ALTERATIONS IN HUMAN VISCERAL SENSATION INDUCED BY NON-INVASIVE CORTICAL AND LUMBOSACRAL MAGNETIC STIMULATION IN HEALTH AND DISEASE

A thesis submitted to the University of Manchester for the degree of Doctor of Medicine in the Faculty of Medical and Human Sciences

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<tr>
<td>ASIC</td>
<td>Acid Sensing Ion Channel</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APB</td>
<td>Abductor Pollicis Brevis</td>
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<tr>
<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
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<tr>
<td>BOP</td>
<td>Baseline Operating Pressure</td>
</tr>
<tr>
<td>CEP</td>
<td>Cortical Evoked Potential</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CN</td>
<td>Cranial Nerve</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CT</td>
<td>Computerised Tomography</td>
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<tr>
<td>DLPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
</tr>
<tr>
<td>EAS</td>
<td>External Anal Sphincter</td>
</tr>
<tr>
<td>EC</td>
<td>Enterochromaffin Cells</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyograph</td>
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<tr>
<td>FGID</td>
<td>Functional Gastrointestinal Disorder</td>
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<tr>
<td>fMRI</td>
<td>functional Magnetic Resonance Imaging</td>
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<tr>
<td>GABA</td>
<td>Gamma amino butyric acid</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>HAD</td>
<td>Hospital Anxiety and Depression</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IAS</td>
<td>Internal Anal Sphincter</td>
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<tr>
<td>IBS</td>
<td>Irritable Bowel Syndrome</td>
</tr>
<tr>
<td>IBS-C</td>
<td>Constipation predominant IBS</td>
</tr>
<tr>
<td>IBS-D</td>
<td>Diarrhoea predominant IBS</td>
</tr>
<tr>
<td>LPC</td>
<td>Left Prefrontal Cortex</td>
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<tr>
<td>LSMS</td>
<td>Lumbosacral magnetic stimulation</td>
</tr>
<tr>
<td>LTD</td>
<td>Long-term Depression</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term Potentiation</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
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<tr>
<td>MEP</td>
<td>Motor Evoked Potential</td>
</tr>
<tr>
<td>MI</td>
<td>Primary Motor cortex</td>
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</table>
MR  Magnetic Resonance
MRI  Magnetic Resonance Imaging
MRS  Magnetic Resonance Spectroscopy
ms  millisecond
MS  Magnetic stimulation
MT  Motor Threshold
µV  microVolt
NHS  National Health Service
NMDA  N-methyl D-aspartate
NSAID  Non-Steroidal Anti-Inflammatory Drug
PET  Positron Emission Tomography
PMC  Premotor Cortex
PPC  Posterior Parietal Cortex
P2X  Purinergic receptor subtype X channel
rLSMS repetitve Lumbosacral Magnetic Stimulation
RMT  Resting Motor Threshold
rTMS repetitve Transcranial Magnetic Stimulation
SI  Primary Somatosensory Cortex
SII  Secondary Somatosensory Cortex
S  Sacral root
SEM  Standard Error of the Mean
SMA  Supplementary Motor Area
SNS  Sacral Nerve Stimulation
SO  Stimulator Output
SERT  Serotonin Reuptake Transporter
TA  Tibialis Anterior
tDCS transcranial Direct Current Stimulation
TMS Transcranial Magnetic Stimulation
TRP Transient Receptor Potential ion channel
UK  United Kingdom
USA  United States of America
VAS  Visual Analogue Scale
VIP  Vasoactive Intestinal Peptide
VDCC Voltage-Dependent Calcium Channel
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ABSTRACT

Background:
Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder (FGID) which can be defined as chronic, relapsing visceral pain with bloating associated with change in bowel habit. It affects up to 10-15% of the adult population in the UK and is more common in females. The cost of IBS in terms of health care utilisation is substantial, exceeding £45.6 million per year in the UK alone, yet its pathophysiology is incompletely understood. Visceral pain is the main and most difficult symptom to manage in IBS and many IBS female sufferers compare it to labour pain in its severity. Modulating visceral pain in healthy volunteers and IBS patients is therefore an important research area. Non-invasive magnetic stimulation may play a crucial role in this respect.

Aim:
The aim of this study is to ascertain whether non-invasive repetitive magnetic stimulation applied to the motor cortex and/or lumbosacrum can modulate gastrointestinal pain originating from the anorectum.

Methods:
Participants: 16 healthy volunteers and 10 IBS patients aged 18 and above were included in the study.

Questionnaires: Healthy volunteers and IBS patients were asked to complete anxiety and depression questionnaire and IBS patients were requested to fill in an IBS severity questionnaire.

Motor measurements in healthy subjects: Single-pulse lumbosacral magnetic stimulation (LSMS) was applied to the lumbosacral area for the anal sphincter where the largest motor evoked potential (MEP) amplitude response was detected. Single-pulse transcranial magnetic stimulation (TMS) was then performed at the pre-determined resting motor thresholds (RMT) for the anal sphincter and the hand.

Sensory measurements in healthy subjects and IBS patients: Electrical stimulation was used to assess the changes in sensory and pain thresholds in the anorectal area. The subjects were asked to score the pain intensity using five-point categorical rating scales. In addition they were asked to describe the pain experienced using a shortened form of the McGill Pain Questionnaire.
**Intervention:** Healthy volunteers received 6 paradigms of magnetic stimulation in a randomised order i.e. 3 repetitive LSMSs (1 Hz, 10 Hz and sham) and 3 repetitive TMSs (1 Hz, 10 Hz and sham) to investigate their modulatory effects on visceral sensitivity and to determine which of these interventions is most effective. The most effective active interventions (1 Hz rLSMS and 10 Hz rTMS) together with one sham were then trialled in a randomised fashion on IBS patients.

**Post intervention:** Motor excitabilities were repeated at 30 min after each intervention. The assessment of sensory and pain thresholds at anal sphincter and rectum were done immediately, 30 and 60 min after each intervention.

**Results:**
Application of 1 Hz rLSMS led to alterations of anal sphincter motor excitabilities and resulted in a significant increase in the amplitude of lumbosacal-anal motor evoked potentials (MEPs) in healthy volunteers recorded at 30 min post intervention.

In healthy volunteers, 1 Hz rLSMS and 10 Hz rTMS caused a significant increase in the rectal pain thresholds experienced immediately, 30 and 60 min after each intervention. 10 Hz rLSMS and 1 Hz rTMS only led to a significant rise in rectal pain thresholds immediately after their application. Furthermore, there was a significant increase in the rectal pain thresholds immediately, 30 and 60 min following 1 Hz rLSMS and 10 Hz rTMS in IBS patients.

**Conclusion:**
The application of magnetic stimulation to the cortical and lumbosacral areas to modulate visceral pain is a new concept, which reduced rectal sensitivity to painful stimuli and offers a much needed new approach in the management of abdominal pain in patients with IBS.
DECLARATION

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PRESENTATIONS AND PUBLICATIONS


4. MODULATION OF THE HUMAN CORTICO-ANAL BRAIN GUT AXIS WITH LUMBOSACRAL NON-INVASIVE MAGNETIC STIMULATION. Tarig Algladi, Mary Louise Harris, Peter J. Whorwell, Shaheen Hamdy. Gut 2011;60:A150

CHAPTER 1

INTRODUCTION
1.1 Introduction

This report will provide an outline of a current clinical problem facing gastrointestinal medicine, which is the driving force behind my research. I aim to review the diagnosis and current treatment of irritable bowel syndrome (IBS), describe the pain matrix, and discuss available methods of neurostimulation.

The rationale behind my research is that non-invasive magnetic stimulation, a safe and well-tolerated technique, which has previously been shown to modulate the nervous system, has the ability to alter pain sensation. I used this technique to attempt to alter visceral pain perception in healthy volunteers and then in a group of IBS patients.

1.2 Irritable Bowel Syndrome (IBS)

IBS is a functional gastrointestinal disorder (FGID) which can be defined by chronic, relapsing visceral pain and bloating associated with changes in bowel habit. It affects up to 15% of the adult population in the UK [1] with a female to male ratio of 2-2.5:1 in healthcare seeking patients [2]. The incidence of IBS is most frequent between 20 to 50 years of age and rarely occurs after 60 years [3]. However, the prevalence is the same amongst young and old adults [3].

The cost of IBS in terms of health care utilisation is substantial, exceeding £45.6 million per year in the UK [4] and $200 billion per year in the USA [5].

1.2.1 Diagnostic criteria

In 1978, Manning et al were the first who found a diagnostic criteria for IBS by evaluating the symptoms of patients with abdominal pain and chose 6 common symptoms in IBS compared with organic gut diseases, including 1) pain relieved by defecation, 2) more frequent stools at onset of pain, 3) looser stools at onset of pain, 4) visible abdominal distension, 5) passage of mucus per rectum and 6) and sense of incomplete evacuation [6].
After that in 1990, a more accurate criteria appeared, the Rome I criteria which was a slight modification to the Manning criteria by clustering items 1 and 3 together [7].

Following this the Rome II criteria were developed in 1999, taking into account that pain can be at the onset of hard stools as well as the loose ones and abdominal pain or discomfort must be for at least 12 weeks in 12 months, associated with two of the following symptoms: 1) relief with defecation, 2) change in stool frequency, or 3) change in stool consistency. Additionally, supportive symptoms include 1) loose, watery, or hard, lumpy stools, 2) stool frequency of less than three times a week or more than three times a day, 3) faecal urgency, or 4) straining with bowel movement [8].

Recently in 2006, the Rome III criteria evolved from the Rome II by specifying the pain duration, stating that recurrent abdominal pain or discomfort must be for at least 3 days a month in the past 3 months, associated with two or more of the following: 1) improvement with defecation, 2) onset associated with a change in frequency of stool, or 3) onset associated with a change in form (appearance) of stool, with the above symptoms begin at least 6 months before the diagnosis [9].

1.2.2 Classification

IBS is classified according to the dominant bowel habit into diarrhoea predominant IBS (IBS-D), constipation predominant IBS (IBS-C) and mixed type. Most studies indicate that IBS can be divided into one third of IBS-D, one third of IBS-C and the remaining for the mixed one [10].

There is also another type which is called alternating IBS because IBS changes with time from one type to another usually from IBS-D and IBS-C into mixed type. It is also reported that around one third of IBS-D converted into IBS-C in one year time [11].
1.2.3 Aetiology

The pathophysiology of IBS is incompletely understood and is likely to be multifactorial where visceral sensation abnormalities play a role with brain-gut axis dysregulation, motility disorders, inflammation, psychological factors, genetic predisposition and others.

1.2.3.1 Visceral hypersensitivity

Visceral hypersensitivity is the prevailing theory of the development of IBS and other functional gastrointestinal disorders (FGIDs). In 1973, Richie et al was the first to find that patients with IBS were hypersensitive to balloon inflation in the colon compared with normal subjects [12]. Then, his finding was confirmed by many researchers [13].

Bouin et al [13] reported that 90% of their IBS patients were suffering from colonic hypersensitivity as studied by electronic balloon distension of the rectum (barostat). Rectal hypersensitivity in IBS (measured by barostat) is characterised by reduced pain threshold, increased responsiveness to repeated inflation and abnormal areas of pain sensation apart from sacral region [14].

It is thought that transient noxious events, such as viral gastroenteritis, lead to long lasting sensitisation of the gut [15]. This sensitisation occurs in the absence of detectable organic disease and results in normal physiological contractions in the gut being perceived as painful [16]. It has been postulated that these symptoms which can be long lasting, are due to either the afferent nerves in the gut becoming sensitised due to previous inflammation or injury and/or the brain processing of gut sensation being altered.

Gut hypersensitivity could arise from the periphery i.e. the pain receptors in the intestine itself and their afferent nerves, and/or centrally by the intensification of normal gut sensation during its journey through spinal cord, brain stem and the brain. The weakness or deficiency of the descending inhibitory pathways from the brain to the gut through spinal cord that regulate the ascending sensory pathways could also be a cause of hypersensitivity.
A- Peripheral sensitisation

After injury, the nociceptors are exposed to various inflammatory and immune mediators like histamine, cytokines, reactive metabolites, serotonin, prostaglandins, leukotrienes and neurotrophic factors [14]. These mediators lead to primary hyperalgesia and allodynia (increased sensitivity and perception to painful and non-painful stimuli, respectively).

Peripheral mechanisms can be supported by the following: a) IBS can occur after gut infection, b) there is inflammatory cells infiltration of the gut of IBS patients and c) rectal sensitivity to balloon distension can be decreased by local anaesthetic and can be increased by local administration of glycerol [14].

B- Central sensitisation

As a result of peripheral sensitisation, the normal visceral areas around the injured ones also develop hypersensitivity in the forms of hyperalgesia and allodynia. This is caused by an increase in receptive field and excitability of the nerves that supply both visceral and somatic structures and converge together at the same spinal level [17].

Central mechanisms can be supported by the following: a) there is an increased sensitivity of proximal GI tract in IBS patients [18], b) fibromyalgia which is a type of somatic hyperalgesia, occurs more frequently in IBS compared with healthy people [19] and c) there is more somatic pain radiation in patients with IBS compared with healthy subjects following colonic stimulation [20].

1.2.3.2 Brain gut-axis dysregulation

Brain-gut interaction plays a very crucial role in regulating gut functions. This bidirectional interaction occurs via afferent and efferent pathways. Afferent pathways that connect the gut to the spinal cord and brain composed of vagal, spinal nerves and neuroendocrine signalling [21]. While efferent pathways that connect the brain and spinal cord to the gut consist of sympathetic, parasympathetic and enteric branches of splanchnic nerves [21].
Visceral and somatic sensation is represented in the same cortical areas i.e. the primary (SI) and the secondary somatosensory cortex (SII). The differences occur between the healthy volunteers and IBS patients in pain processing in different parts of the brain especially the prefrontal cortex, insula, cingulate cortex and thalamus as confirmed by applying functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) [22].

Additionally, during balloon inflation, IBS patients showed increased activity in cortical areas responsible for attention and affect, subcortical areas responsible for autonomic reaction and abnormalities in areas responsible for pain regulation [23].

These differences between IBS patients and healthy volunteers have been confirmed recently by Tillisch et al in a quantitative meta-analysis identifying the brain regions activated during balloon distension [24].

1.2.3.3 Gastrointestinal motility

Gastrointestinal motility abnormalities are present in IBS patients. Two studies have shown a relationship between intestinal contractions and visceral pain attacks in patients with IBS [25-26]. Another study reported an increase in rectal motor reactivity to distension besides the well-known sensory hypersensitivity in IBS patients [27].

1.2.3.4 Inflammatory changes

Inflammation may play an important part in the development of at least a subgroup of IBS (post-infectious IBS). Gut dysfunction can develop after acute or chronic inflammation of the gut. Acute gastrointestinal infections have been reported before the development of IBS by about 6-17% of patients [28] with a relative risk of 1.1 [29]. Patients with post-infectious IBS have increased numbers of T cells and enteroendocrine cells in colonic mucosa [30]. Furthermore, inflammatory bowel disease patients in a long-lasting remission reported IBS like symptoms 2-3 times more common compared with general population [31].
Other inflammatory mechanisms are also suspected to be involved with the aetiology of IBS such as the increase in gut permeability [30], the change in gut normal flora [32] and food allergies [1].

1.2.3.5 Neurotransmitters

A number of neurotransmitters and molecules could be involved in the pathogenesis of IBS, partly due to their effects on sensitivity and motility of the gut like serotonin [33], vasoactive intestinal peptide (VIP) [34], moilin [35], cholecystokinin, stress response hormones, substance P [36], peptide YY and neuropeptide Y [37].

Serotonin is the major neurotransmitter in the gut and affects its sensation, motion and secretion. Serotonin is secreted by EC (Enterochromaffin) cells in response to different stimuli within the gut and its effect is stopped by SERTs (serotonin reuptake transporters) [33]. There is a change in serotonin signalling and bioavailability in patients with IBS and therefore serotonin has become the focus of the development of new treatments for IBS. Serotonin secretion increased after meals and its reuptake decreased in diarrhoea-dominant IBS and post-infectious IBS patients. In contrast, serotonin release decreased in constipation-dominant IBS patients [33].

1.2.3.6 Psychological factors

There is a clear relationship between the severity of IBS and psychological factors such as anxiety and depression but the link between psychological factors and the development of IBS is still controversial.

Two population-based studies found that psychological distress can predict future development of IBS [38-39]. However, another study found no relationship between psychological distress and the change in abdominal symptoms in 1 year but showed a connection between chronic abdominal symptoms and frequent medical health attendance [40].
Again the relationship between physical and sexual abuse and the development of IBS is another controversial topic. Some studies indicate that women with IBS more often report different types of physical and sexual abuse compared with normal population [41-42].

On the other hand, Hobbis et al showed no relationship between the prevalence of abuse between IBS patients, Crohn’s disease patients and normal subjects, although they noticed a higher psychological distress in people with a history of abuse irrespective of their functional gut symptoms [43].

1.2.3.7 Genetic factors

Although it is known that IBS is more common in certain families [3], twin studies indicated that the environmental factors are much stronger than the genetic ones in the development of IBS [44]. There are few genetic abnormalities that can be associated with IBS, these include:

A- Serotonin-transporter polymorphisms are shown to be important for determining the subtype of IBS and its response to treatment [45].

B- Alpha 2-adrenergic receptors polymorphisms are related to the change of bowel habits and the severity of IBS symptoms [45].

C- Cytokine gene polymorphisms, such as tumour necrosis factor alpha (TNF-α) and interleukin 10 (IL-10) are also more widespread among patients suffering from IBS [46].

1.2.3.8 Hormonal factors

Hormonal changes could play a role in IBS pathogenesis since IBS is more common in women than men and IBS symptoms get worse during the menstrual cycle [47]. In addition, the visceral sensitivity in the rectum changes during menstrual cycle in IBS patients but not in healthy subjects [48].
Some studies suggest a role of gender in the perception of rectal distension and this concept is supported by an animal model which showed an effect of hormonal levels on colorectal distension sensitivity [49]. There is also a gender effect on the response of IBS patients to medical treatment.

1.2.4 Exacerbating factors

Stress and psychological factors are generally regarded as exacerbating factors rather than a cause of IBS [1]. In addition, meals can enhance the symptoms of patients with IBS especially fatty food as Simren et al have shown that administration of lipids into duodenum led to increased rectal sensitivity [50]. Antibiotic use (erythromycin) and non-steroidal anti-inflammatory drugs (NSAIDs) can also increase the severity of IBS symptoms [1].

1.2.5 Management

The management of IBS is a great challenge because of the variability of symptoms between patients and even in the same patient over time and the difference in meeting the diagnostic criteria. There are also other conditions which may overlap with the diagnosis of IBS like lactose intolerance, coeliac disease and microscopic colitis [51]. Furthermore, extra intestinal symptoms, gender and genetic variations complicate the IBS therapy.

Even though most IBS medications target specific symptoms, it is now accepted that the global symptoms and quality of life improvements are the most important end points for IBS patients [51].

1.2.5.1 Dietary management

IBS patients often feel intolerant to specific food items, such as raw vegetables, fruits, caffeinated drinks, chocolate, or sweeteners like sorbitol [1]. This is may be only due to increased symptoms of IBS patients after eating as there is no strong evidence to support a possible role of IgE mediated dietary reaction in IBS and little evidence indicating a possible connection between IBS and foods associated
with IgG antibodies [1]. Therefore, elimination of certain foods should be tailored to individual patients with strong associations with severe symptoms.

1.2.5.2 Psychological management

The rationale for psychological management is that psychological symptoms, such as depression and anxiety are widespread in IBS and related to gut symptoms as mentioned before. There are three psychological modalities, including relaxation therapies, cognitive behavioural therapy, and psychodynamic interpersonal therapy [52]. The availability of these psychological therapies and patient’s preferences dictate which one of these to choose from. Clinical trials showed clear benefits of psychological therapies in the form of behavioural therapies and psychotherapy in the management of IBS symptoms [53-55].

1.2.5.3 Hypnotherapy

In 1984, the first hypnotherapy study was conducted on patients with IBS refractory to other treatments and showed a significant improvement after 3 months compared with supportive treatment with placebo [56]. After that, accumulating evidence confirms the value of hypnotherapy in the management of IBS [57]. Additionally, early studies suggested that a home hypnosis programme might be helpful but not as good as those in programmes delivered by therapists [58]. Other advantages of hypnotherapy are that it can improve many aspects of IBS, such as psychological condition and quality of life and its helpful effects last longer and in some cases for about 5 years [59].

