while the brain's processing of oesophageal sensation has been investigated little is known about the brain's processing of human ano-rectal sensation. Previous studies using different techniques have given conflicting results. The cause of these differences is unknown, but could relate to differences in the methodology used. It therefore remains important to investigate the cortical representation of the ano-rectum in greater detail.

My studies in chapter 5 have demonstrated a network of cortical areas that are activated following rectal stimulation. This network includes areas involved in both the spatial discriminative aspects of sensation (SI, SII and the sensory association cortex), affect (anterior cingulate and pre-frontal cortex) and

autonomic areas involved in regulating gastrointestinal function (anterior cingulate and peri-orbital cortex). While a direct comparison with the cortical representation of oesophageal sensation has not been performed, there are similarities with my results and previous reports of the oesophageal representation (43, 45-47, 93, 117, 118, 151, 156).

Studies of somatic pain have identified two ascending pathways, the medial and lateral pain pathways (32). The lateral pain pathway projects to the somatosensory cortex and is involved in spatial discrimination. The medial pain pathway projects to the limbic system and is involved with the affective aspects of pain. My results suggest that as with somatic pain, GIT sensations are mediated via both the medial and lateral pain pathways. The demonstration of anterior cingulate activation with non painful rectal and oesophageal (151) stimulation suggests greater activation of the medial pain pathway with GIT sensations than somatic sensations. This could in part explain the greater autonomic responses seen following visceral stimulation, in comparison to somatic stimulation (5).

There are subjective differences in the sensations from the viscera and somatic structures, including the anal canal. The skin has a rich supply of afferent nerves and a highly organised representation in SI, which results in skin sensations being precisely localised and sensitive to a wide range to sensory modalities. In contrast, the afferent innervation of the gut is sparse, relative to the skin, resulting in sensations that are poorly localised and often referred to somatic structures. I have demonstrated differences in the primary somatosensory cortex (SI) representation of the rectum and anal canal (chapter 5). Anal canal stimulation resulted in activation of the SI cortex at a level superior to the hand representation. In comparison the SI activation with rectal stimulation was in a similar area to that previously observed with oesophageal stimulation (40, 43, 118, 151) in the inferior portion of SI. This is in keeping with the demonstration of convergence of afferents from multiple visceral organs onto the same cortical cell in the inferior SI (38). The similarity of the oesophageal and rectal SI representation suggest that the inferior SI is adapted to

representing the whole gut, while somatic structures are represented in the more superior SI. The differences in the visceral and somatic sensations could be explained by the differences in their SI representation.

Previous studies have suggested that irritable bowel syndrome is due to abnormal cortical processing of rectal sensation based on the lack of anterior cingulate (ACC) activation and the additional pre-frontal activation in IBS (44). However, my studies shown that activation of pre-frontal cortex is a normal component in the cortical processing of rectal sensation, as previously suggested by animal studies (153, 154). My speculation that the lack of ACC activation with painful rectal stimulation in my studies was due to anticipation of pain in the resting state could also explain the lack of ACC activation in IBS patients.

6.2 Future studies

My studies in chapter 4 have suggested differences in the afferent pathways or cortical representation of different gut organs. However, little can currently be said about these differences due to the lack of studies comparing the central processing of sensations from these organs in the same subjects using tools with good spatial resolution. To expand the knowledge of these differences requires studies to be performed using MEG and / or fMRI. Both of these tools have good spatial resolution, so could be used to compare the cortical regions processing sensations from different gut organs and investigate the "visceral homunculus". MEG has the additional benefit of good temporal resolution and will therefore allow the sequence of cortical activation to be studied.

The goal of research into gut sensation is to understand the pathophysiology of functional gastrointestinal disorders (FGD) and develop a useful clinical tool for investigating large groups of patients. In order to achieve this goal it is necessary to understand how the possible pathophysiology of FGD

will affect the central processing of GIT sensation. To investigate sufficient patients with FGD and to control for the wide range of patient characteristics a tool that is widely available and cheap is required. Cerebral evoked potentials (CEP) is the only functional imaging tool that fulfils this role, as the equipment required for CEP recording is cheap and already widely available in most hospitals. The major limitation of CEP, however, is its poor spatial resolution, which limits its accuracy in identifying the neuronal areas generating each CEP component. This disadvantage could be overcome by combined studies with a technique with greater spatial resolution such as MEG, so that the neuronal sources of each CEP component can be identified.

6.2.1 Peripheral and central hyperalgesia:

Sarker et al. have developed a model of peripheral and central oesophageal sensitisation based on distal oesophageal acid infusion. This has been shown to lower the pain threshold not only at the site of acid infusion, but also the non-acid exposed proximal oesophagus and the somatic site of pain referral on the anterior chest wall (158). This demonstrates both primary hyperalgesia in the acid exposed distal oesophagus and secondary hyperalgesia in the non-acid exposed proximal oesophagus and the anterior chest wall. These models could be used to study the effect of both primary and secondary hyperalgesia on the cortical processing of gut sensation, by performing MEG studies before and after GIT sensitisation.

6.2.2 Cognitive and emotional factors:

The level of vigilance to sensations will affect their perception, and central processing. It has been suggested that hypervigilance could play a role in the aetiology of pain in FGD. Therefore, the effect of attention and anticipation on the central processing of GIT sensations needs to be investigated. This could be achieved by distraction studies in which two stimuli are presented

simultaneously and the subject instructed to attend to each in turn, while monitoring the level of attention during the study.

The other important consideration with this aspect of sensory modulation is demonstrated in my fMRI studies (chapter 5), which has highlighted the possible effect of anticipation on the results of standard block design fMRI studies. Therefore, studies will have to be carefully designed so that the effect of anticipation does not generate false or misleading results.

6.3 Conclusions

I have demonstrated the feasibility of studying ano-rectal sensation using both CEP and fMRI. I have demonstrated that in comparison to mechanical stimulation, electrical rectal stimulation produces more reliable and robust CEP mediated via similar afferent pathways. I have also demonstrated that the differences in the anatomy, physiology and function of the rectum in comparison to proximal gut organs are reflected by differences in their central processing. I have also identified a wide cortical network responsible for processing ano-rectal sensation, with differences in the somatosensory cortex representation of the visceral and somatic aspects of ano-rectal sensation. Finally I have suggested how further continuation of this work could lead to an increased understanding of the pathophysiology of functional gastrointestinal disorders, which account for 30% to 50% of all Gastrointestinal referrals. The development of CEP as a clinical tool could led to the tailoring of management strategies to individual patients.

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