The mechanisms underlying the beneficial effects of hypnotherapy in IBS are not fully elaborated. However, some evidence suggests that hypnotherapy may reduce colonic contractions and normalise gut hypersensitivity in IBS patients [60]. Hypnotherapy may also work by modifying the central response of IBS patients to their pain [57] as it is reported that activation of certain cortical areas, especially the anterior cingulate cortex in response to rectal pain stimuli, is increased in patients with IBS compared with normal subjects and the reduction of somatic pain by hypnosis is also linked with a reduction in activation in the same cortical area [61].
1.2.5.4 Traditional pharmacological medications

A- Antidiarrhoeal agents like loperamide are very effective in controlling diarrhoea in IBS-D.

B- Antispasmodic agents like mebeverine are highly used for abdominal pain control.

C- Laxatives are used in the treatment of constipation in IBS-C

D- Antidepressants, such as tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI) are similar to psychological therapies and helpful only with good IBS patients' compliance.

1.2.5.5 New pharmacological medications

A- Serotonin receptor agonists (5-HT4 receptor agonists)
   Tegaserod is a partial 5-HT4 receptor agonist and showed a beneficial effect in the management of IBS-C by increasing gastric emptying, small intestine and colonic transits, as well as improving global IBS symptoms [62]. This effect has been confirmed by several large double-blind placebo controlled trials [62], [63], [64].

B- Serotonin receptor antagonist (5-HT3 receptor antagonists)
   Alosetron is a 5-HT3 receptor antagonist which induces adequate visceral pain relief and improves bowel motion, urgency, stool consistency and global IBS symptoms of IBS-D as shown in many double-blind placebo controlled trials [65], [63].

C- Probiotics
   There is a growing interest in the use of probiotic agents as a treatment for IBS. In several clinical trials, probiotic agents, such as Lactobacillus rhamnosus plantarum, Bifidobacterium infantis and VSL#3 (a mixture of lactobacilli, bifidobacteria, and a streptococcus) have shown benefit in IBS symptoms improvement especially bloating and flatulence [66], [67], [68].
1.3 Pain

1.3.1 Definition of pain

Pain has been defined as unpleasant sensory and emotional experiences related to actual or potential injury [69]. According to this definition, pain is a constantly unpleasant subjective sensation.

Chronic pain refers to pain which lasts for 3 months or longer due to progressive pathological conditions or the persistence of pain after the subsidence of the immediate effects of the damage and the passing of the expected healing time [69]. A significant behavioural response and variable degrees of disability always accompany chronic pain.

1.3.2 Peripheral pathways

1.3.2.1 Nociceptors

Nociceptors are the polymodal afferent sensory fibres which tend to respond to noxious and strong stimuli including mechanical stimuli, heat and chemical stimuli such as capsaicin. Sensory nerves are classified according to their degree of myelination and diameter into Aα, -β, -δ and C-fibres [70].

New research advances have explored the molecular mechanisms on how different types of stimuli which are encoded as neural signals, are transmitted from pain receptors to the brain. Ion channels in sensory nerves were studied to characterise their genetic structures, biophysical and molecular properties [71]. These channels can be activated by generating inward or outward currents of ions which lead to increase or decrease in sensory nerves excitability by depolarising or hyperpolarising the nerve membrane [72].

The inward current generation or outward current inhibition is responsible for depolarising the nerve terminals and the release of nociceptive signals. Voltage-activated sodium and calcium channels are the main inward membrane current, while potassium ions are the major outward channels in nociceptors. Additionally,
excitation of sensory nerves can also be done by activation of non-selective cation channels. By modulating the activity of these channels and regulating their expression, the excitability of the nerves can be controlled [72].

A- Voltage gated Na+ channels

Sodium channels are thought to be responsible for action potential by rapid depolarisation of the membrane. In addition, there is strong evidence that some Na+ channels are associated with neuropathic pain where nerves are injured partially [73].

B- Voltage-dependent Ca2+ channels

Voltage-dependent calcium channels (VDCC) sensory transduction works in response to depolarisation by increasing the concentration of intracellular Ca2+. They play a role in different cellular functions like membrane excitability, gene expression and neurotransmitter release [74]. For example, substance-P which is a neurotransmitter related to nociception, is released from sensory nerves by activation of specific types of calcium channels [74].

VDCCs are made from α1, β, α2δ, and, γ subunits [75]. The α1 subunit plays the major channel functions such as Ca2+ permeation, ion selectivity and voltage sensing by being the pore of the channel and also the essential subunit for VDCCs classification [74].

There are ten subtypes of α1 subunit and L-type is known to be required in nociception because its blockers inhibit the substance-P release by inflammation and reduce thermal and mechanical pain [76]. On the other hand, the N-type which is present in some nerves, appears to be involved in synaptic transmission in the central nervous system [77].

C- K+ channels

K+ channels lead to stabilisation of the membrane potential by hyperpolarisation through outward currents. They are hugely diverse in structure and function due to the difference in membrane characteristics of different cells [78]. In sensory nerves, K+ channels can be used to
modulate pain and suppress nociceptors by hyperpolarising their membranes [79].

D- Transient receptor potential (TRP) ion channels
TRP channels, originally called due to their role in Drosophila phototransduction, represent a large family of ion channels with different physiological functions [80]. They are part of a superfamily of voltage-gated proteins because they are composed of 6 subunits and most of TRPs are non-selective cation channels with variable permeability to Ca2+ [81].

In humans, there are 28 TRP genes, grouped into 6 families and present in various cell types, including sensory nerves [81]. TRP channels serve crucial roles as molecular sensors to detect a variety of stimuli in specific sensations, such as smell, vision, hearing, taste, thermosensation, mechanosensation and pain [82].

E- Serotonin ionotropic receptors (5-HT3R)
Serotonin or 5-HT (5-hydroxy-tryptamine) has one ionotropic receptor (5-HT3R) and various G protein-coupled metabotrophic receptors. 5HT3R is the specific subtype which is expressed in nociceptors and involves activation of nociceptors by serotonin [83]. Recently, odansetron, 5-HT3R antagonist, was shown to reduce neuropathic pain in studies performed on humans [84].

F- Acid sensing ion channels (ASIC)
ASIC channels are distributed widely in a variety of sensory nerves and are activated by extracellular acid. They are composed of two transmembrane subunits and a large extracellular cysteine-rich loop [85]. They are expected to have a role in nociception because they are activated by extracellular acid which is one of the main noxious stimuli in inflammation [72].

G- Purinergic receptor subtype X channels (P2X)
P2X channels are part of ATP-gated channel family and their genes have 7 different subtypes. P2X3 receptors are one of these subtypes and are
highly expressed in nociceptors [86]. Extracellular ATP appears to induce pain and P2X levels are increased in carrageenan induced inflammation and play an important role in neuropathic pain [86].

1.3.3 Central pathways

1.3.3.1 Spinal cord

The dorsal root ganglia near the spinal cord have the cell bodies of the first order nerve fibres which supply nociceptors [21]. Then, the first order nerves synapse with second order nerves in the dorsal horn of the spinal cord which their axons cross to the contralateral side and ascend to synapse with third order nerves. Finally, the third order nerves send fibres to the brain cortex to where conscious awareness of the nociceptive signals happens [21].

The interneurons within the spinal cord transmit nociceptive signals to the cerebral cortex in addition to modulating these signals before ascending to a number of higher cortical areas [70]. Some inputs from interneurons can lead to sensitisation of projection nerves and increase pain transmission. Other inputs can lead to inhibition of projection nerves and decrease pain transmission. The balance between excitatory and inhibitory processes acts like a gate for pain transmission [87].

1.3.3.2 Brain

From the spinal cord, sensory signals are conducted by spinothalamic, spinomesencephalic and spinoreticular pathways to the brain (Figure 1.1). The spinothalamic tract ends in medial and posterior thalamus, and then projects via thalamocortical fibres to the primary somatosensory cortex (SI) and for visceral pain mainly to the secondary somatosensory cortex (SII) [88].

Sensory discrimination of the pain experience is the basic function of the spinothalamic tract. In contrast, the spinomesencephalic and spinoreticular tracts end on the medial thalamus and then ascend via thalamocortical fibres to the anterior cingulate cortex and insula where encoding of affective, motivational and
reflective characteristics of the stimulus occur. Furthermore, the integration of sensory and motor activities takes place solely in the insula [88].

The dorsal column is well-known to conduct fine touch and vibration stimuli, has been recently suggested to be involved with visceral pain. An MRI study showed a lesion in the dorsal column at T10 led to an acute visceral pain and a great decrease in brain activation by colorectal distension in a primate model [89]. Surprisingly in the same study, a lesion in the spinothalamic tract at T10 did not show the same effects.

In addition to the ascending pathways, a number of inhibitory descending pathways are involved in the perception of normal visceral sensation. The inhibitory descending pathways originate from the opioid rich anterior cingulate cortex and travel directly or indirectly from the amygdala to periaqueductal grey area.

Then synaptic connections are made in the other midbrain areas, such as the rostral ventral medulla and locus coeruleus [90]. Finally, serotoninergic, opioidergic and noradrenergic nerves synapse with the dorsal horn nerves where they can modulate and inhibit the ascending afferent signals [90].
1.3.4 Visceral pain

The gut can tolerate a large variety of food and digest and absorb them without symptoms because of complex regulatory mechanisms that work via reflex pathways [17].

Most of the gut stimuli activate afferent nerves which relay on reflex arcs within the enteric or autonomic nervous system (sympathetic and parasympathetic) without travelling up to the cortex [17]. Though in specific circumstances, some gut stimuli activate afferent nerves which project to the cortex and lead to a conscious awareness of abdominal sensations. Functional digestive dysfunction is usually reflected as a perception of abdominal symptoms without a clear organic cause [92].
This is a very important clinical problem because a large number of the patients who attend clinical services complain of abdominal symptoms which cannot be attributed to a clear organic cause by conventional diagnostic tests. These gut conditions are termed functional gastrointestinal disorders (FGIDs) and include several syndromes, such as irritable bowel syndrome and functional dyspepsia [90]. Because the underlying pathophysiology of FGIDs is not completely established yet, diagnosis is mostly based on symptom based criteria.

Rectal and colonic hypersensitivity in IBS patients and gastric hypersensitivity in functional dyspeptic patients are thought to be a pivotal mechanism underlying their pathophysiology [14]. Additionally, various functional gynaecological, urological and thoracic disorders show increased visceral perception. This may be explained by a sensory dysfunction that makes unperceived normal physiological stimuli activate cortical pathways and produce symptoms [17, 93].

1.3.4.1 Peripheral sensitisation

Nociceptors on the peripheral nerve terminal have been characterised by new biological techniques and include sodium channels, P2X3 channels, acid sensitive ion channels and others [94]. The sensitivity of these receptors is enhanced by the release of local inflammatory mediators, such as bradykinin, cytokines, prostaglandin PGE2, and neurotrophins. These local inflammatory mediators also induce the expression of new nociceptors by blocking the local inhibitory mechanisms (Figure 1.2).

Bradykinin, for example, activates protein kinase C (PKC) and consequently increases the sensitisation of sodium and TRPV1 channels [72]. PGE2 activates G protein coupled adenylate cyclase and increases the expression of cAMP (cyclic adenosine monophosphate) which in turn leads to sensitisation of ion channels and expression of new nociceptors. Nerve growth factor (NGF) stimulates the expression of new TRPV1 channels [72].

Susceptibility to gut inflammation could also be determined by the genetic variability of secondary messengers following activation of the immune cells. G proteins, for example, are involved in signal transduction from about 80% of
membrane receptors and important in intracellular effector systems like ion channels, protein kinases, transcription factors, the phosphoinositide system and the adenylcyclases [91].

One study showed that in patients with FGID, there may be an increase in polymorphism of one specific G protein gene [95]. The defective G protein coupled signal transduction was speculated to interfere with normal immune response by reducing pathogen clearance or directly compromising local inhibitory nerves and causing visceral hypersensitivity [95].

![Fig 1.2 The molecular mechanisms of peripheral sensitisation [96].](image-url)
1.3.4.2 Central sensitisation

Central sensitisation is caused by the increased afferent nerves signals bombardment to the dorsal horn nerves of the spinal cord, which in turn due to increased presynaptic secretion of glutamate, substance P and brain derived neurotrophic factor (BDNF) and prostaglandins. These lead to activation of N-methyl-D-aspartate (NMDA), NK1 and tyrosine kinases and to an increase in intracellular calcium [97]. Subsequently, this leads to activation of calcium-dependent enzymes such as protein kinase A, protein kinase C and tyrosine kinases and phosphorylation of the NMDA receptors [97]. This significantly alters NMDA-receptor kinetics and reduces its magnesium (Mg2+) block [91] and leads to increase in the responsiveness to glutamate and enhance the synaptic strength (Figure 1.3).

The increased responsiveness of the dorsal horn nerves makes the initial injury last longer. In addition, the activation of the surrounding nerves leads to the enlargement of original injured area of the gut [91].

The NMDA receptors, a major excitatory ligand-gated ion channel in the central nervous system, are normally blocked by Mg2+ ions on their channel pores. The activation of the NMDA receptors depends on both, the binding of its natural ligand (glutamate) and the depolarisation which will result in the removal of Mg ions from the channel pores [98].

One study showed that NMDA antagonist could reverse the acid induced gut hypersensitivity and this indicates a possible role of the NMDA receptors in human visceral hypersensitivity mediated by central sensitisation [98].
Fig 1.3 The molecular mechanisms of central sensitisation [97].

1.4 Anorectum

After discussing the pain matrix in the previous section, this section will focus on the anatomy of the human anorectal area and the available methods to assess its sensory and motor functions.

Anal canal is about 4-4.5 cm long and extends from anal verge to the rectum as demonstrated in figure 1.4. Its upper 10 mm is lined by columnar epithelium (like the rectum), then the next 15 mm is lined by stratified, modified columnar epithelium and the next lower 10 mm is lined by stratified epithelium. Finally the most distal 5-10 mm of anal canal is covered by hairy skin.

Anal sphincter consists of two types of muscles i.e. internal anal sphincter (IAS) and external anal sphincter (EAS). Both work in harmony to constrict the anus.
IAS is composed of smooth muscle and works involuntarily and mainly at rest, while EAS is composed of striated muscle and works voluntarily during squeezing process.

IAS is about 4 cm long and located in the upper part of the anal canal and runs in a circular fashion. It is innervated by autonomic nerves i.e. the sympathetic thoracolumbar and parasympathetic sacral pathways.

EAS encircles mainly the distal part of the anal canal and is innervated predominantly by the pudendal nerve which originates from the second, third and fourth sacral nerve roots (S2, S3 and S4) [99].
The rectum extends from the rectosigmoid junction proximally at the level of third sacral vertebra to the anal canal distally and is lined by columnar epithelium (Figure 1.2). The rectum can be divided into two parts separated by a horizontal fold; lower rectum, which is derived embryologically from cloaca, is surrounded with thick extra peritoneal connective tissue. In healthy subjects, lower rectum is generally empty except during defecation. The upper rectum, on the other hand, is derived from hind-gut and contains faeces generally and can extend towards the peritoneal cavity.

The rectum is surrounded by smooth muscle and innervated by sympathetic and parasympathetic pathways similar to IAS. In humans, rectum has less enteric ganglia compared with colon, whereas anal canal contains only few enteric ganglia [100].

1.4.1 Evaluation of anorectal sensation

Thorough assessment of anorectal sensation is a complex process owing to the mixture of nerves that supply the anorectum (somatosensory pudendal nerve to the anus and visceral afferents to the rectum) and because of the use of mainly artificial stimuli to resemble normal physiological processes in the anorectum to generate conscious sensation by activating certain sensory afferent pathways [101]. Moreover, the anorectal sensory measurements rely mainly on the subjects’ own accuracy and reliability to report and describe their feelings in a descriptive way.

Different techniques have been applied to produce visceral sensation; these contain the following: electrical stimulation, rapid balloon distension, barostat, manual balloon inflation and thermal stimulation.

A- Electrical stimulation

Electrical stimulation can be used to evaluate anorectal sensation by introducing a dual electrode stimulating catheter and positioned in the rectum and anal canal. The catheter is placed such that the two electrode pairs are positioned 1 cm and 10 cm from the anal verge. Sensory and pain thresholds are then determined by
using electrical stimulation as the amount of electricity that cause the subjects to first feel a sensation in the anus and rectum and that required to feel pain [101]. The subjects score the sensation intensity by using pain categorical rating scales. In addition they describe the experienced pain by using a short form of the McGill Pain Questionnaire [102].

**B- Rapid balloon distension**

Rapid balloon distension can be used to evaluate anorectal sensation by introducing a tube with a balloon attached at one end. The tube is connected to a specially designed inflator device which is capable of rapidly distending the balloon by enabling pressurised air to be pumped rapidly into the balloon catheter at a preset rate and pressure [101].

Similar to the electrical stimulation technique, the amount of air required to cause the subjects to first feel a sensation in the rectum and that required to feel pain are recorded as sensory and pain thresholds. Following the delivery of random balloon inflations, the subjects score the sensations experienced using pain categorical rating scales and McGill Pain Questionnaire.

**C- Rectal barostat**

Rectal barostat has been used for the assessment of rectal sensation in healthy volunteers and patients with IBS [103]. Barostat is used by inserting a rectal barostat catheter attached to a balloon (length, 22 cm; diameter, 15 cm; capacity 600 ml) into the rectum and the balloon is usually positioned 10 cm from the anal verge [104].

A conditioning distension of the rectum is then performed by transiently inflating the balloon with 150 ml air and then deflating it completely and the subjects are allowed to rest for 20 minutes.

After that, the catheter is connected to a barostat and the pressure in the balloon is increased by 1 mmHg for 1 minute per step until respiratory excursions will be observed.
Then measuring the baseline operating pressure (BOP) which is defined as 2 mmHg above the minimal distension pressure at which respiratory excursions are clearly recorded from the barostat tracing, if respiratory variation is not obvious, set at 12 mmHg. This pressure is then maintained for 15 minutes to give a measure of rectal tone.

Rectal compliance and sensory thresholds are measured by ramp inflation, starting at 0 mmHg and increasing in steps of 4 mmHg for 1 minute per step to a maximum of 60 mmHg. Thresholds for first sensation, urgency, discomfort and pain are indicated by the subjects and inflation is terminated as soon as the subjects reported the first sensation of pain.

**1.4.2 Evaluation of anorectal motor excitabilities**

The cortical topography of human anorectum muscles were studied by Turnbull and his colleagues [105] by applying figure of 8 coil to generate single-pulse TMS over a 12 X 8 cm grid, with rows of points 1 cm apart mediolaterally, and 2 cm apart anteroposteriorly was superimposed onto a transparent plastic sheet and attached to a surgical hood that was securely fastened to the head of each subject. An anal plug and a rectal catheter were used to record anal and rectal EMG, respectively from 9 healthy volunteers.

They found that the anorectal cortical representation is located at the most medial aspect of the superior motor cortex, adjacent to the interhemispheric fissure (Figure 1.5). They also found that there was no significant difference between anal and rectal EMG latencies and a subtle difference between anal and rectal EMG amplitudes.
In contrast to the anorectal sensory evaluation, there is limited information regarding the evaluation of anorectal descending motor pathway. A novel approach is to examine the motor evoked potentials (MEPs) of the anorectum following single-pulse transcranial magnetic stimulation (TMS).

Recently, Remes-Troche and his team [106] studied bi-directional brain-anorectal axis on 26 healthy subjects by using motor evoked potentials (MEP) for the efferent pathway following transcranial magnetic stimulation (TMS) over paramedian motor cortices bilaterally and cortical evoked potentials (CEP) for the afferent pathway following electrical stimulation of anus and rectum. They found that both methods showed no gender differences and their reproducibility were excellent and concluded that both methods are simple, economical and valid for evaluating brain-anorectal axis.
1.5 Magnetic stimulation

1.5.1 Plasticity

The human nervous system has the ability to reorganise and adapt to the changes in the surrounding environment, as well as to conditioning stimuli and pathological lesions. The term plasticity refers to any lasting changes in functional or morphological characteristics of the brain cortex and can be studied in two levels, including: 1) body representation level as changes in cortical representation of body parts and 2) neuronal level as changes in the efficacy of the existing synapses and the generation of new ones. Cortical maps are dynamic and can be modulated by central or peripheral changes of inputs and in reaction to behavioural experiences [107].

Cortical plasticity can occur at the level of sensory or motor representation of body regions [107]. Plasticity at the sensory representation can be expressed as alteration in cortical receptive areas. The loss of afferent input can lead to rearrangement of the cortical representation of the skin, cornea and retina. The rearrangement of cortical regions is also in the somatosensory system after peripheral nerve injury and repair, limb amputation and reconnection. Furthermore, plasticity at the motor representation indicates the changes in the cortical motor output maps and can happen after amputation, stroke, and special repetitive motor exercise tasks [108].

1.5.1.1 Hebbian theory

Hebbian theory which is introduced by Donald Hebb in 1949 tried to explain the mechanisms behind the neuroplasticity. It proposes that synaptic strengthening occurs as a result of temporal correlation of pre and postsynaptic activity, when there is lack of such correlation synapses weaken. At the synaptic level, the synaptic strength is increased between neurons that fire together [109].
Neuroplasticity can be shown in various ways:

A- Modification of the efficacy of existing synapses by activity-dependent modification that includes long-term potentiating (LTP) and long-term depression (LTD), and when the synaptic activity does not have the specificity of LTP and LTD, it leads to generalised changes in postsynaptic excitability [110].

B- Morphological changes associated with plasticity, such as dendritic branching, generation of new synaptic contacts or larger number of axons collaterals of the horizontal pathways.

1.5.1.2 Molecular mechanisms in neuronal plasticity

The molecular mechanisms in neuronal plasticity include up-regulation and down-regulation of specific genes and modulation of neurotransmitters and growth factors.

NMDA (N-methyl D-aspartate) receptors have an important role in LTP and LTD by causing some forms of long term synaptic changes. NMDA receptors activate Ca2+-dependent protein kinases such as Ca2+/calmodulin-dependent kinase II (CaMK II) by allowing Ca2+ influx [111]. In animal models, the knock-out of α subunit Ca2+/calmodulin-dependent kinase II mice do not show cortical plasticity [111].

GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter, while glutamate is the main excitatory neurotransmitter in the human brain [108]. Regulation of these two neurotransmitters i.e. GABA and glutamate, in the cerebral cortex may play a critical role in the re-organisational process in both experimentally induced plasticity and after focal cortical injury [111]. BDNF (brain-derived neurotrophic factor), neurotrophin-4/5, and neurotrophin-3 are trophic factors which thought to be involved in cortical plasticity. For example, BDNF which is produced by neocortex, plays an important role in plasticity by increasing the chemical and morphological differentiation of neurons containing GABA [111].
1.5.2 Nerve stimulation

1.5.2.1 Electrical stimulation

Muscles and nerves have been known to be stimulated by external electrical current since Galvani and Volta’s works in 1790s [112]. Electrical stimulation can be effective by using surface electrode or through needle reaching near the target organ but cannot reach deep nerves from the surface. The charge goes into the target cell membrane and leading to a change in the membrane potential, causing a depolarisation of the membrane and starting of an action potential, which spreads by the nerve conduction ways [113].

1.5.2.2 Magnetic stimulation

Magnetism is a phenomenon associated with the motion of electric charges. The basis for magnetism relates to the presence of electro-magnetic fields and their effects on matter. The magnetic field can be seen as the magnetic force deflecting the particles without changing their speed and it is measured in units of tesla (T) [113]. The magnetic force is similar to electric force in strength and direction, however, the magnetic force has north and south poles, instead of positive and negative charge and is always found in pairs.

The concept of electromagnetism was discovered in 1831 by Michael Faraday in showing that a changing electric field can produce a magnetic field and a changing magnetic field can also produce an electric current [114] (Figure 1.6). In contrast to electrical stimulation, the magnetic current flows deep into the tissue by the use of a pulse of magnetic field. In 1985, non-invasive transcranial magnetic stimulation was demonstrated for the first time at the Royal Hallamshire Hospital and the University of Sheffield by Barker and colleagues [114] (Figures 1.7 to 1.9). Thereafter, its use as a clinical tool spread worldwide.
Fig 1.6 Michael Faraday discovered electromagnetic induction in 1831.

Fig 1.7 In 1985, transcranial magnetic stimulation (TMS) was demonstrated for the first time at the University of Sheffield by Barker and his colleagues.
Fig 1.8 Transcranial magnetic stimulation (TMS).

Fig 1.9 The depth of rTMS penetration.
A- Advantages of magnetic stimulation

Magnetic stimulation has many advantages over electrical stimulation. These comprise the ability of magnetic stimulation to stimulate human cerebral cortex, spinal roots, and cranial and peripheral nerves without introducing needles or causing pain and therefore can be used as a routine technique on patients and healthy volunteers [113]. The reason behind the lack of pain is due to the deep penetration of magnetic pulses without attenuation and no current passage over the skin [113].

B- Disadvantages of magnetic stimulation

There are only 3 disadvantages of magnetic stimulation compared with electrical stimulation. First, magnetic stimulators are very expensive and large. Second, it is difficult to attain a high rate compared to electrical stimulus. Third, there is a less well demarked site of stimulation. These setbacks are now minimised by new advances in engineering design [113].

1.5.2.3 The magnetic simulation equipment and process

The magnetic stimulators usually are made of capacitor discharge systems connected with switching elements and a coil [113] (Figures 1.10 to 1.14). The intensity of the magnetic field is associated with the current flowing around the coil and the number of turns of the wire within the coil and its dimensions. The magnetic effects depend on the strength of the electric field induced within the brain by the stimulator. The intensity of the magnetic stimuli decreases by increasing the distance from the coil.

Magnetic stimulation is administered by placing a stimulating coil upon the target area (Figure 1.10). The coil then generates a magnetic field in the direction parallel to the central axis of the coil that can pass through bones and tissues without interruption and therefore can stimulate sites beyond these structures. The magnetic intensity required to induce motor movement by cortical stimulation is called motor threshold (MT) and it is variable from one individual to another [114].
The nervous tissue activity can be activated or inhibited depending on the frequency, intensity, direction and the duration of the magnetic stimulation. Fast magnetic stimulation (>1 Hz) is generally excitatory and on the other hand, slow (≤1 Hz) is inhibitory [115]. Magnetic stimulation is termed single-pulsed when the coil is discharged once which used for neurodiagnosis and repetitive when the coil is discharged in trains several times per second and usually applied in clinical research and therapeutic functions [113].

![Figure 1.10](image1.jpg)

Fig 1.10 Figure of 8 coils (Source: Magstim Ltd).

![Figure 1.11](image2.jpg)

Fig 1.11 Single-pulse TMS (Source: Magstim Ltd).
Fig 1.12 Repetitive TMS (Source: Magstim Ltd).

Fig 1.13 Applying transcranial magnetic stimulation (TMS).
Fig 1.14 Applying lumbosacral magnetic stimulation (LSMS).

1.5.2.4 Safety of magnetic stimulation

Single-pulse TMS, according to the literature, is very safe and a useful technique in neurophysiological investigations in both healthy subjects and patients. Similarly, repetitive TMS (rTMS) appears to be safe in general and well tolerated. The long term risks of both single pulse and repetitive TMS in adults are not significant according to most of the available data [116].

Low rate repetitive TMS is safe and has been used by thousands of healthy subjects and patients with few side effects. However, a rapid rate repetitive TMS should be applied with caution because at intensities close to motor threshold has been reported to cause seizures without long lasting consequences [117]. Adverse effects related to the rTMS use can be reduced or avoided by choosing carefully the stimulation frequencies, duration and amplitudes [116].

1.5.2.5 Precautions

The international society for transcranial stimulation consensus on rTMS [118] recommended that:

A- Healthy volunteers and patients with family history and past medical history of seizures must be excluded from magnetic stimulation especially rTMS.
B- Patients and healthy subjects must be always informed about the possible side effects and assessed for risk factors.

C- Informed consent forms must be obtained.

D- Magnetic stimulation especially rTMS should always be applied under the supervision of trained doctors and in areas close to medical facilities.

E- Risk of scalp burning must be avoided by using appropriate procedures like covering the head of patients and healthy subjects.

F- During rTMS stimulation, the spread of excitability should warn the investigators about the risk of development of seizures and stimulation must be stopped.

G- Subjects and patients must be monitored during and after rTMS, and

H- Wearing earplugs when rTMS frequencies $> 1$Hz.

1.5.2.6 Therapeutic applications of magnetic stimulation

Magnetic stimulation as a non-invasive technique has been used in many clinical conditions as diagnostic as well as therapeutic methods.

A- Peripheral nerves

Peripheral nerves can be stimulated by magnetic stimulation with patient’s ease and comfort in contrast to traditional electrical stimulation [119]. Single-pulse magnetic stimulation can be used for diagnosis and monitoring of peripheral nerve disorders like monitoring respiratory muscle strength in pulmonary diseases [120]. In addition, repetitive magnetic stimulation can be used as a treatment modality like in peripheral pain management [121].

B- Cranial nerves

Magnetic stimulation has a role in assessing facial nerve function both transcranial and extracranial [122]. Trigeminal, hypoglossal and accessory nerves are possible to be assessed by central or peripheral magnetic
stimulation but difficult to evaluate the results because of cross muscle contamination[123].

C- Spinal nerve roots
Magnetic stimulation is a safe and painless method to measure spinal nerve roots’ conduction times to the target muscles and lead to the diagnosis of any lesion.

It is also used successfully over the lumbosacral area for the management of faecal incontinence [124].

D- Coma
TMS shows valuable diagnostic and prognostic results in the early assessment of patients in coma [125].

E- Motor neurone disease
TMS is the most sensitive method for evaluation and monitoring the electrophysiological changes in motor neuron disease, especially respiratory muscle involvement [126].

F- Parkinson’s disease
TMS is proving to be an important tool for the assessment of pathophysiological changes in Parkinson’s disease and rTMS may play a role as a possible therapeutic method as well [127].

G- Multiple sclerosis
Magnetic stimulation methods made possible the measurement of motor disability in patients with multiple sclerosis and therefore they are very useful in regular monitoring of patients, assessing the therapeutic effectiveness and predicting a relapse [128]. Moreover, there is a role of rTMS in therapeutic intervention for the management of spasticity in patients with multiple sclerosis [129].
H- Stroke
Magnetic stimulation can be used to try to compensate for the loss of brain function after a stroke. Cortical reorganisation and brain recovery has been enhanced by applying neurorehabilitation programmes such as rTMS [130].

Dysphagia is a common problem after stroke and rTMS in one study has shown promising results in improving the swallowing and preventing aspiration in patients with dysphagia [115].

I- Depression
Several studies using high frequency rTMS showed an efficacy in the management of depression with various degree of success [131].

J- Schizophrenia
Few studies have been conducted to evaluate the effect of magnetic stimulation in the management of schizophrenia in contrast to depression. However, these studies showed an improvement of symptoms of patients with schizophrenia following the application of rTMS [131].

1.5.2.7 Cortical magnetic stimulation

A- Cortical magnetic stimulation and chronic pain
Cortical stimulation has been used to treat pain for a while but its development in this respect was slow compared with its other usages. From 1990s the direct surgical implanted electrodes stimulation of the deep brain of the thalamus and periventricular grey region was the only method used for the treatment of the intractable pain [132]. Its application was very limited because of its high risk and cost.

Later on, a non-invasive technique called epidural motor cortical stimulation was invented for the treatment of chronic pain [132]. Its utilization not only decreased the risk of surgical intervention but also opened a new era for the use of non-invasive techniques for cortical stimulation.
Transcranial magnetic stimulation (TMS) was developed in 1985 [113]. It is a non-invasive, painless and well tolerated technique used to stimulate the human motor cortex. It works by discharging a capacitance through a figure of eight copper wire stimulating coil, when placed directly to the scalp over the area of brain cortical area intended for study. Repetitive TMS (rTMS) has been used widely in human studies using the pharynx and oesophagus [133]. Furthermore, depending on the frequency of stimulation, both excitation and inhibition of the excitability of the corticobulbar projections to the visceral structures can be induced. For example, equal or less than 1 Hz produced inhibition and more than 5 Hz produced excitation [115].

There is however a limited number of human studies that having attempted to use TMS or magnetic stimulation to the spinal cord in order to modulate chronic pain (summarised in table 1). Most studies used rTMS to modulate pain mainly on primary motor cortex with very promising results in patients with neuropathic pain and fibromyalgia [132]. Its pain reduction effects are likely mediated via the activation of horizontal fibres in the superficial layers of primary motor cortex [134]. Its only limitation to clinical use is the short duration of action which last for few hours compared to transcranial direct current stimulation (tDCS) effects which last for weeks [132]. This obstacle can be overcome by applying rTMS every day for several weeks [132].

Lefaucheur et al were the first group to apply rTMS on patients with chronic pain. They did a placebo controlled trial involving 18 patients with neurogenic pain and demonstrated that 10 Hz rTMS over the motor cortex led to a significant pain relief compared with sham as assessed by visual analogue scale [135]. The same group did a similar placebo controlled trial 3 years later with a larger sample size of 60 patients with intractable central pain and showed the same results [136]. Another group demonstrated that multiple consecutive sessions of 20 Hz rTMS were associated with a significant pain relief as assessed by visual analogue scale [137].

Fregni et al did a pilot crossover, sham-controlled study in 5 patients with chronic visceral pain due to pancreatitis. They applied six sessions of
rTMS, with different parameters of stimulation on right and left secondary somatosensory cortex stimulation (SII) with 1 Hz, 20 Hz, and sham. They demonstrated that 1 Hz rTMS stimulation of left and right secondary somatosensory cortex led to a significant pain reduction (36% and 31%, respectively) as assessed by pain and medication reduction. However, 20 Hz rTMS stimulation on left secondary somatosensory cortex led to an increase in pain [138]. Saitoh et al showed that 10 Hz rTMS stimulation of precentral gyrus has a better effect than 5 Hz in pain reduction in patients with spinal cord or peripheral lesion [139]. In the same study 1 Hz stimulation did not show any effect.
### Table 1.1 Neurostimulation and pain studies.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Technique</th>
<th>Target, origin and intensity of stimulation</th>
<th>Study design</th>
<th>Number</th>
<th>Cause of pain</th>
<th>Percentage of pain relief</th>
</tr>
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<tbody>
<tr>
<td>Lefaucheur et al [135]</td>
<td>2001</td>
<td>rTMS</td>
<td>M1, 80% MT, 0.5/10 Hz, 1000 pulses</td>
<td>Cross-over</td>
<td>14</td>
<td>Trigeminal neuralgia, thalamic stroke</td>
<td>10Hz-rTMS: 20%</td>
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<td></td>
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<td>(sham: 4-7%), P = 0.001</td>
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<tr>
<td>Lefaucheur et al [140]</td>
<td>2001</td>
<td>rTMS</td>
<td>M1, 80% MT, 10 Hz, 1000 pulses</td>
<td>Cross-over</td>
<td>18</td>
<td>Thalamic stroke, brainstem lesion, brachial plexus lesion</td>
<td>10Hz-rTMS: 30%</td>
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<td>(sham: 0%), P = 0.01</td>
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<tr>
<td>Rollnik et al [141]</td>
<td>2002</td>
<td>rTMS</td>
<td>M1, 80% RMT, 20 Hz, 800 pulses</td>
<td>Cross-over</td>
<td>12</td>
<td>Spinal cord lesion, osteomyelitis, peripheral nerve lesion, complex regional pain syndrome, and phantom limb pain</td>
<td>20Hz-rTMS: 4%</td>
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<td>(sham: 2%), P &gt; 0.05</td>
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<tr>
<td>Lefaucheur et al [136]</td>
<td>2004</td>
<td>rTMS</td>
<td>M1, 80% MT, 10Hz 1000 pulses</td>
<td>Cross-over</td>
<td>60</td>
<td>Thalamic stroke, brainstem lesion, brachial plexus lesion, spinal cord lesion, trigeminal nerve lesion</td>
<td>10Hz-rTMS: 21%</td>
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<td></td>
<td>(sham: 9%), P = 0.0002</td>
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<tr>
<td>Pleger et al [142]</td>
<td>2004</td>
<td>rTMS</td>
<td>M1, 110% RMT, 10Hz 120 pulses</td>
<td>Cross-over</td>
<td>10</td>
<td>Minor trauma, radial fracture, luxation of 2nd and 3rd fingers, fracture of navicular</td>
<td>10Hz-rTMS: 21%</td>
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<td></td>
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<td>(sham: pain increase); P = 0.02</td>
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<tr>
<td>Fregni et al [138]</td>
<td>2005</td>
<td>rTMS</td>
<td>Right/left S2, 90% RMT, 1/20 Hz, 1600 pulses</td>
<td>Cross-over</td>
<td>5</td>
<td>Chronic pancreatitis (visceral pain)</td>
<td>1Hz-rTMS: 62%</td>
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<td>(S2: 54%), P = 0.037</td>
</tr>
<tr>
<td>Khedr et al [137]</td>
<td>2005</td>
<td>rTMS</td>
<td>M1, 80% RMT, 20 Hz, 2000 pulses</td>
<td>Parallel</td>
<td>48</td>
<td>Trigeminal neuralgia, post-stroke</td>
<td>20Hz-rTMS: 45%</td>
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<td>(sham: 5%), P &lt; 0.001</td>
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<tr>
<td>Lefaucheur et al [143]</td>
<td>2006</td>
<td>rTMS</td>
<td>M1, 90% MT, 1/10 Hz, 1200 pulses</td>
<td>Cross-over</td>
<td>22</td>
<td>Thalamic stroke, brainstem lesion, brachial plexus lesion, spinal cord lesion</td>
<td>10Hz-rTMS: 33%</td>
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<td>(sham: 11%), P = 0.002</td>
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<tr>
<td>Lefaucheur et al [144]</td>
<td>2006</td>
<td>rTMS</td>
<td>M1, 90% MT, 10 Hz, 2000 pulses</td>
<td>Cross-over</td>
<td>36</td>
<td>Thalamic, brainstem stroke, trigeminal nerve, brachial plexus, nerve trunk and spinal cord lesion</td>
<td>10Hz-rTMS, best cortical target (face/hand pain): 27/37%</td>
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<td>(M1): 28%, P &lt;</td>
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<tr>
<td>Hirayama et al [145]</td>
<td>2006</td>
<td>rTMS</td>
<td>M1/S1/PMC/SMA, 90% MT, 5 Hz, 500 pulses</td>
<td>Cross-over</td>
<td>20</td>
<td>Post-stroke, spinal cord lesion, trigeminal</td>
<td>5Hz-rTMS, best cortical target (M1): 28%</td>
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<td>(M1): 28% P &lt;</td>
</tr>
</tbody>
</table>
DLPFC: dorsolateral prefrontal cortex; LPC: left prefrontal cortex; MI: primary motor cortex; PMC: premotor cortex; PPC: posterior parietal cortex; MT: motor threshold; rTMS: repetitive transcranial magnetic stimulation; SI: primary sensory cortex; SII: secondary sensory cortex; S3 and S4: sacral foramina 3 and 4; SMA: supplementary motor area; SNS: sacral nerve stimulation; SO: stimulator output; tDCS: transcranial direct current stimulation.

### B- Motor cortex and pain

Recently, many studies have shown that stimulating primary motor cortex with high frequency rTMS results in pain reduction in people who suffer from both peripheral and central pain [137] [152]. The exact mechanism behind that is still unknown but may be due to a direct effect on the primary motor cortex or more likely by an indirect effect through transsynaptic connections to the pain perception and modulating systems [153] i.e. anterior cingulate, insula and secondary sensory cortex areas as shown by neuroimaging studies [154] and the extensive connection between the motor cortex and thalamic nuclei [155]. In healthy volunteers, there were similar results as the application of both low and high frequency rTMS for
short duration has shown to reduce acutely induced pain by different forms for several minutes.

Yoo et al [156] have demonstrated that high frequency rTMS (10 Hz, total 900 stimuli) applied over the primary motor cortex area resulted in a significant increase in pain tolerance threshold of the right thumb of 8 healthy volunteers induced by electrical current compared with sham and in the contrary, 10 Hz rTMS (total 900 stimuli) applied over the medial frontal cortex made a significant reduction of pain tolerance threshold of another 8 healthy subjects compared with sham.

In addition, Summers et al [157] have demonstrated that low (1 Hz) and high (20 Hz) frequency rTMS (total 500 stimuli) significantly decreased cold sensation threshold compared with sham. However, cold pain threshold was only reduced significantly by 20 Hz rTMS compared with 1 Hz rTMS and sham in 40 healthy volunteers. Furthermore, Tamura et al [158] have reported that 1 Hz (total 300 stimuli) stimulation of the motor cortex has resulted in a significant relief of acute pain induced by injection of capsaicin intradermally compared with sham in seven healthy volunteers associated with functional changes in caudal anterior cingulate cortex and medial prefrontal cortex.

This concept has led to the development of implanted motor cortex stimulation (MCS) technique to effectively treat different chronic pain syndromes by the use of electrical stimulation of motor cortex via surgically implant electrodes [159].

1.5.2.8 Peripheral stimulation

A- Lumbosacral stimulation

Non-invasive MS over lumbosacral area is still under researched area though few studies tried successfully to use it to modulate anal sphincter in healthy volunteers and pelvic muscles in patients suffering from bladder dysfunction.
In animal models study, Lin et al applied a short course of magnetic stimulation over the rat's lumbosacral spine and that led to a long and very significant pain reduction effect (80–90%) to both mechanical and heat stimuli [160].

Harris and colleagues [124] have studied eight healthy volunteers and showed that 15 Hz rLSMS (with total of 2250 pulses) increased anal sphincter cortical EMG response amplitude in the first hour post intervention and on the other hand, 5 Hz rLSMS (with total of 2250 pulses) and sham did not show any change. Also in the same study there were no changes in anal sphincter latency after 5, 15 Hz rLSMS or sham. Hand and leg muscle excitabilities were not changed after those interventions.

In patients with intractable neurogenic urinary bladder dysfunction secondary to lumbosacral nerve injuries, Khedr et al [161] have used 15 Hz rLSMS (with total of 1500 pulses per session for 10 sessions per patient) and resulted in a significant reduction in the mean number of voids and incontinence per 24 hours compared to the sham. This positive effect was maintained up to one month after the last treatment session. Patients also reported an improvement of their visual analogue scale (VAS) to assess lower back pain and showed an improvement of posterior tibial nerve F-wave and H-reflexes after receiving the real treatment compared with sham.

B- Sacral nerve stimulation

Sacral nerve stimulation (SNS) is a surgical technique, applied with minimally invasive way. It uses chronic low level electrical stimulation of the sacral nerve roots to produce a clinically useful physiological effect on the organs innervated by sacral plexus nerves. Therefore, SNS can alter anal sphincter function, pelvic floor, colonic motility and afferent sensation and urinary bladder. This is achieved in two phases: a first brief test stage to evaluate symptomatic response and then insertion of a permanent implant in patients considered to have adequate response.

The exact mechanism of action of SNS remains unclear, though it appears to work by affecting multiple sacral plexus nerves including efferent nerves
to the pelvic floor musculature and somatic pudendal nerves. Contrary to the non-invasive rLSMS, SNS has been frequently used to try to improve anal sphincter’s function in patients with faecal incontinence and symptoms in patients with IBS, constipation, and urinary bladder dysfunction.

- **SNS and pain**
  Sacral nerve stimulation (SNS) in one study demonstrated a considerable effect in neuropathic pain reduction and showed at same time a significant modulation of cortical excitability. The group concluded that spinal cord stimulation mediates its clinical effect at supraspinal levels and at least cortical in origin to a certain extent [166].

  SNS has also been used to try to treat pelvic pain syndromes including functional anorectal pain disorders. In one centre [167], nine patients with functional anorectal pain disorder due to various causes and not responding to conventional treatments were tested with temporary SNS. There were four respondents who underwent permanent SNS. All four patients showed a long lasting pain improvement up to 24 months during the follow ups.

  In a relatively larger series [168], 11 patients with chronic anal and perianal pain syndromes with poor response to other treatments were treated with SNS and showed a significant improvement in their visual analogue pain scores and quality of life questionnaire (SF-36) after 15 months of average follow up and this was not associated with any manometric changes.

- **SNS in IBS**
  Temporary SNS has been used to treat six patients with diarrhoea-predominant IBS. It has provided a significant reduction in diarrhoea-predominant irritable bowel symptoms including a substantial relief in diarrhoea, bloating and pain. SNS also has improved the quality of life of the treated IBS patients [151].
• SNS in constipation
SNS has been shown to increase colonic motility and therefore has been used to treat intractable constipation.

In one study [169], SNS at 14 Hz was used to treat eight patients with slow transit constipation. In the trial period, SNS at the level of S3 caused a significant increase in pan-colonic antegrade propagating sequence. Moreover, following three weeks of continuous stimulation, 6 out of 8 patients reported an increase in their bowel habit and a reduction in their laxative consumption.

In a larger prospective study at 5 European centres [170], 62 patients with intractable constipation underwent a temporary SNS stimulation and 45 patients who achieved more than 50% of symptoms improvements had permanent SNS implantation. After about 28 months, patients reported a significant improvement of defecation frequency, time spent toileting, straining, perception of incomplete evacuation, successful evacuations, abdominal pain, bloating and quality of life. In patients with slow baseline colonic transit, SNS normalised the colonic transit of half of them.

• SNS in faecal incontinence
SNS has been used extensively to treat patients with faecal incontinence with good results in many medical centres.

Sheldon and colleagues [163] have treated 10 intractable faecal incontinence patients with temporary SNS (15 Hz, pulse width 210 µs) and showed a significant improvement of global faecal incontinence symptom scores associated with a reversible reduction in corticoanal excitability. This indicates that SNS may exert its effects partly through a dynamic brain changes.

In another study, Kenefick et al [171] have used SNS to treat 19 patients with resistant faecal incontinence for 6 years and 4 patients with resistant idiopathic constipation for 8 years and more. All faecal
incontinence patients showed improved continence at 24 months and 14 patients became fully continent. There were also statistically significant improvements in quality of life, urgency, anal squeeze pressure and rectal sensation. In addition, all constipated patients had their symptoms improved with 3 fully improved at 8 months of stimulation. Bowel frequency increased by 6 to 28 evacuations per 3 weeks with quality of life and symptoms score improvements.

In a randomised, controlled trial [172] involving 120 patients with severe faecal incontinence, 60 SNS treated patients showed significant improvement in faecal incontinence quality of life index compared with the other 60 patients who treated with optimal medical therapy.

Furthermore, Chan and colleagues [173] compared the effect of SNS on 21 faecal incontinence patients with external anal sphincter defect compared with 21 faecal incontinence patients with intact sphincter. Both groups showed significant improvement in functional outcomes and there was no significant difference between the two groups. SNS effect was successful even with the presence of pudendal nerve neuropathy.

Finally, a large study of 200 faecal incontinence patients who underwent SNS has found that the severity score was significantly reduced during stimulation [174]. It has identified that low stimulation intensity and loose stool consistency are predictive factors for a favourable SNS short term outcome.

- **SNS in urinary bladder dysfunction**

  There is promising results in the use of SNS to manage variable urinary bladder disorders.

  Lavano and colleagues [175] have used SNS to treat 6 patients with neurogenic bladder and 3 patients with urinary retention via an implanted neuroprosthesis to chronically stimulate S3. They have
successfully treated 2 patients with neurogenic bladder and 1 patient had an 80% control after the procedure. Moreover, 2 out of 3 patients of urinary retention obtained complete recovery.

SNS is also used to treat interstitial cystitis with good results as shown by Peters [176] who treated 22 refractory interstitial cystitis patients with a permanent implantation. After about 6 months of follow up, patients showed 56% overall improvement in symptoms with significant improvements in the number of day voids (44%) and nocturia (55%).

C- Pudendal nerve stimulation

Pudendal nerve electrical stimulation is also used in healthy volunteers and patients to try to modulate anal sphincter and pelvic organs.

Hamdy and colleagues [177] studied the effect of non-invasive temporary short term pudendal nerve stimulation (by positioning a pair of electrodes on a glove over the index finger through the rectum to the ischial spine adjacent to the pudendal nerve) and non-invasive lumbosacral magnetic stimulation on 11 healthy subjects and compared the effects of 2 methods on the non-invasive anorectal motor cortex stimulation response. They observed that both lumbosacral and pudendal nerve stimulation facilitate the corticoanal responses at interval of 5-20 ms and shortened the latency of the corticoanal responses. However, lumbosacral but not pudendal nerve stimulation provoked a second, delayed increase in the corticoanal response amplitudes at interval of 50–100 ms without further effect on latency and they concluded that the lumbosacral magnetic stimulus is more diffuse and stimulates a greater area of neural tissue and excites more pelvic afferents than the pudendal stimulus.

Spinelli et al [178] have used a minimally invasive technique to stimulate pudendal nerve in 12 patients with neurogenic bladder dysfunction who did not respond to SNS and resulted in a significance reduction in their incontinence.
Additionally, another team [179] have used minimally invasive pudendal nerve stimulation method as a proof of concept to treat 2 patients with refractory faecal incontinence and showed a very promising results.

However, the main limitations in the peripheral stimulation studies, and in particular SNS, are the fact that these were non-blinded both with respect to investigators or patients and there was no control/sham. Some of these studies have been performed in a small number of patients and the effects of SNS were evaluated based on self-reported clinical improvement by the patients.

1.6 Summary

This introduction has provided some background on irritable bowel syndrome, the pain matrix, and magnetic stimulation and its role in the management of chronic pain. Modulating visceral pain in healthy volunteers and IBS patients is an important research area.

Non-invasive magnetic stimulation may play an important role in this respect, since it is recognised that sensory and motor cortical regions of the brain are activated during artificial stimulation of the anorectum [180]. The application of magnetic stimulation to the cortical and lumbosacral areas might alter the perception of sensation following painful stimulation of the anorectum.

1.7 Aims

The purpose of the study is to ascertain whether non-invasive repetitive magnetic stimulation applied to the motor cortex and lumbosacrum can modulate gastrointestinal pain originating from the anorectum in healthy volunteers and IBS patients?

The protocols have been chosen to answer the following questions:

**Question 1**
Does repetitive magnetic stimulation over the lumbosacrum and the anorectal motor cortex modulate anal sphincter motor excitability in healthy volunteers?
Question 2
Does repetitive magnetic stimulation over the lumbosacrum and the anorectal motor cortex modulate visceral pain in healthy volunteers?

Question 3
Does repetitive magnetic stimulation over the lumbosacrum and the anorectal motor cortex modulate visceral pain in IBS patients?

1.8 Hypotheses

Hypothesis 1
Non-invasive repetitive magnetic stimulation over the anorectal motor cortex and/or lumbosacral area can modulate anal sphincter motor excitability in healthy volunteers.

Hypothesis 2
Non-invasive repetitive magnetic stimulation over the anorectal motor cortex and/or lumbosacral area can modulate anorectal sensation and pain in healthy volunteers.

Hypothesis 3
Non-invasive repetitive magnetic stimulation over the anorectal motor cortex and/or lumbosacral area can modulate anorectal sensation and pain in IBS patients.
CHAPTER 2

MATERIALS AND METHODS
2.1 Ethical approval

Experiments were approved by local research ethics committees and were performed in accordance with the Declaration of Helsinki.

Informed consent was gained prior to commencing the studies and all healthy subjects and IBS patients were given the opportunity of discontinuing studies at any point if they so desired.

All studies were conducted in the Gastrointestinal Sciences department of the University of Manchester at the Salford Royal NHS Foundation Trust.

2.2 Subjects

Sixteen healthy volunteers and 10 IBS patients participated in this project.

Detailed anthropometric data will be provided in chapters 3, 4 and 5.

Healthy subjects for this study were recruited via poster advertisement, which was displayed on notice boards at the University of Manchester’s buildings and on registered websites, allowed by the University of Manchester. IBS patients were recruited from medical outpatients at the discretion of the consultant in charge of their care. All volunteers were given sufficient time to consider their participation and given the opportunity to tour the research facility prior to participation. Suitable volunteer fees were provided, and no coercion was practised.

- **Inclusion criteria**
  All healthy volunteers who did not meet Rome III criteria for IBS and IBS patients, who fulfilled Rome III criteria for IBS, aged 18 and above, able to give written consent and who did not have the following exclusion criteria were included.
• **Exclusion criteria**
  A history of epilepsy, a cardiac pacemaker or an implantable cardiac defibrillator, previous brain surgery, implanted metal in the head, eyes or lumbosacral spine, taking medication which is active in the central nervous system and pregnancy.

• **Studies**
  Up to 6 studies for each healthy volunteer and 3 studies for IBS patients in total, each study lasted for about 3 hours with one week apart. The average total duration of participation in these studies were 18 hours for each healthy volunteer and 9 hours for each IBS patient.

Healthy subjects and IBS patients who had taken part in recent neurophysiological studies were encouraged to have a two week period free of such studies before participation in this research, in order to maintain scientific validity and to reduce the burden to volunteers.

2.3 **Questionnaires**

Healthy volunteers and IBS patients were asked to complete the Hospital Anxiety and Depression (HAD) rating scale [181]. HAD scale used to obtain both Anxiety (A) and Depression (D) scores from 0-21. HAD scale results were interpreted as following: 0-7=Normal, 8-10=Mild, 11-14=Moderate and 15-21=Severe (Appendix 1).

In addition, IBS patients were asked to complete IBS severity questionnaire [182]. IBS severity questionnaire maximally scores 500 and its results analysed as following: 75-175=Mild, 175-300=Moderate and >300=Severe (Appendix 2). This questionnaire was used to give me an idea about the psychological and physical status of the IBS patients.
2.4 Physiological techniques

2.4.1 Lumbosacral and cortical magnetic stimulation

The magnetic stimulator is made of capacitor discharge system connected with switching element and a coil [113]. The coil generates a magnetic field in the direction parallel to the central axis of the coil that can pass through bones and tissues to stimulate sites beyond these structures [113].

The magnetic effects depend on the strength of the electric field induced within the nervous system by the magnetic stimulator and the intensity of the electric field is associated with the current flowing around the coil and the number of turns of the wire within the coil and its dimensions and decreases by increasing the distance between the stimulating coil and the target organ.

Monophasic single-pulse LSMS of the sacral nerve roots was performed using a magnetic stimulator (Magstim model 200; Magstim Co. Ltd) attached to a branding iron figure of 8 coil with 120 mm outer diameter (figure 2.2) positioned in the midline over the lumbosacral vertebrae at about 5 cm above the natal cleft.

Monophasic single-pulse transcranial magnetic stimulation (TMS) of the cerebral cortex for the anal sphincter and tibialis anterior (TA) muscles were achieved by using a magnetic stimulator (Magstim model 200; Magstim Co. Ltd) attached to a double cone coil with 130 mm outer diameter (maximum output of 2.2 Tesla) as shown in figure 2.1. Additionally, single-pulse TMS for abductor pollicis brevis (APB) was performed by using a flat figure of 8 coil with 70 mm outer diameter hold at an angle of 45° to the scalp. APB and TA muscles were used as controls.

Biphasic repetitive TMS (rTMS) and lumbosacral magnetic stimulation (rLSMS) were achieved by using a Magstim Super Rapid stimulator (The Magstim Company Limited, Whitland, Wales, UK).

On each subject, Single-pulse lumbosacral magnetic stimulation (LSMS) was performed and the best position (largest motor evoked potential “MEP” amplitude
A disposable cotton hood was fastened securely on the scalp and the cranial vertex were identified and marked on the cap. Next, the double-cone coil was discharged over the anal sphincter motor cortex (a deep motor area located in the inter-hemispheric fissure) as validated by Turnbull et al [105] and the best site was identified and marked on the scalp. This site was then stimulated using a single-pulse transcranial magnetic stimulation (TMS), commencing at sub-threshold intensity and increasing by 5% steps until intensity was found that evoked an electromyographic (EMG) response exceeding 50 µV on at least five of 10 consecutive stimulations. This was defined as the resting motor threshold (RMT) for the anal sphincter. Tibialis anterior (TA) EMG response was concurrently measured during anorectal motor cortex stimulation and hand (APB) RMT was measured by using the flat figure-of-8 coil evoking an EMG response exceeding 50 µV on at least five of 10 consecutive stimulations.

To assess pre-stimulation anal sphincter, TA and APB motor cortex excitabilities, the anal sphincter and hand sites were independently stimulated at intensity of RMT plus 10% using TMS with 10 stimuli. To avoid any unintentional facilitation of the cortically evoked responses, all healthy subjects were encouraged to remain as relaxed as possible during stimulation.
Fig 2.1 Single-pulse TMS application with cortical anal sphincter EMG recording.

Fig 2.2 Single-pulse LSMS application with lumbosacral anal sphincter EMG recording.
2.4.2 Electromyogram

Electromyogram (EMG) responses were recorded from the anal sphincter, non-dominant hand (APB) and leg (TA) muscles. Anal sphincter EMG responses were recorded using an anal plug (specially designed and manufactured for this study by the medical engineering department of the University of Manchester at Salford Royal) that housed three silver/silver chloride plate electrodes arranged radially 120° apart as shown in figure 2.3. Silver/silver chloride surface electrodes (Arbo Medical, Stratford, CT, USA) were used to record APB and TA EMG. The electrodes were positioned vertically over the belly of the muscles, 1 cm apart with the reference electrode placed on the tip of the index finger and the posterior aspect of the knee.

A preamplifier (CED 1902; Cambridge Electronic Design, Cambridge, UK) was used to pass through the signals of electromyogram response with filter settings of 5 to 2000 Hz. Then, a laboratory interface (CED 1401 plus; Cambridge Electronic Design, Cambridge, UK) was used to collect these signals at a sampling rate of 5 kHz. Data were displayed on a computer screen which allowed immediate display and storing of traces to file for later analysis.

2.4.3 Anorectal sensory measurements

Sensory measurements were assessed by electrical stimulation, a minimally invasive method of applying a brief stimulus and can be repeated after intervention in a time dependent manner in contrast to thermal stimulation, manual balloon inflation or barostat. Furthermore, it can be combined with the measurement of motor evoked potential (MEP) of the anorectum. Harris et al [101] found that electrical stimulation was the most reproducible method for evaluating pain in the anorectum in comparison to rapid and manual balloon distension techniques.

- Electrical stimulation

An electrode stimulating catheter (Gaeltec, Dunvegan, Isle of Skye, IV55 8GU, UK) with two circumferential electrodes was passed through the central hole of the anal plug (specially designed and manufactured for this study by the
medical engineering department of the University of Manchester at Salford Royal) housing three plate electrodes (Figures 2.3 to 2.5). The anal plug was positioned in the anal sphincter, while the catheter was placed in the rectum such that the electrodes positioned at 10 cm from the anal verge. Stimulating the rectum at 10 cm from anal verge is well established by many studies using electrical stimulation and barostat [101, 103-104]. The anal plug and the rectal catheter were secured in place by applying adhesive tapes attaching them to the skin of the surrounding area. Sensory and pain thresholds of the anal sphincter and rectum were then determined using electrical stimulation from the anal plug and rectal catheter, respectively [101].

The anal plug and then the rectal catheter were connected to a constant-current stimulator (model DS7A, Digitimer Ltd, Hertfordshire, UK) (Figure 2.6) where the available current was limited by a pre-set (safety) value of 200 V. The Digitimer stimulator was attached to a trigger generator (model DG2; Digitimer Ltd, Hertfordshire, UK) (figure 2.7) that enabled a pre-set stimulation frequency to be delivered. For both anal and rectal electrical stimuli were delivered at a frequency of 0.5 Hz, with square wave pulses of 500 µs duration, at intensities between 0 and 100 mA. These parameters were validated by Harris et al [101] who found that electrical current with frequencies above 0.5 Hz led to more variable results and led to intolerable pain and increased urge perception.

The voltage required for a single electrical stimulus to cause the subject to first feel a sensation in the anal sphincter and rectum and that required to feel pain were established (this was confirmed by repeating 3 times and taking a mean value).

The subjects were asked to score pain intensity, unpleasantness and urge using categorical visual analog scores (VAS) (Appendices 3-5). Word anchors for pain and urge were listed from 1 to 6 as faint, weak, mild, moderate, strong, and intense. The word anchors for the unpleasantness were categorized from 1 to 5 as mild, discomforting, distressing, horrible and excruciating.
In addition the subjects were asked to describe the multidimensional qualities of the anorectal pain by using the short form of the McGill Pain Questionnaire [102] including describing the experienced anorectal pain from a list of words and scoring the pain on a scale from 0 to 10 where 0 indicates there was no pain and 10 refers to the worst pain they ever experienced (Appendix 6).

All sensory measurements were repeated after the interventions to allow re-quantification of sensory perception in each individual.

Fig 2.3 The anal plug for electrical stimulation and EMG recording.
Fig 2.4 The Gaeltec rectal catheter with built in bipolar ring stimulating electrodes.

Fig 2.5 The rectal catheter passes through the central hole of the anal plug.
2.5 Interventions

Healthy volunteers underwent four active magnetic stimulation interventions (two repetitive LSMS and two repetitive TMS sessions) and two sham interventions to
assess modulation of visceral sensation. By contrast, IBS patients only underwent the two most effective active repetitive MS on rectal pain of healthy volunteers and one sham to reduce the number of visits and avoid any unnecessary discomfort.

2.5.1 Repetitive lumbosacral magnetic stimulation (rLSMS)

Repetitive LSMS was achieved using a magnetic stimulator connected to a 130 mm outer diameter double-cone coil (maximum output of 2.2 Tesla) as shown in figure 2.8. Lumbosacral stimulation was delivered at 1 and 10 Hz, or sham stimulation (comprising a 90° coil tilt) to the marked lumbosacral area. To control for total energy delivered a total of 600 pulses were given for each intervention. Each subject received all two rLSMS stimulations and sham; each being delivered on separate days, at least 1 week apart and the order of stimulus delivery were randomised as following:

(a) 10 trains of 1 Hz stimulation, each lasting 1 min with an inter-train interval of 10 sec.
(b) 10 trains of 10 Hz stimulation, each lasting 6 sec with an inter-train interval of 60 sec.
(c) 10 trains of sham stimulation, each lasting 6 sec with an inter-train interval of 60 sec.

Sensory measurements were then assessed immediately post-rLSMS and were repeated at 30 and 60 min. Moreover at 30 min post-rLSMS anal sphincter, leg and hand excitabilities were recorded using single-pulse MS at lumbosacral and TMS at intensities RMT plus 10%, with 10 stimuli.
Fig 2.8 Repetitive LSMS application.

2.5.2 Repetitive transcranial magnetic stimulation (rTMS) over motor cortex

Repetitive TMS was performed using a Magstim Super Rapid stimulator connected to a figure of eight coil with a 70mm outer diameter (maximum output of 1.8 Tesla), held in the motor cortex area representing the anorectum (Figure 2.9). From previous studies the optimal orientation was known to be an antero-posterior direction with the plane of the coil parallel to the scalp surface and the handle/axis of the coil approximately 45° to the midsagittal line [105]. The optimal location of the coil was determined by the location on the scalp where magnetic stimulation produced the largest MEPs from the target muscle when the subject was relaxed (the 'motor hot-spot').
Repetitive TMS at 1 and 10 Hz, or sham (comprising a 90° coil tilt) was delivered at 80% anal sphincter threshold (capped at 90% thenar threshold). To control for total energy delivered a total of 600 pulses were given for each intervention. Each subject received two rTMS stimulations and sham; each being delivered on separate days, at least 1 week apart and the order of stimulus delivery were randomised as following:

(a) 10 trains of 1 Hz stimulation, each lasting 1 min with an inter-train interval of 10 sec.
(b) 10 trains of 10 Hz stimulation, each lasting 6 sec with an inter-train interval of 60 sec.
(c) 10 trains of sham stimulation, each lasting 6 sec with an inter-train interval of 60 sec.

Fig 2.9 Repetitive TMS application.

Sensory measurements were then assessed immediately post-rTMS and were repeated at 30 and 60 min. Moreover at 30 min post-rTMS anal sphincter, leg and hand excitabilities were recorded.
2.6 Acquisition, storage and analysis of data

2.6.1 Acquisition

In this thesis, all data for measurement of muscle contractility were obtained using Signal software (Version 4, Cambridge Electronic Design, UK) and all data measurements of anorectal sensation and pain were obtained using a constant-current stimulator (model DS7A, Digitimer Ltd, Hertfordshire, AL7 3BE, UK).

2.6.2 Data handling

The research data were anonymised with the use of an ID identifier. The contact details were kept on a University of Manchester placed computer database which is password protected. Anonymised data were transferred from the laboratories to the University of Manchester's computers over the University of Manchester intranet.

Personal data of research participants and the consent forms were kept as hard copies in separate files in a locked filing cabinet in a secured office at Salford Royal NHS Foundation Trust site of the University of Manchester. Access to this office & filing cabinet is limited to the members of the research team.

2.6.3 Statistical analysis

Recorded data were transferred to StatsDirect (Version 2.7, StatsDirect Ltd, Cheshire, UK) statistical program for analysis. For anal sphincter, abductor pollicis brevis (APB) and tibialis anterior (TA) muscles, the mean amplitude of the 10 MEPs were calculated for resting motor threshold (RMT) + 10% at each time point. The electrical output in milliamps (mA) was taken as a measure of the sensory and pain thresholds and each sensory and pain measurement were repeated three times and the mean value used for the analysis of the results. The process gives a single amplitude value per individual for each time point. Similarly, the mean value of the three sensory measurements was recorded for each individual at each time point. Data were then analysed with analysis of variance (ANOVA). Categorical data from visual analogue scores were analysed with Pearson’s chi-
squared test. Data in the tables are shown as mean ± standard error of mean (SEM) and in the graphs as a percentage change from baseline. The level of significance for all calculations was set at the 95% confidence level ($P < 0.05$).

### 2.7 Summary

The techniques of anorectal electrical stimulation, cortical-anal and lumbosacral MEP measurements by non-invasive magnetic stimulation, and rTMS and rLSMS were uncomplicated and straightforward. Electrical stimulation is a quick procedure and with high reproducibility and repeatability and superior to other anorectal sensory evaluation methods [101]. Moreover, the application of single-pulse TMS and LSMS to evaluate motor excitabilities at MEP + 10% demonstrated a great deal of repeatability and reproducibility [101] [124] and they are applied in this study as objective surrogate measurements to support any subjective sensory and pain alterations experienced by the subjects.
CHAPTER 3

THE EFFECTS OF NON-INVASIVE REPETITIVE MAGNETIC STIMULATION ON ANAL MOTOR EXCITABILITY IN HEALTHY VOLUNTEERS
3.1 Introduction

It has been established that the human central nervous system (CNS) is capable of undergoing changes in response to the surrounding environment, as well as to conditioning stimuli and pathological lesions [107]. This process is called plasticity. The capacity of the CNS to respond to various stimuli may be of great importance to overcome pathological processes such as stroke [183] and this phenomenon also has the potential to be enhanced therapeutically in conditions where there is a possible defect in the brain-gut axis such as in IBS.

Non-invasive repetitive magnetic stimulation (MS) is a well-known non-invasive neurostimulatory technique, widely applied in research and neurosychiatric diagnosis and therapy. Non-invasive repetitive MS has the ability to alter the properties of the nervous tissues and has been used before to modulate the excitabilities of striated muscles [133, 184-186].

Repetitive transcranial magnetic stimulation (rTMS) applied to human motor cortex has demonstrated in general that rates of 1 Hz and below reduce motor excitability and 5 Hz and above increase motor excitability of cortical projection of the hand muscle (APB) with maximum effect at 30 min or later post intervention [185-186].

This effect has also been observed by Gow and colleagues [184] who studied the effect of rTMS (100 pulses) on 12 healthy subjects and found that 5 Hz rTMS increased the excitability of the corticobulbar projection to the pharynx in the first hour. Additionally, Mistry et al [133] showed that 1 Hz rTMS (600 pulses) significantly suppressed pharyngeal motor cortex immediately and for up to 45 min post intervention.

There is limited literature regarding the effect of rTMS on anorectal motor function. Aizawa and colleagues [187] have used low frequency (0.1 Hz) rTMS and high frequency (10 Hz) rTMS over the right dorsolateral prefrontal cortex (DLPFC) on 11 healthy males to modulate anxiety induced by electrical stimulation of the rectum and rectal tone as measured by a rectal barostat. They found that only 0.1 Hz rTMS significantly reduced the anxiety induced by electrical stimulation of the rectum whereas neither frequency had an effect on rectal motility.
Non-invasive repetitive MS over the lumbosacral area is still an under researched area although few studies tried successfully to use it to modulate the anal sphincter in healthy volunteers and pelvic muscles in patients suffering from bladder dysfunction.

Harris et al [124] studied eight healthy volunteers and showed that 15 Hz rLSMS (with total of 2250 pulses) increased anal sphincter MEP (motor evoked potential) amplitude following transcranial magnetic stimulation (TMS) in the first hour post intervention. Five Hz rapid rate lumbosacral magnetic stimulation (rLSMS) (with total of 2250 pulses) and sham did not show any change. Also in the same study there were no changes in anal sphincter latency after 5, 15 Hz rLSMS or sham. Hand and leg muscles excitabilities showed no change with either intervention.

In patients with intractable neurogenic urinary bladder dysfunction secondary to lumbosacral nerve injuries, 15 Hz rLSMS (with total of 1500 pulses per session for 10 sessions per patient) resulted in a significant reduction in the mean number of voids and incontinence per 24 hours compared to the sham [161]. This positive effect was maintained up to one month after the last treatment session. Patients also reported an improvement of their visual analogue scale (VAS) to assess lower back pain and showed an improvement of posterior tibial nerve F-wave and H-reflexes after receiving the real treatment compared with sham.

The mechanisms behind these effects are incompletely understood and may involve changes in long-term potentiation (LTP) and/or depression (LTD) at cortical synapses [113]. At a cellular level, it has been found recently that repetitive MS increased cAMP levels and phosphorylation of transcription factor CREB when applied on a human-derived neuronal cell culture (SH-SY5Y neuroblastoma cells) [188]. This effect was potentiated with pre-treatment with ketamine and inhibited by pre-treatment with lithium.

Due to the importance of the anorectal area and the lack of research in the use of non-invasive cortical and lumbosacral repetitive MS to modulate this area, it was considered of interest to investigate whether the application of low frequency (1 Hz) and high frequency (10 Hz) rLSMS and rTMS can produce any significant
changes in anal sphincter excitability in healthy volunteers as a prelude to applying these parameters therapeutically in patients with anorectal dysfunction.

3.2 Aim

The purpose of the study is to ascertain whether non-invasive repetitive magnetic stimulation applied to either the motor cortex or lumbosacrum can modulate anal sphincter excitability.

3.3 Hypothesis

Non-invasive repetitive MS over the anorectal motor cortex and/or lumbosacral area can modulate anal sphincter motor excitability.

3.4 Methods

Ethics

The research protocol was approved by Salford and Trafford Research Ethics Committee and all experiments were undertaken in the clinical laboratories of the Gastrointestinal Sciences Department at Salford Royal NHS Foundation Trust, UK, in accordance to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Sample size calculation, randomisation and analysis

The sample size calculation for this study was performed by the Medical Statistics department at Salford Royal Foundation Trust, UK, based on previous work performed within the department of GI Sciences using rTMS [133] and rLSMS [124] on healthy subjects (StatsDirect (Version 2.7, StatsDirect Ltd Cheshire, UK).

Sixteen healthy volunteers were therefore needed to achieve a power of 80% and statistical significance of 1% in order to find a difference in the amplitude of MEP in µV of at least 0.8 standard deviation (SD) for a paired t-test (applying the Bonferroni correction).
The six different conditions (1 Hz, 10 Hz and sham rLSMS and 1 Hz, 10 Hz and sham rTMS) were randomised for all subjects’ visits using the block randomisation option of the statistical software StatsDirect (Version 2.7, StatsDirect Ltd Cheshire, UK).

**Participants**

Sixteen healthy volunteers (n=16) were recruited (nine females, age range 20-56 years). All volunteers were well and did not fulfil Rome III criteria for IBS (to exclude any undiagnosed IBS patient). Exclusion criteria were a history of epilepsy, a cardiac pacemaker, previous brain surgery, implanted metal in the head, eyes or lumbosacral spine, taking medication which is active in the central nervous system and pregnancy. Informed consent was obtained before the start of the experiments.

**Protocol**

- Bowel preparation was avoided to prevent any irritation to the anorectal area. However, subjects were encouraged to empty their rectum before starting the study.
- The anal plug was lubricated with KY Jelly (Johnson and Johnson, New Brunswick, NJ, USA), inserted and positioned in the anal canal and attached to the recording system.
- Subjects were left to rest for 10 min to allow baseline EMG activity to stabilise.
- To avoid any inadvertent facilitation of the cortically evoked responses, all subjects were encouraged to remain as relaxed as possible during stimulation.
- Single-pulse lumbosacral magnetic stimulation (LSMS) was performed to determine lumbosacral motor threshold (MT) and the position marked on the skin. LSMS was then delivered at MT+10% and recorded on ten occasions.
• Subjects were then comfortably seated and surface electrodes (Arbo Medical, Stratford, CT, USA) were placed over the thenar area of the hand (APB) and tibialis anterior muscle of the leg (TA). A disposable cotton hood was fastened securely on the scalp and the cranial vertex was identified [124] and marked on the cap. The double-cone magnetic stimulating coil was discharged over the anal sphincter motor cortex (a deep motor area located in the inter-hemispheric fissure) [105] and the best site was identified and marked on the scalp. This site was then stimulated using a single-pulse transcranial magnetic stimulation (TMS), commencing at sub-threshold intensity and increasing by 5% steps until an intensity that evoked an electromyographic (EMG) response exceeding 50 µV was found on at least five of 10 consecutive stimulations. This was defined as the resting MT (RMT) for the anal sphincter. TMS was then delivered at MT+10% and recorded on ten occasions.

• TA MEP responses were concurrently measured during sphincter motor cortex stimulation.

• Hand muscle (APB) MT was determined by stimulating thenar motor cortex area using the flat figure of 8 coil evoking an EMG response exceeding 100 µV on at least five of 10 consecutive stimulations. This site was then stimulated using single-pulse TMS on ten occasions at MT+10%.

• The randomised intervention was then delivered (Figure 3.1).
Fig 3.1 Study design chart.

Healthy volunteers

Lumbosacral stimulation

Baseline measurements:
• single pulse TMS
• single pulse LSMS

Randomisation: 600 pulses rLSMS

Motor cortical stimulation

Baseline measurements:
• single pulse TMS
• single pulse LSMS

Randomisation: 600 pulses rTMS

1 Hz  10 Hz  sham

1 Hz  10 Hz  sham

Post intervention anal sphincter motor measurements at 30 min
• Anal sphincter MEPs following TMS were recorded for 30 min following each intervention to prevent any discomfort associated with frequent application of TMS. This time interval has shown a significant change in EMG from previous studies [124, 133].

• In addition to single-pulse TMS over anorectal cortical area, single-pulse LSMS over lumbosacral area was also used to record peripheral nerve effects after each intervention which was not done in previous work [124].

• Furthermore, in order to assess how topographically focal the effects of rLSMS and rTMS applications to the anal sphincter, changes of non-dominant hand and leg motor responses were investigated from APB and TA muscles, as a control for the current project. The use of the hand and leg muscles, as a control, for the studies would strengthen any conclusions made upon the results for any changes of anal sphincter cortical or lumbosacral excitabilities, since especially the cortical representation of the TA muscle being topographically close to the motor representation of the anorectum.

**Data handling**

Recorded data were transferred to the statistical software StatsDirect (Version 2.7, StatsDirect Ltd Cheshire, UK) for analysis.

The peak-to-peak amplitude of MEPs in microvolts (µV) evoked by magnetic stimulation was used as a measure of motor cortex excitability. MEP’s of cortico-anal, lumbosacral-anal, cortico-TA and cortico-APB excitabilities were repeated 10 times and the means were used in the results. Data were then analysed with analysis of variance (ANOVA).

Individual MEPs (10 EMG traces for each muscle group) were reviewed with Signal Software (Version 4, Cambridge Electronic Design, UK) and an average trace was created at each time-point. Then the latencies and amplitudes of individual’s averaged MEPs were determined.
Inter-individual factors such as age and sex, which might conceivably alter these results, were therefore equalised, and not considered for any additional analysis, since each subject acted as his/her own control.

Data in the tables are shown as mean ± standard error of mean (SEM) and in the graphs as a percentage of change from the baseline. The level of significance for all calculations was set at the 95% confidence level ($P < 0.05$).

3.5 Results

Twenty two subjects were recruited. One subject was excluded due to lack of any cortical EMG response at 100% of single-pulse magnetic stimulation output. Another five subjects did not finish the six studies; one due to intolerability to the basket coil used for single-pulse cortical MEP measurement and four as a result of being unable to regularly attend additional studies. Thus, all six subjects who did not complete the studies were excluded from the data analysis. Sixteen subjects finished the whole study (6 visits) without any adverse events.

Cortical and lumbosacral MEP’s were observed in all participants. The optimal site of cortical measurements was 2-3 centimetres in the midline in front of the vertex and that for the lumbosacral measurements was 2-3 centimetres below the line between the two iliac crests.

Mean (SEM) values of cortical and lumbosacral MT’s for the anorectal area were 60.6 ± 3%, 29.3 ± 2.3% of magnetic stimulator output, respectively. Mean (SEM) values of thenar MT’s were 46.1 ± 1.2%.

Baseline anorectal lumbosacral and cortical excitabilities (MEP’s) and of APB and TA representations (MEP’s), prior to the application of the different interventions, was similar across all arms, as shown with Friedman’s test (anorectum lumbosacral representation: Chi squares: 2.904, p=0.726, anorectum cortical
Example lumbosacral and cortically induced anal MEP’s as well as cortically evoked leg and hand MEP’s from a representative individual are shown in the figures 3.2 and 3.3.

Fig 3.2 Sphincter EMG response by single-pulse TMS at MT + 10% (upper trace) and single-pulse LSMS at MT + 10% (lower trace).
3.5.1 Effects of peripheral stimulation on anal sphincter excitability

1 Hz rLSMS led to a statistically significant increase in lumbosacral-anal amplitude of MEP (38.5 ± 5.7 vs. 58 ± 12.3 µV, p=0.04) as shown in table 3.1 and figure 3.5.

There were no significant effects with 10 Hz rLSMS and sham rLSMS on LS MEP.

There was no significant change in cortical motor excitability response (MEP) from baseline at 30 min following 1 Hz, 10 Hz rLSMS and sham as demonstrated in table 3.1 and figure 3.4.

Latencies of both cortical-anal and lumbosacral-anal MEPs demonstrated no change from baseline after all 6 interventions (Table 3.2).
Table 3.1 Amplitudes (µV) following TMS for each frequency of rLSMS, across each time point for anal sphincter.

<table>
<thead>
<tr>
<th>Anal Sphincter Muscle</th>
<th>Frequency</th>
<th>Site</th>
<th>Baseline</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rLSMS sham</td>
<td>Cortical</td>
<td>39.3 ± 8.2</td>
<td>35.8 ± 5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>41.8 ± 8</td>
<td>43 ± 6</td>
</tr>
<tr>
<td></td>
<td>rLSMS 1 Hz</td>
<td>Cortical</td>
<td>45.8 ± 8</td>
<td>34.1 ± 4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>38.5 ± 5.7</td>
<td>58 ± 12.3*</td>
</tr>
<tr>
<td></td>
<td>rLSMS 10 Hz</td>
<td>Cortical</td>
<td>37.9 ± 7.2</td>
<td>34.5 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>49.4 ± 8.2</td>
<td>58.8 ± 9.7</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects. Significant changes are shown in bold. * p=0.04.

Table 3.2 Motor latencies (ms) following TMS with each frequency of rLSMS and rTMS across each time point for anal sphincter.

<table>
<thead>
<tr>
<th>Anal Sphincter Muscle</th>
<th>Frequency</th>
<th>Site</th>
<th>Baseline</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rLSMS sham</td>
<td>Cortical</td>
<td>20.6 ± 0.6</td>
<td>20.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>rLSMS 1 Hz</td>
<td>Cortical</td>
<td>20.4 ± 0.7</td>
<td>21.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>3.1 ± 0.2</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>rLSMS 10 Hz</td>
<td>Cortical</td>
<td>20.4 ± 0.5</td>
<td>20.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>2.9 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.
Fig 3.4 Effects of rLSMS on cortico-anal excitability. Data are presented as group % change in cortically evoked sphincter MEPs amplitudes 30 min following 1 Hz, 10 Hz rTMS and sham.

Fig 3.5 Effects of rLSMS on lumbosacral-anal excitability. Data are presented as group % change in lumbosacrally evoked sphincter MEPs amplitudes 30 min following 1 Hz, 10 Hz rLSMS and sham.
3.5.2 Effects of cortical stimulation on anal sphincter excitability

Repetitive TMS in the form of 1 Hz, 10 Hz and sham demonstrated no significant change in motor amplitudes of both cortical-anal and lumbosacral-anal MEPs (table 3.3 and figures 3.6 and 3.7).

There were no changes in motor latencies of both cortical-anal and lumbosacral-anal MEPs to the effects of 1 Hz rTMS, 10 Hz rTMS and sham rTMS as shown in table 3.4.

Table 3.3 Amplitudes (µV) following TMS for each frequency of rTMS, across each time point for anal sphincter.

<table>
<thead>
<tr>
<th>Anal Sphincter Muscle</th>
<th>Frequency</th>
<th>Site</th>
<th>Baseline</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rTMS sham</td>
<td>Cortical</td>
<td>52.8 ± 12</td>
<td>45.5 ± 8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>40.4 ± 5</td>
<td>43.9 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>rTMS 1 HZ</td>
<td>Cortical</td>
<td>48.6 ± 10</td>
<td>49.4 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>40 ± 9.6</td>
<td>51.1 ± 9</td>
</tr>
<tr>
<td></td>
<td>rTMS 10 Hz</td>
<td>Cortical</td>
<td>53.4 ± 13.2</td>
<td>59.6 ± 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>47.4 ± 7.9</td>
<td>39.5 ± 6.2</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.

Table 3.4 Motor latencies (ms) following TMS with each frequency of rTMS across each time point for anal sphincter.

<table>
<thead>
<tr>
<th>Anal Sphincter Muscle</th>
<th>Frequency</th>
<th>Site</th>
<th>Baseline</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rTMS sham</td>
<td>Cortical</td>
<td>20.6 ± 0.5</td>
<td>20.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>2.9 ± 0.1</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>rTMS 1 HZ</td>
<td>Cortical</td>
<td>21.4 ± 0.6</td>
<td>19.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>2.7 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>rTMS 10 Hz</td>
<td>Cortical</td>
<td>20.2 ± 0.4</td>
<td>20.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumbosacral</td>
<td>2.9 ± 0.1</td>
<td>3 ± 0.1</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.
Fig 3.6 Effects of rTMS on cortico-anal excitability. Data are presented as group % change in cortically evoked sphincter MEPs amplitudes 30 min following 1 Hz, 10 Hz rTMS and sham.

Fig 3.7 Effects of rTMS on lumbosacral-anal excitability. Data are presented as group % change in lumbosacrally evoked sphincter MEPs amplitudes 30 min following 1 Hz, 10 Hz rTMS and sham.
3.5.3 Effects of peripheral and cortical stimulation on APB and TA excitabilities

Amplitudes and latencies of both APB (Table 3.5) and TA (Table 3.6) MEPs demonstrated no change from baseline after all 6 interventions.

Table 3.5 Amplitudes (µV) following TMS for each frequency of rLSMS and rTMS, across each time point for APB and TA muscles.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Frequency</th>
<th>Site</th>
<th>Baseline</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>APB</td>
<td>rLSMS sham</td>
<td>Cortical</td>
<td>290.5 ± 123.5</td>
<td>191.1 ± 46.8</td>
</tr>
<tr>
<td></td>
<td>rLSMS 1 Hz</td>
<td>Cortical</td>
<td>244.8 ± 56.3</td>
<td>299 ± 86</td>
</tr>
<tr>
<td></td>
<td>rLSMS 10 Hz</td>
<td>Cortical</td>
<td>277.2 ± 105.2</td>
<td>322.2 ± 108.9</td>
</tr>
<tr>
<td></td>
<td>rTMS sham</td>
<td>Cortical</td>
<td>187 ± 92.1</td>
<td>200.4 ± 70.4</td>
</tr>
<tr>
<td></td>
<td>rTMS 1 Hz</td>
<td>Cortical</td>
<td>283 ± 76.3</td>
<td>249 ± 69.7</td>
</tr>
<tr>
<td></td>
<td>rTMS 10 Hz</td>
<td>Cortical</td>
<td>232.9 ± 95</td>
<td>353.9 ± 158.7</td>
</tr>
<tr>
<td>TA</td>
<td>rLSMS sham</td>
<td>Cortical</td>
<td>382.8 ± 147.7</td>
<td>239.2 ± 84.5</td>
</tr>
<tr>
<td></td>
<td>rLSMS 1 Hz</td>
<td>Cortical</td>
<td>341.1 ± 92.9</td>
<td>235.5 ± 61.2</td>
</tr>
<tr>
<td></td>
<td>rLSMS 10 Hz</td>
<td>Cortical</td>
<td>191.1 ± 67.9</td>
<td>272.4 ± 93.2</td>
</tr>
<tr>
<td></td>
<td>rTMS sham</td>
<td>Cortical</td>
<td>245.4 ± 124.5</td>
<td>329.8 ± 137.7</td>
</tr>
<tr>
<td></td>
<td>rTMS 1 Hz</td>
<td>Cortical</td>
<td>207.5 ± 83.7</td>
<td>308.9 ± 103.7</td>
</tr>
<tr>
<td></td>
<td>rTMS 10 Hz</td>
<td>Cortical</td>
<td>204.9 ± 82</td>
<td>196.7 ± 76.7</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.

Table 3.6 Motor latencies (ms) following TMS with each frequency of rLSMS and rTMS across each time point for APB and TA muscles.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Frequency</th>
<th>Site</th>
<th>Baseline</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>APB</td>
<td>rLSMS sham</td>
<td>Cortical</td>
<td>20.7 ± 0.4</td>
<td>20.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>rLSMS 1 Hz</td>
<td>Cortical</td>
<td>20.1 ± 0.4</td>
<td>20.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>rLSMS 10 Hz</td>
<td>Cortical</td>
<td>20.9 ± 0.4</td>
<td>21 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>rTMS sham</td>
<td>Cortical</td>
<td>21.1 ± 0.4</td>
<td>21 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>rTMS 1 Hz</td>
<td>Cortical</td>
<td>21 ± 0.4</td>
<td>20.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>rTMS 10 Hz</td>
<td>Cortical</td>
<td>21 ± 0.5</td>
<td>21.1 ± 0.6</td>
</tr>
<tr>
<td>TA</td>
<td>rLSMS sham</td>
<td>Cortical</td>
<td>29.1 ± 0.6</td>
<td>29.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>rLSMS 1 Hz</td>
<td>Cortical</td>
<td>27.9 ± 1</td>
<td>28.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>rLSMS 10 Hz</td>
<td>Cortical</td>
<td>28.6 ± 0.5</td>
<td>28.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>rTMS sham</td>
<td>Cortical</td>
<td>29.1 ± 0.6</td>
<td>28.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>rTMS 1 Hz</td>
<td>Cortical</td>
<td>28.9 ± 0.6</td>
<td>28.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>rTMS 10 Hz</td>
<td>Cortical</td>
<td>28.9 ± 0.6</td>
<td>28.8 ± 0.6</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.
3.6 Discussion

In summary, these experiments explored the effects of peripheral (rLSMS) and cortical (rTMS) excitatory paradigm (10 Hz) and inhibitory paradigm (1 Hz) and sham stimulation on cortical-anal and lumbosacral-anal motor excitabilities recorded 30 min after each intervention. Hand and leg motor excitabilities recorded from APB and TA muscles were also studied as controls and to show if any positive or negative effects of rTMS’s and/or rLSMS’s are localised to anal area or extend to other muscles.

These data showed that 1 Hz rLSMS caused an increase in amplitude of lumbosacral-anal MEP with no effect on latency. Interestingly, 1 Hz rLSMS did not lead to any significant change in cortical-anal MEP amplitude and latency.

There were no effects of 10 Hz rLSMS, 1 Hz rTMS, 10 Hz rTMS and shams on both lumbosacral-anal and cortical-anal amplitudes and latencies. In addition, APB and TA amplitudes and latencies show no change after 30 min following all interventions.

This study demonstrated that short-term repetitive MS delivered over the lumbosacrum induces a post stimulus effect on the excitability of the anal sphincter motor lumbosacrum that was both frequency and muscle specific in healthy control subjects.

To date, there is one systematic, randomised, crossover study that assessed the effects of non-invasive MS on anal sphincter excitability [124]. Harris et al showed that 15 Hz rLSMS but not 5 Hz rLSMS caused an increase in a cortical-anal excitability at 30 min and beyond using the same methodology but they failed to measure lumbosacral-anal MEP’s.

However, in another study [163], temporary SNS set at 15 Hz applied over two weeks on patients with faecal incontinence showed a decrease in cortical-anal excitability. Even though, Harris et al concluded that anal sphincter needs 15 Hz rLSMS or higher to stimulate contraction. In my study, 10 Hz and 1 Hz rLSMS were used because they have not been studied before and additionally, the final
aim of my project is to try to modulate anorectal sensation which may require lower intensities.

The exact mechanism behind the excitability changes observed after 1 Hz rLSMS remains uncertain. This post stimulus effect may be attributed to long-term potentiation (LTP) of neural synapses observed after the activation of the cortex by rTMS [189].

It is difficult to ascertain exactly whether the change in the excitability occurred within the spinal cord or anal sphincter itself or influenced by afferent or efferent pathways from the cortex. It is unlikely that a direct effect of 1 Hz rLSMS on pudendal nerve efferent alone to the anal sphincter could cause the excitability change observed in this study, as the 1 Hz rLSMS (inhibitory paradigm) would show a decrease, not an increase, in the amplitude of lumbosacral-anal sphincter MEP if it stimulates pudendal nerve alone [177].

Another explanation that this lumbosacral-anal excitability effect was secondary to 1 Hz rLSMS stimulation of peripheral afferents and this induces local reflex activity at the spinal cord. However, no change in the latency of the lumbosacral-anal MEP would probably contradict this theory as the excitability alterations of the anal neurones would have led to a change in the motor latency [163]. Thus, there is one possible theory that these effects occurred mainly in the central nervous system level (spinal cord and cortex).

Although, previous studies using rTMS to the human motor cortex has shown decreased excitability with low frequency stimulation (1 Hz) to both the pharyngeal [133] and hand motor cortical areas [186], the 1 Hz rLSMS has shown an increase in lumbosacral-anal excitability. One possibility is, therefore, that 1 Hz rLSMS might be a stimulation frequency that favours cortical excitation over inhibition when applied to pelvic nerves but this excitation is not strong enough to elicit any immediate cortical-anal excitability change. Rather, the lack of an increase in cortico-anal excitation to 1Hz LSMS, despite an increase in LS-anal responses, suggests the cortex might be inhibited while the peripheral spinal system is excited. Such a differential effect requires further study to establish why such a mechanism would exist.
It is important to know that the brain representation of anal sphincter function goes beyond the motor cortex and incorporates a number of other cerebral areas. Kern and colleagues [190] performed a brain imaging study in healthy subjects and demonstrated multifocal functional magnetic resonance imaging activity in sensory/motor, anterior cingulate, prefrontal, parietal, occipital and insular regions of the cerebral cortex. Therefore it is possible that, while the cortical-anal excitability to 1 Hz rLSMS did not show any changes, other brain regions relevant to anal sphincter control may also have changed, either in a similar or in a contrary manner. Future experiments using whole-brain imaging of recruited cerebral regions are warranted to allow a better understanding of the relationship between rLSMS and cortical changes.

**Why did 1 Hz, 10 Hz rTMS and 10 Hz rLSMS not show any significant effect on anal sphincter motor excitability?**

Even though, studies on pharynx and hand muscles have generally demonstrated that 1 Hz rTMS and below are inhibitory whereas 5 Hz and above are excitatory it is also known that the robustness and the long lasting effect of rTMS depend on the higher number of pulses applied [186, 191]. Moreover, several hundreds or even thousands of pulses must be used in order to achieve reproducible effect especially when stimuli under the motor threshold have been applied as in this experiments [191]. In the present studies, the use of stimuli limited to 600 pulses (relatively low) at sub-threshold intensity for safety and tolerability, may be the reason behind there was no measurable rTMS effect on anal sphincter excitability.

In addition, although EMG recording at 30 min only after different rLSMS and rTMS interventions was done for tolerability and also based on previous studies when maximum response where measured at 30 min [185-186], it deprived measuring any possible changes before or after 30 min from each intervention.

In conclusion, this study showed that 1 Hz rLSMS led to changes in sphincter lumbosacral excitability that was measured 30 min after stimulation. This effect seems to be central in origin and may be related to synaptic cortical plasticity.
This paradigm could be translated into patients with functional GI and defecation disorders as a future therapeutic intervention.
CHAPTER 4

THE EFFECTS OF NON-INVASIVE REPETITIVE MAGNETIC STIMULATION ON ANORECTAL SENSATION IN HEALTHY VOLUNTEERS
4.1 Introduction

Chapter 3 demonstrated that rLSMS was able to modulate anal sphincter motor excitability in a frequency specific manner in healthy volunteers. It is of interest to know whether this form of stimulation may also modulate anorectal sensation and pain.

Repetitive TMS (rTMS) is known to modulate chronic somatic pain arising from different human body parts [141-142]. Most studies used rTMS to reduce pain in patients suffering from neuropathic pain and fibromyalgia [143, 192]. Primary motor cortical area was mainly stimulated in these studies with promising results from specifically targeting parietal and prefrontal areas [132, 150].

Hirayama et al [145] compared the effect of 5 Hz rTMS (500 pulses) over different cortical areas (primary motor, primary sensory, premotor and supplementary motor) to try to relieve pain in 20 patients suffering from intractable neurogenic pain. Only stimulating the primary motor cortex resulted in significant and beneficial pain reduction in 50% of patients which lasted for three hours.

The effective rTMS parameters in pain reduction were high frequency rTMS (5, 10 and 20 Hz) rather than low frequency rTMS (1 Hz or below) at or above the motor threshold or near it (80% or 90%) with high intensity (with number of pulses range from 120 to 2000) [132]. In one study [135], 10 Hz rTMS but not 0.5 Hz rTMS over motor cortex (1000 pulses at 80% of motor threshold) caused a significant transient pain reduction compared with sham in 18 patients with intractable chronic pain due to trigeminal neuralgia and thalamic stroke.

Moreover, Khedr and colleagues [137] applied five daily sessions of 20 Hz rTMS (2000 pulses at 80% of RMT) over primary motor cortex in a larger cohort of 48 patients with chronic pain syndromes (Trigeminal neuralgia and post-stroke). They showed that 20 Hz rTMS application resulted in a significant transient pain relief compared with sham.

Surprisingly, 1 Hz (total 300 stimuli) stimulation of the primary motor cortex in another study [158] resulted in a significant relief of acute pain induced by injection
of capsaicin intradermally compared with sham in seven healthy volunteers. This was associated with functional changes in caudal anterior cingulate cortex and medial prefrontal cortex. The pain reduction effects of rTMS are likely mediated via the activation of horizontal fibres in the superficial layers of primary motor cortex [134].

In contrast to the effect of rTMS on somatic pain, there are few attempts to use rTMS to relieve visceral pain. In a small pilot study, Fregni and his team [138] used six sessions of rTMS on right and left secondary somatosensory cortex stimulation (SII) with 1 Hz, 20 Hz, and sham on five patients with chronic visceral pain due to pancreatitis. They demonstrated that 1 Hz of right and left secondary somatosensory cortex led to a significant pain reduction (36% and 31%, respectively) as assessed by pain and medication reduction. However, 20 Hz stimulation on left secondary somatosensory cortex led to an increase in pain.

Recently, the same team [193] used 10 days of a daily session of 1 Hz rTMS over the right secondary somatosensory cortex in 17 patients with chronic severe visceral pain due to chronic pancreatitis. They showed that 1 Hz rTMS caused significant relief in visceral pain compared with sham and this effect lasted for at least three weeks and was associated with a bilateral increase in excitatory neurotransmitters i.e. glutamate and N-acetyl aspartate measured in secondary somatosensory cortex by magnetic resonance spectrometry (MRS).

Another group [187] have used low frequency (0.1 Hz) rTMS and high frequency (10 Hz) rTMS over the right dorsolateral prefrontal cortex (DLPFC) on 11 healthy males to modulate anxiety induced by electrical stimulation of the rectum. They found that only 0.1 Hz rTMS led to a significant reduction in the anxiety induced by electrical stimulation of the rectum but there was no direct effect of both 0.1 and 10 Hz on rectal pain.

Non-invasive repetitive MS over lumbosacral area is still under researched area although few studies tried successfully to use it to reduce pain. In an animal model study, Lin et al applied a short course of high intensity magnetic stimulation (20 Hz) over the rat's lumbosacral spine and that led to a long and very significant pain reduction effect (80–90%) to both mechanical and heat stimuli [160].
Recently, Lo and his colleagues [194] have used a high intensity non-invasive magnetic stimulation at 10 Hz (1000 pulses) over the sacral area of 10 patients suffering from lumbosacral region pain localised or radiating down to lower limbs. They showed that a single session of 10 Hz MS resulted in a significant reduction of pain immediately and at four days after this intervention. There was no similar improvement of pain following the sham application.

Similarly, when patients with intractable neurogenic urinary bladder dysfunction secondary to lumbosacral nerve injuries were treated with 15 Hz rLSMS (with total of 1500 pulses per session for 10 sessions per patient), Khedr et al [161] noticed a significant improvement in the mean number of voids and incontinence per 24 hours, as well as a significant reduction in the lower back pain of these patients as assessed by visual analogue scale (VAS).

Sacral nerve stimulation (SNS) has also been used to try to treat pelvic pain syndromes including functional anorectal pain disorders. In one centre [167], nine patients with functional anorectal pain disorder due to various causes and not responding to conventional treatments were tested with temporary SNS. There were four respondents who underwent permanent SNS. All four patients showed a long lasting pain improvement up to 24 months during the follow ups.

In a relatively larger series [168], 11 patients with chronic anal and perianal pain syndrome with poor response to other treatments were treated with SNS and showed a significant improvement in their visual analogue pain scores and quality of life questionnaire (SF-36) after 15 months of average follow up and this was not associated with any manometric changes.

Visceral pain is a major clinical problem being a primary feature of functional gastrointestinal disorders such as IBS. Treatments for this condition are limited but neural stimulation could play a significant role. Therefore, as a proof of concept, it is of interest to investigate whether the application of low frequency (1 Hz) and high frequency (10 Hz) rLSMS and rTMS can produce any significant changes in the perception of sensation and pain from anus and rectum in healthy volunteers.
I used electrical stimulation to assess sensory and pain thresholds as described in detail in chapter two. I chose electrical stimulation as a method for sensory and pain threshold measurements because electrical stimulation is a quick procedure, has good tolerability, repeatability and is preferable to other anorectal sensory evaluation methods [101].

4.2 Aim

The purpose of the study is to ascertain whether non-invasive repetitive magnetic stimulation applied to either the motor cortex or lumbosacrum can modulate gastrointestinal sensation and pain originating from the anorectum.

4.3 Hypothesis

The hypothesis is that non-invasive repetitive MS over the anorectal motor cortex and/or lumbosacral area can modulate anorectal sensation and pain.

4.4 Methods

Ethics

The research protocol was approved by Salford and Trafford Research Ethics Committee and all experiments were undertaken in the clinical laboratories of the Gastrointestinal Sciences Department at Salford Royal NHS Foundation Trust, UK, in accordance to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Sample size calculation, randomisation and analysis

The number of participants in this study was determined through the results from a power calculation performed by the Medical Statistics department at Salford Royal Foundation Trust, UK, which was in turn based on previous data taken from work performed within the department of GI Sciences using anorectal electrical stimulation measurements on healthy subjects [101].

Sample size calculation on StatsDirect for a paired t-test (applying the Bonferroni correction) revealed that 16 healthy volunteers would be a sufficient size for
finding a significant difference of 1 standard deviation (SD) in anorectal pain thresholds measured in mA.

The six different conditions (1 Hz, 10 Hz and sham rLSMS and 1 Hz 10 Hz and sham rTMS) were randomised for all subjects’ visits using the block randomisation option of the statistical software StatsDirect (Version 2.7, StatsDirect Ltd, Cheshire, UK).

**Participants**
Sixteen healthy volunteers (n=16) were recruited (nine females, age range 20-56 years). All volunteers did not fulfil Rome III criteria for IBS (to exclude any undiagnosed IBS patient). Exclusion criteria were a history of epilepsy, a cardiac pacemaker, previous brain surgery, implanted metal in the head, eyes or lumbosacral spine, taking medication which is active in the central nervous system and pregnancy. Informed consent was obtained before the start of the experiments.

**Questionnaire**
Healthy volunteers were asked to complete the Hospital Anxiety and Depression Scale (HAD) [181]. HAD scale used to obtain both Anxiety (A) and Depression (D) scores from 0-21. HAD scale results were interpreted as following: 0-7=Normal, 8-10=Mild, 11-14=Moderate and 15-21=Severe. This questionnaire was used to quantify the general wellbeing of the subjects and was used to help ensure that the volunteers fall within the healthy adult range for anxiety and depression.

**Protocol**
- Bowel preparation was avoided to prevent any irritation to the anorectal area. However, subjects were encouraged to empty their rectum before starting the study.
- Subjects were positioned in the left lateral position with knees and hips flexed to 90°.
• An anal plug (Figure 2.3) was positioned in the anal canal and a rectal catheter (Gaeltec, Dunvegan, Isle of Skye, UK) was passed through a hole in the anal plug into the rectum and positioned 10 cm from the anus.

• Subjects were left to rest for 10 minutes to allow for any irritation or anorectal contractions to subside.

• The anal plug and then the rectal catheter were connected to a constant-current stimulator (model DS7A, Digitimer, Hertfordshire, UK).

• For both anal and rectal electrical stimuli were delivered to the subjects at a frequency of 0.5 Hz, with square wave pulses of 500 µs duration, at intensities between 0 and 100 mA.

• Sensory threshold is the stimulus intensity required for a single electrical stimulus to cause the subject to first feel a definite sensation in the anal sphincter and rectum using an ascending method (0.2 and 2 mA increments, respectively). This was repeated three times for each set of measurements and the mean sensory threshold was calculated for each subject.

• Pain threshold is the stimulus intensity required for the electrical stimulation to cause the subject to feel the maximum tolerable pain in the anal sphincter and rectum using an ascending method (0.2 and 2 mA increments, respectively). This was repeated three times for each set of measurements and the mean pain threshold was calculated for each subject.

• After each set of measurements, the subjects were also asked to score the pain intensity, unpleasantness and urge intensity using categorical rating scales (visual analogue score “VAS”) as shown in the appendices 3-5. The descriptions for the pain and urge were marked as faint, weak, mild, moderate, strong, and intense. The descriptions for the unpleasantness were labelled as mild, discomforting, distressing, horrible, and excruciating.
• In addition, the subjects were asked to describe the quality of the anorectal pain experienced by using a list of words from the short form of the McGill Pain Questionnaire [102] and by scoring the pain on scale from 0 to 10, where 0 means no pain and 10 indicates the worst pain ever they had experienced before (Appendix 6).

• The randomised intervention was then delivered (Figure 4.1).

• The assessment of anorectal sensation and pain was repeated immediately, 30 min and 60 min after each intervention.
Fig 4.1 Study design chart.

Healthy volunteers

Lumbosacral stimulation

Motor cortical stimulation

Baseline measurements:
- Anal sensory and pain thresholds
- Rectal sensory and pain thresholds

Randomisation: 600 pulses rLSMS

Randomisation: 600 pulses rTMS

Baseline measurements:
- Anal sensory and pain thresholds
- Rectal sensory and pain thresholds

Post intervention anorectal sensory and pain threshold measurements immediately and at 30 and 60 min

1 Hz 10 Hz sham 1 Hz 10 Hz sham
Data handling

Recorded data were transferred to the statistical software StatsDirect (Version 2.7, StatsDirect Ltd, Cheshire, UK) for analysis.

The electrical output in milliamps (mA) was taken as a measure of sensory and pain thresholds both in anal sphincter and rectum. Each sensory and pain measurements were repeated 3 times and the means used for the analysis of the results. Data were then analysed with analysis of variance (ANOVA). Categorical data from visual analogue scores were analysed with Pearson's chi-squared test.

Data in the tables are shown as mean ± standard error of mean (SEM) and in the graphs as a percentage of change from the baseline. The level of significance for all calculations was set at the 95% confidence level ($P < 0.05$).

4.5 Results

Sixteen subjects finished the whole study (6 visits) without any adverse events. Their average HAD anxiety and depression scores were 3.1 ± 0.4 and 1.5 ± 0.6, respectively, which is considered to be within the normal range of anxiety and depression.

Baseline rectal sensory threshold, rectal pain threshold, anal sensory threshold and anal pain threshold representations, prior to the application of the different interventions, was similar across all arms, as shown with Friedman's test (rectal sensory representation: Chi squares: 8.19, p=0.14, rectal pain representation: Chi squares: 3.89, p=0.58, anal sensory representation: Chi squares: 6.99, p=0.22, anal pain representation: Chi squares: 6.17, p=0.29).

4.5.1 Effects of peripheral stimulation on rectal sensory function

One Hz rLSMS: There was a significant increase in pain thresholds in the rectum immediately, at 30 min and 60 min following 1 Hz rLSMS (p=0.005, 0.02 and
0.007, respectively) as shown in table 4.1 and figure 4.2. In addition, there was also an increase in sensory thresholds in the rectum following 1 Hz rLSMS only at 60 min (p=0.04).

**Ten Hz rLSMS:** There was a significant increase in sensory and pain thresholds in the rectum only immediately following 10 Hz rLSMS (p=0.02 and 0.007, respectively) as illustrated in table 4.1 and figures 4.1 and 4.2.

**Sham:** Sham had no measureable effect on sensory and pain thresholds.

There was no significant change in pain characteristics as measured by visual analogue scores following all interventions (Table 4.2).

**Table 4.1 Sensory stimulation of the rectum values (mA) across each time point for 1 Hz, 10 Hz rLSMS and sham stimulation.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rLSMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>14.6 ± 1.3</td>
<td>15.9 ± 1.5</td>
<td>16.6 ± 1.9</td>
<td><strong>18.4 ± 2.2</strong>*</td>
</tr>
<tr>
<td>P</td>
<td>25.5 ± 1.5</td>
<td><strong>29.6 ± 2</strong>*</td>
<td><strong>30.5 ± 2.5</strong>*</td>
<td><strong>32.8 ± 2.8</strong>*</td>
</tr>
<tr>
<td><strong>rLSMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>14.3 ± 2</td>
<td><strong>16.9 ± 2.1</strong>*</td>
<td>16.4 ± 1.9</td>
<td>16.6 ± 2.2</td>
</tr>
<tr>
<td>P</td>
<td>27.4 ± 2.4</td>
<td><strong>30.3 ± 2.6</strong>*</td>
<td>29.8 ± 2.5</td>
<td>29.2 ± 2.9</td>
</tr>
<tr>
<td><strong>rLSMS Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>19.8 ± 3.9</td>
<td>19.3 ± 3.5</td>
<td>19.1 ± 3.5</td>
<td>17.4 ± 2.8</td>
</tr>
<tr>
<td>P</td>
<td>26.9 ± 2.9</td>
<td>27 ± 3</td>
<td>27.5 ± 3.1</td>
<td>27.7 ± 2.8</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects, sensory threshold (ST) and pain (P). Significant changes are shown in bold. * p<0.05.
Table 4.2 rectal pain characteristics at baseline and immediately, 30 min and 60 min following rLSMS 1 Hz, 10 Hz and sham.

<table>
<thead>
<tr>
<th>Rectum</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rLSMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>5.4 ± 0.8</td>
<td>4.9 ± 0.8</td>
<td>4.3 ± 0.8</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.5 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.5 ± 0.6</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>Urge</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.5</td>
<td>1.4 ± 0.5</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td><strong>rLSMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>4.8 ± 0.7</td>
<td>4.6 ± 0.7</td>
<td>3.7 ± 0.7</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.4 ± 0.4</td>
<td>4.5 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td><strong>rLSMS Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>4 ± 0.8</td>
<td>3.6 ± 0.8</td>
<td>3.2 ± 0.8</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.6 ± 0.3</td>
<td>4.1 ± 0.4</td>
<td>4.1 ± 0.3</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Urge</td>
<td>1.7 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.

Fig 4.1 Effects of rLSMS on rectal sensory threshold. Data are represented as group % change following different interventions.
Fig 4.2 Effects of rLSMS on rectal pain threshold. Data are represented as group % change following different interventions.

4.5.2 Effects of cortical stimulation on rectal sensory function

One Hz rTMS: 1 Hz rTMS only caused a significant increase in pain threshold only immediately after its application (p=0.02) but no effect on sensory thresholds (Table 4.3 and figures 4.3 and 4.4).

Ten Hz rTMS: There was a significant increase in pain threshold following 10 Hz rTMS immediately and at 30 and 60 min, (p=0.01, 0.003 and 0.005, respectively) as demonstrated in table 4.3 and figure 4.4. There was no increase in sensory threshold following 10 Hz rTMS (Figure 4.3).

Sham: Sham had no effect on both sensory and pain thresholds (Table 4.3 and figures 4.3 and 4.4).

There was no significant change in pain characteristics as measured by visual analogue scores following all interventions (Table 4.4).
Table 4.3 Sensory stimulation of the rectum values (mA) across each time point for 1 Hz, 10 Hz rTMS and sham stimulation.

<table>
<thead>
<tr>
<th>Rectum</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rTMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>15.4 ± 1.7</td>
<td>17.8 ± 2.5</td>
<td>18.1 ± 2.4</td>
<td>18 ± 2.7</td>
</tr>
<tr>
<td>P</td>
<td>25.8 ± 2.9</td>
<td><strong>29.9 ± 3.2</strong></td>
<td>32.4 ± 4.8</td>
<td>32.3 ± 5.1</td>
</tr>
<tr>
<td><strong>rTMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>14.5 ± 1.5</td>
<td>15.5 ± 1.3</td>
<td>16.3 ± 1.5</td>
<td>16.2 ± 1.6</td>
</tr>
<tr>
<td>P</td>
<td>27.8 ± 1.1</td>
<td><strong>28.7 ± 2.4</strong>*</td>
<td>29.4 ± 2.7*</td>
<td>30.3 ± 3*</td>
</tr>
<tr>
<td><strong>rTMS sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>19.4 ± 2.8</td>
<td>18.2 ± 2.3</td>
<td>17.3 ± 2.5</td>
<td>18.7 ± 2.5</td>
</tr>
<tr>
<td>P</td>
<td>30.6 ± 3.6</td>
<td>29 ± 2.9</td>
<td>28.2 ± 3.1</td>
<td>29.7 ± 2.9</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects, sensory threshold (ST) and pain (P). Significant changes are shown in bold. * p<0.05.

Table 4.4 rectal pain characteristics at baseline and immediately, 30 min and 60 min following rTMS 1 Hz, 10 Hz and sham.

<table>
<thead>
<tr>
<th>Rectum</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rTMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>4.8 ± 0.8</td>
<td>4.1 ± 0.8</td>
<td>3.5 ± 0.7</td>
<td>3.9 ± 0.8</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.4 ± 0.3</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.6 ± 0.3</td>
<td>4.4 ± 0.4</td>
<td>4.4 ± 0.4</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>Urge</td>
<td>1.8 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td><strong>rTMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>4.3 ± 0.8</td>
<td>4.3 ± 0.8</td>
<td>4.6 ± 0.8</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 ± 0.1</td>
<td>3.4 ± 0.3</td>
<td>3.4 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.5</td>
<td>4.5 ± 0.4</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>1.5 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td><strong>rTMS Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.1 ± 0.7</td>
<td>2.7 ± 0.6</td>
<td>2.8 ± 0.6</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>3.4 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.5 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>1.3 ± 0.4</td>
<td>1 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>1 ± 0.4</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.
Fig 4.3 Effects of rTMS on rectal sensory threshold. Data are represented as group % change following different interventions.

Fig 4.4 Effects of rTMS on rectal pain threshold. Data are represented as group % change following different interventions.
4.5.3 Effects of peripheral stimulation on anal sphincter sensory function

One Hz rLSMS: 1 Hz rLSMS had no significant change in anal sphincter pain (table 4.5 and figure 4.6) but it led to a significant increase in sensory threshold immediately, 30 and 60 min following its use (p=0.02, 0.03 and 0.03, respectively) as demonstrated in table 4.5 and figures 4.5.

Ten Hz rLSMS: 10 Hz rLSMS showed no significant change in anal sphincter pain and sensation (Tables 4.5 and 4.6 and figure 4.6).

Sham: Sham had no effects on sensory and pain thresholds (table 4.5 and figures 4.5 and 4.6).

There was no significant change in pain characteristics as measured by visual analogue scores following all interventions (Table 4.6).

Table 4.5 Sensory stimulation of the anal sphincter values (mA) across each time point for 1 Hz, 10 Hz rLSMS and sham stimulation

<table>
<thead>
<tr>
<th>Sphincter</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rLSMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>3.4 ± 0.2</td>
<td>4.5 ± 0.4*</td>
<td>4.5 ± 0.5*</td>
<td>4.4 ± 0.4*</td>
</tr>
<tr>
<td>P</td>
<td>9.7 ± 0.9</td>
<td>10.9 ± 0.8</td>
<td>11 ± 0.9</td>
<td>10.8 ± 0.8</td>
</tr>
<tr>
<td><strong>rLSMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>3.9 ± 0.7</td>
<td>5.2 ± 1.3</td>
<td>4.4 ± 0.9</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>P</td>
<td>10.8 ± 1.9</td>
<td>12.2 ± 2</td>
<td>11.6 ± 2</td>
<td>11.3 ± 1.7</td>
</tr>
<tr>
<td><strong>rLSMS sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>4.1 ± 0.3</td>
<td>4.4 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>P</td>
<td>11.4 ± 0.9</td>
<td>11.2 ± 0.9</td>
<td>11.2 ± 0.7</td>
<td>10.5 ± 0.8</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects, sensory threshold (ST) and pain (P). Significant changes are shown in bold. * p<0.05.
Table 4.6 Anal sphincter pain characteristics at baseline and immediately, 30 min and 60 min following rLSMS 1 Hz, 10 Hz and sham.

<table>
<thead>
<tr>
<th>Anal sphincter</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rLSMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>2.9 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>3.4 ± 0.4</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.6 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>4.5 ± 0.5</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>Urge</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td><strong>rLSMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.3 ± 0.4</td>
<td>4.1 ± 0.6</td>
<td>3.9 ± 0.6</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.3 ± 0.4</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.4</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td><strong>rLSMS Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.4 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>3.6 ± 0.6</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2.1 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.2 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>0.7 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.

Fig 4.5 Effects of rLSMS on anal sphincter sensory threshold. Data are represented as group % change following different interventions.
4.5.4 Effects of cortical stimulation on anal sphincter sensory function

One Hz rTMS: 1 Hz rTMS had no effect on anal sphincter sensation but it showed an increase in pain thresholds at 60 min (p<0.5) (Table 4.7 and figures 4.7 and 4.8).

Ten Hz rTMS: 10 Hz rTMS also showed no effect on anal sphincter sensation but increased pain thresholds immediately and 30 min following its application (p=0.03 and 0.02, respectively) as illustrated in table 4.7 and figures 4.7 and 4.8.

Sham: Sham had no effects on sensory and pain thresholds (Table 4.7 and figures 4.7 and 4.8).

There was no significant change in pain characteristics as measured by visual analogue scores following all interventions as shown in table 4.8.
Table 4.7 Sensory stimulation of the anal sphincter values (mA) across each time point for 1 Hz, 10 Hz rTMS and sham stimulation.

<table>
<thead>
<tr>
<th>Sphincter</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>rTMS 1 Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>4 ± 0.4</td>
<td>4 ± 0.6</td>
<td>4 ± 0.6</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>P</td>
<td>9.9 ± 0.9</td>
<td>10.8 ± 1.1</td>
<td>10.7 ± 1.2</td>
<td>12.2 ± 1.3*</td>
</tr>
<tr>
<td>rTMS 10 Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>3.4 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>4 ± 0.4</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>P</td>
<td>9.8 ± 0.9</td>
<td>12.3 ± 1.3*</td>
<td>12.2 ± 1.1*</td>
<td>11.1 ± 0.8</td>
</tr>
<tr>
<td>rTMS sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>3.8 ± 0.3</td>
<td>4.2 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>P</td>
<td>10.8 ± 1</td>
<td>11.7 ± 0.9</td>
<td>11.7 ± 0.9</td>
<td>11.3 ± 0.9</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects, sensory threshold (ST) and pain (P). Significant changes are shown in bold. * p<0.05.

Table 4.8 Anal sphincter pain characteristics at baseline and immediately, 30 min and 60 min following rTMS 1 Hz, 10 Hz and sham.

<table>
<thead>
<tr>
<th>Anal sphincter</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>rTMS 1 Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.1 ± 0.4</td>
<td>3.2 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.4 ± 0.4</td>
<td>4.4 ± 0.5</td>
<td>4.6 ± 0.5</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>Urge</td>
<td>0.6 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>rTMS 10 Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.6 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>3.9 ± 0.4</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.3 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.4 ± 0.4</td>
<td>4.5 ± 0.5</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>rTMS Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.4 ± 0.3</td>
<td>3.4 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.1 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>1.9 ± 0.1</td>
<td>2 ± 0.2</td>
<td>2 ± 0.2</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>4.4 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 subjects.
Fig 4.7 Effects of rTMS on anal sphincter sensory threshold. Data are represented as group % change following different interventions.

Fig 4.8 Effects of rTMS on anal sphincter pain. Data are represented as group % change following different interventions.

* p<0.05
4.6 Discussion

In summary, these experiments studied the effects of peripheral (rLSMS) and cortical (rTMS) excitatory (10 Hz) and inhibitory (1 Hz) paradigms and sham stimulation on sensory and pain thresholds of the anal sphincter and rectum recorded immediately, 30 and 60 min after each intervention in healthy volunteers. Electrical stimulation was used to measure sensation (sensory threshold and pain) arising from the anorectal area.

These data demonstrate that 1 Hz rLSMS and 10 Hz rTMS causes an increase in rectal pain thresholds immediately, 30 and 60 min after each intervention. 10 Hz rLSMS and 1 Hz rTMS only led to a significant reduction in rectal sensitivity immediately after its application. Furthermore, there was a significant increase in rectal sensory threshold following 1 Hz and 10 Hz rLSMS only at 60 min and immediately, respectively. There was no significant change in rectal sensory threshold following 1 Hz and 10 Hz rTMS.

By contrast, the application of 1 Hz and 10 Hz rLSMS did not show any effects on anal sphincter pain threshold. Only 1 Hz rLSMS led to a significant increase in anal sphincter sensory threshold immediately, 30 and 60 min post intervention. However, there was a significant rise in anal sphincter pain thresholds immediately and at 30 min following 10 Hz rTMS. In addition, 1 Hz rTMS caused a rise in anal sphincter pain threshold only at 60 min post intervention. Both 10 Hz and 1 Hz rTMS had no effect on anal sphincter sensory thresholds. All sham rTMS and rLSMS did not demonstrate any change in sensory and pain thresholds of anus and rectum.

This study demonstrated that short-term repetitive MS delivered over the lumbosacrum and anorectal motor cortex induces a post stimulus effect on the sensory pathway of the anorectum that is both frequency and time dependent in healthy subjects.

The use of high frequency rTMS over the primary motor cortex (especially 10 Hz and above) to reduce chronic pain is well documented in the literature [136-137, 140-142, 145]. Also there were similar results when other cortical brain regions
were stimulated like the left prefrontal cortex which led in one study [150] to
increase in thermal pain thresholds when stimulated by 10 Hz rTMS in healthy
volunteers. There have been some promising results with the use of low
frequency rTMS (1 Hz and below) in pain relief as stimulating right secondary
somatosensory region by 1 Hz rTMS caused a significant pain relief in patients
suffering from chronic pancreatitis [193].

The pain reduction effects of rTMS over the motor cortical area was also noted to
be enhanced and last longer with the increase in the frequency and intensity of the
simulation and with longer durations and more frequent therapeutic sessions [132].
Additionally, Hirayama and his team [145] found that stimulation primary motor
cortical area with 5 Hz rTMS resulted in a significant and better pain control
compared with the use of 5 Hz rTMS over other cortical areas (primary sensory
area, premotor cortex and supplementary motor area).

The exact mechanism behind the pain relief effect of rTMS on motor cortical area
is still unclear but the putative mechanism is thought to be related to the effect of
rTMS on the superficial layers of the motor cortex which have a wide spread
connections to other areas of the brain including thalamo cortical, cortico cortical
and local cortical projections which will result finally to a series of synaptic events
leading to modulation in an widespread neural network that includes thalamic
nuclei, limbic system, brainstem nuclei, and spinal cord [132, 195].

By contrast, there was not much work on the use of rLSMS to modulate sensation
and pain. There was one animal study [160] that proved that high intensity
magnetic stimulation (20 Hz) over the lumbosacral area of rats resulted in
reduction in pain induced by both mechanical and heat stimuli. These pain relief
effects required an intact supraspinal pathway and were opioidergic mediated as
these effects were abolised by the use of naloxone (a well-known opiate
antagonist) [160]. Similarly in a double blind placebo controlled trial, 10 Hz non-
invasive MS over the sacral region of 10 lumbosacral spondylotic patients resulted
in a significant pain improvement up to 4 days after its use compared with sham
[194].
The differential effects of pain vs. sensation

The results of these studies may indicate that rTMS effects mainly 10 Hz on anorectal pain is widespread and pain specific, both visceral and somatic and has no effect on anorectal sensation while rLSMS especially 1 Hz is more specific to the visceral pain originating from rectal area compared with more somatic type of pain originating from anus, as well as affecting sensation in both rectum and anus. It seems that pain might be a more useful and responsive perception to manipulate. These observations may be explained by the following putative mechanisms of action of rTMS and rLSMS.

Mechanisms of Action of rTMS

Repetitive TMS over anorectal primary motor cortex reduced artificially induced acute anorectal pain but the mechanisms underlying its modulatory effects remain poorly understood. Some suggestions resulted from electrophysiological and PET studies [154-155, 196] showed that cerebral blood flow was found to increase mainly in thalamus following the stimulation of the motor cortex and in the orbitofrontal and anterior cingulate cortex, the anterior insula and upper brainstem adjacent to the periacqueductal grey matter. Activation of cingulate and orbitofrontal area would play a part in a modulation of affective and emotional element of pain, whereas descending stimulation of the brainstem would prevent the transmission of discriminative nociceptive signals [154-155, 196].

Moreover, there is growing evidence that motor cortex stimulation using implanted electrodes [197] and rTMS [198] might implicate endogenous opioids systems in the pain reduction effects. Additionally, it was demonstrated that naloxone reverses the pain relief effects of epidural motor cortex stimulation in the rat [199] and significantly reduced the antinociceptive effects of rTMS of motor cortex stimulation [198]. However, naloxone did not change the effects of rTMS of the dorsolateral prefrontal cortex or sham [198]. This difference in the effects of naloxone on rTMS stimulation of motor cortex and dorsolateral prefrontal cortex indicates that the pain relieving effects induced by stimulating the two cortical areas are implicated by different mechanisms.
Mechanisms of Action of rLSMS

Repetitive LSMS modulated artificially induced acute rectal sensation and pain but the mechanisms underlying these responses remain unknown although effects may occur at both spinal and/or supraspinal levels. Stimulation of lumbosacral region could lead to direct activation of the peripheral afferent fibers and induce sensory input to spinal cord. Furthermore, these sensory inputs could also spread to the supraspinal level, where it might modulate the excitability of cortico-rectal pathways and evoke compensatory changes within the cerebral cortex. It was reported that the local application of repetitive magnetic stimulation successfully led to a decrease in musculoskeletal pain for several days [121] and a long period of peripheral magnetic stimulation application resulted in modulation of the response of primary and secondary somatosensory cortices to afferent input [200].

The results of my studies were limited to the parameters of rLSMS and rTMS and the duration of application as until now there was no “golden rule” to choose the perfect intensity, number of pulses and the duration for optimal neuromodulation effects. Moreover, although I used anal sphincter and rectal first sensation and pain as a way of comparing between the effects of rTMS and rLSMS on modulating somatic and visceral pain, however, a use of leg and hand sensory and pain thresholds as other controls could illustrate more the specificity of rTMS and rLSMS sensory and pain modulating effects to anorectal region or beyond that to a generalised effect reaching leg and/or hand as well.

In conclusion, this study showed that rLSMS and rTMS led to changes in the anorectal sensation and pain which are frequency and time dependent. These paradigms could be translated into patients with chronic visceral pain such as IBS as a future therapeutic intervention.
CHAPTER 5

THE EFFECTS OF NON-INVASIVE
REPETITIVE MAGNETIC STIMULATION ON
ANORECTAL SENSATION IN IBS PATIENTS
5.1 Introduction

In chapter 4, I reported that rLSMS and rTMS over the anorectal motor area have frequency-specific and time-dependent effects on the anorectal sensation and pain thresholds in healthy volunteers. One Hz rLSMS and 10 Hz rTMS consistently increased the rectal pain thresholds up to 60 min following their application.

In this chapter, I explored the effects of 1 Hz rLSMS and 10 Hz rTMS on anorectal sensory and pain modulation in IBS patients as this potentially may provide a safe method to manage visceral pain in IBS patients.

Visceral pain is the main and most difficult symptom to manage in IBS and many IBS female sufferers compare it to labour pain in its severity [1]. In 2006, Rome III diagnostic criteria for IBS was published and stated that recurrent abdominal pain or discomfort must be for at least 3 days a month in the past 3 months, associated with two or more of the following: 1) improvement with defecation, 2) onset associated with a change in frequency of stool, or 3) onset associated with a change in form (appearance) of stool, with the above symptoms beginning at least 6 months before the diagnosis [9].

Visceral and somatic sensation and pain are represented in the same cortical areas; the primary (SI) and the secondary somatosensory cortex (SII). Differences occur between healthy volunteers and IBS patients in pain processing in the prefrontal cortex, insula, cingulate cortex and thalamus as confirmed by functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) [22].

Additionally, during balloon inflation, IBS patients showed increased activity in cortical areas responsible for attention and affect, subcortical areas responsible for autonomic reaction and abnormalities in areas responsible for pain regulation [23].

Recently, Arebi et al [201] measured rectal sensory and pain thresholds to electrical stimulation and cerebral evoked potentials (CEPs) to evaluate the neurophysiological patterns in 33 IBS patients and 21 healthy volunteers. They showed significant differences in 3-day mean rectal pain threshold (using electrical
stimulation) and cortical evoked potential (CEP) amplitudes and latencies between IBS patients and controls.

Repetitive TMS was able to alter resistant neurogenic pain of the limbs [135] and help in the management of intractable chronic pain syndromes [141]. More recently, repetitive magnetic stimulation to the cortex has been used as a method to alleviate visceral pain due to chronic pancreatitis [193].

Additionally, non-invasive MS applied over the sacral area resulted in a significant reduction of pain immediately and at four days after this intervention in patients suffering from lumbosacral pain [194].

Similarly, sacral nerve stimulation (SNS) was used successfully to treat pelvic pain syndromes including functional anorectal pain disorders [167-168].

Lundby et al showed that temporary percutaneous magnetic stimulation of sacral nerves provided a significant reduction in diarrhoea-predominant IBS including a substantial pain relief and improved quality of life [151]. Additionally, stimulation of the sacral nerves in another study reduced the symptoms in selected people with constipation and showed a reduction in abdominal pain and bloating from 79% of the time without stimulation to 33% during the stimulation period [202].

Visceral pain is a major clinical problem being a primary feature of IBS. Treatments for this condition are limited but neural stimulation could play part in this regard. Therefore, it is of interest to investigate whether neurostimulation has a role in the management of visceral pain and hypersensitivity. Thus, having determined the optimum magnetic stimulation rates to alter pain sensation in healthy volunteers, in this chapter, I decided to use the same stimulation techniques over the anorectal cortical area and lumbosacrum to try to alter anorectal sensation and pain experienced by IBS patients. It is of great interest to know if IBS patient will respond to rTMS and rLSMS in as same manner as in healthy volunteers.
5.2 Aim

The purpose of the study is to ascertain whether non-invasive repetitive magnetic stimulation applied to either the motor cortex or lumbosacrum can modulate gastrointestinal pain originating from the anorectum in IBS patients.

5.3 Hypothesis

My hypothesis is that non-invasive repetitive MS over the anorectal motor cortex and/or lumbosacral area can modulate anorectal pain sensation in IBS patients.

5.4 Methods

Ethics

The research protocol was approved by the Northwest 8 Research Ethics Committee - Greater Manchester and all experiments were undertaken in the clinical laboratories of the Gastrointestinal Sciences Department at Salford Royal NHS Foundation Trust, UK, in accordance to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Sample size calculation and randomisation

The number of IBS patients required to participate in this study was determined according to a power calculation performed by the Medical Statistics department at Salford Royal Foundation Trust, UK, based on the results of the healthy volunteers’ study demonstrated in chapter 4. Sample size calculation on StatsDirect for a paired t-test (applying the Bonferroni correction) revealed that 10 IBS patients would be needed to achieve a power of 80% and statistical significance of 1.25% (with at least 1 standard deviation change in anorectal pain thresholds in mA), given that these parameters provided substantial results in healthy volunteers.

The three different conditions (1 Hz rLSMS, 10 Hz rTMS and sham) were randomised for all IBS patients’ visits using the block randomisation option of the statistical software StatsDirect (Version 2.7, StatsDirect Ltd, Cheshire, UK).
**Participants**

Ten IBS patients (n=10) were recruited (nine females, age range 22-54 years). All IBS patients fulfilled Rome III criteria for IBS. They classified into three diarrhoea predominant and seven constipation predominant IBS patients. Exclusion criteria were a history of epilepsy, a cardiac pacemaker, previous brain surgery, implanted metal in the head, eyes or lumbosacral spine, taking medication which is active in the central nervous system and pregnancy. Informed consent was obtained before the start of the experiments.

**Questionnaire**

IBS patients were asked to complete the Hospital Anxiety and Depression Scale (HAD) [181] and IBS severity questionnaire [182]. HAD scale used to obtain both Anxiety (A) and Depression (D) scores from 0-21. HAD scale results were interpreted as following: 0-7=Normal, 8-10=Mild, 11-14=Moderate and 15-21=Severe (Appendix 1). IBS severity questionnaire maximally scores 500 and its results analysed as following: 75-175=Mild, 175-300=Moderate and >300=Severe (Appendix 2). These questionnaires were used to give me an idea about the psychological and physical status of the IBS patients.

**Protocol**

- Bowel preparation was avoided to prevent any irritation to the anorectal area. However, IBS patients were encouraged to empty their rectum before starting the study.

- IBS patients were positioned in the left lateral position with knees and hips flexed to 90°.

- An anal plug (Figure 2.3) was positioned in the anal canal and a rectal catheter (Gaeltec, Dunvegan, Isle of Skye, UK) was passed through a hole in the anal plug into the rectum and positioned 10 cm from the anus.
• IBS patients were left to rest for 10 minutes to allow for any irritation or anorectal contractions to subside.

• The anal plug and then the rectal catheter were connected to a constant-current stimulator (model DS7A, Digitimer, Hertfordshire, UK).

• For both anal and rectal electrical stimuli were delivered to the IBS patients at a frequency of 0.5 Hz, with square wave pulses of 500 μs duration, at intensities between 0 and 100 mA.

• Sensory threshold is the stimulus intensity required for a single electrical stimulus to cause the IBS patient to first feel a definite sensation in the anal sphincter and rectum using an ascending method (0.2 and 2 mA increments, respectively). This was repeated three times for each set of measurements and the mean sensory threshold was calculated for each IBS patient.

• Pain threshold is the stimulus intensity required for the electrical stimulation to cause the IBS patient to feel the maximum tolerable pain in the anal sphincter and rectum using an ascending method (0.2 and 2 mA increments, respectively). This was repeated three times for each set of measurements and the mean pain threshold was calculated for each IBS patient.

• After each set of measurements, the IBS patients were also asked to score the pain intensity, unpleasantness and urge intensity using categorical rating scales (visual analogue score “VAS”) as shown in the appendices 3-5. The description anchors used for the pain and urge were faint, weak, mild, moderate, strong, and intense. The description anchors for the unpleasantness were mild, discomforting, distressing, horrible, and excruciating.
• In addition, the IBS patients were asked to describe the quality of the anorectal pain experienced by using a list of words from the short form of the McGill Pain Questionnaire [102] and by scoring the pain on scale from 0 to 10, where 0 means no pain and 10 indicates the worst pain ever they had experienced before (Appendix 6).

• The randomised intervention was then delivered (Figure 5.1).

• The assessment of anorectal sensation and pain was repeated immediately, 30 min and 60 min after each intervention.
Fig 5.1 Study design chart.

IBS patients

Lumbosacral stimulation

Baseline measurements:
- Anal sensory and pain thresholds
- Rectal sensory and pain thresholds

Randomisation: 600 pulses rLSMS

1 Hz

Motor cortical stimulation

Baseline measurements:
- Anal sensory and pain thresholds
- Rectal sensory and pain thresholds

Randomisation: 600 pulses rTMS

sham

10 Hz

Post intervention anorectal sensory and pain threshold measurements immediately and at 30 and 60 min
**Data handling**

Recorded data were transferred to the statistical software StatsDirect (Version 2.7, StatsDirect Ltd, Cheshire, UK) for analysis.

The electrical output in milliamps (mA) was taken as a measure of sensory and pain thresholds both in anal sphincter and rectum. Each sensory and pain measurements were repeated 3 times and the means used for the analysis of the results. Data were then analysed with analysis of variance (ANOVA). Categorical data from visual analogue scores were analysed with Pearson's chi-squared test.

Data in the tables are shown as mean ± standard error of mean (SEM) and in the graphs as a percentage of change from the baseline. The level of significance for all calculations was set at the 95% confidence level ($P < 0.05$).

**5.5 Results**

Ten IBS patients finished the whole study (3 visits) without any adverse events (Table 5.1). Their average HAD anxiety and depression scores were 5.1 ± 0.5 and 3.9 ± 1.3, respectively, which is considered to be within the normal range of anxiety and depression. Furthermore, their average IBS severity score was 206 ± 18.7, which is regarded as moderate in severity.

Baseline rectal sensory threshold, rectal pain threshold, anal sensory threshold and anal pain threshold representations, prior to the application of the different interventions, were similar across all arms, as shown with Friedman’s test (rectal sensory representation: Chi squares: 0.2, $p=0.91$, rectal pain representation: Chi squares: 2.4, $p=0.32$, anal sensory representation: Chi squares: 1.8, $p=0.43$, anal pain representation: Chi squares: 0.2, $p=0.91$).
Table 5.1 IBS patients’ profile.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender</th>
<th>Age</th>
<th>IBS</th>
<th>Abdominal pain</th>
<th>IBS score</th>
<th>HAD A</th>
<th>HAD D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>35</td>
<td>C</td>
<td>mild</td>
<td>210</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>47</td>
<td>D</td>
<td>moderate</td>
<td>275</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>43</td>
<td>D</td>
<td>moderate</td>
<td>290</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>35</td>
<td>C</td>
<td>mild</td>
<td>170</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>35</td>
<td>C</td>
<td>mild</td>
<td>160</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>40</td>
<td>C</td>
<td>moderate</td>
<td>260</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>45</td>
<td>D</td>
<td>mild</td>
<td>195</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>22</td>
<td>C</td>
<td>mild</td>
<td>145</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>32</td>
<td>C</td>
<td>mild</td>
<td>115</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>54</td>
<td>C</td>
<td>moderate</td>
<td>240</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

All IBS patients involved are shown. C stands for constipation predominant IBS, D stands for diarrhoea predominant IBS, A stands for anxiety and D stands for depression.

5.5.1 Effects of peripheral and cortical stimulation on rectal sensory function

One Hz rLSMS: There was a significant increase in pain thresholds in the rectum immediately, at 30 and 60 min following 1 Hz rLSMS (p=0.015, 0.048 and 0.022, respectively) as shown in table 5.2 and figure 5.3. In addition, there was an increase in sensory thresholds in the rectum following 1 Hz rLSMS immediately and at 30 and 60 min (p=0.014, 0.004 and 0.012, respectively) (Table 5.2 and figure 5.2).

Ten Hz rTMS: There was a significant increase in pain thresholds in the rectum immediately, 30 and 60 min following 10 Hz rLSMS (p=0.046, 0.041 and 0.017, respectively) as illustrated in table 5.2 and figures 5.3. Ten Hz rTMS also caused a significant increase in sensory thresholds immediately and at 30 and 60 min (p=0.005, 0.02 and 0.007, respectively), as shown in table 5.2 and figure 5.2.

Sham: Sham had no measurable effect on sensory and pain thresholds.

There was no significant change in pain characteristics as measured by visual analogue scores and the short-form McGill Pain Questionnaire following all interventions (Table 5.3).
### Table 5.2 Sensory stimulation of the rectum values (mA) across each time point for 1 Hz rLSMS, 10 Hz rTMS and sham stimulation.

<table>
<thead>
<tr>
<th>Rectum</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rLSMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>22.6 ± 2.8</td>
<td>27.1 ± 3.4*</td>
<td>28.8 ± 3.5*</td>
<td>29.6 ± 3.6*</td>
</tr>
<tr>
<td>P</td>
<td>45.9 ± 6.4</td>
<td>53.8 ± 6.5*</td>
<td>53.8 ± 6.8*</td>
<td>56.9 ± 8.2*</td>
</tr>
<tr>
<td><strong>rTMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>24.4 ± 2.7</td>
<td>27.9 ± 3*</td>
<td>29.1 ± 3.8*</td>
<td>29.3 ± 3.6*</td>
</tr>
<tr>
<td>P</td>
<td>44.1 ± 5.5</td>
<td>52.9 ± 8.7*</td>
<td>54.6 ± 8.9*</td>
<td>57.1 ± 8.9*</td>
</tr>
<tr>
<td><strong>Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>21.3 ± 3</td>
<td>23.3 ± 3</td>
<td>24.1 ± 3.3</td>
<td>23.2 ± 2.8</td>
</tr>
<tr>
<td>P</td>
<td>38.7 ± 3.4</td>
<td>38.6 ± 3.2</td>
<td>39.8 ± 3.6</td>
<td>40.4 ± 3.5</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 10 IBS patients, sensory threshold (ST) and pain (P). Significant changes are shown in bold. * p<0.05.

### Table 5.3 Questionnaire and VAS based rectal pain characteristics at baseline and immediately, 30 min and 60 min following 1 Hz rLSMS, 10 Hz rTMS and sham.

<table>
<thead>
<tr>
<th>Rectum</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rLSMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3 ± 0.9</td>
<td>2.3 ± 0.7</td>
<td>2 ± 0.7</td>
<td>3 ± 0.9</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.9 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>1.9 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>5.6 ± 0.4</td>
<td>5.7 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>5.9 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>2.5 ± 0.5</td>
<td>2.9 ± 0.6</td>
<td>2.9 ± 0.6</td>
<td>3 ± 0.6</td>
</tr>
<tr>
<td><strong>rTMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.8 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2 ± 0</td>
<td>2 ± 0.02</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>5.6 ± 0.4</td>
<td>5.7 ± 0.3</td>
<td>5.6 ± 0.3</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>1.9 ± 0.4</td>
<td>2 ± 0.5</td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td><strong>Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.2 ± 0.9</td>
<td>3.1 ± 0.9</td>
<td>3.1 ± 0.9</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td>Intensity</td>
<td>4.2 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2.1 ± 0.2</td>
<td>2 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>5.8 ± 0.4</td>
<td>6 ± 0.4</td>
<td>5.7 ± 0.4</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>2 ± 0.6</td>
<td>2.3 ± 0.6</td>
<td>1.6 ± 0.5</td>
<td>2 ± 0.5</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 10 IBS patients.
Fig 5.2 Effects of 1 Hz rLSMS, 10 Hz rTMS and sham on rectal sensory threshold. Data are represented as group % change following different interventions.

Fig 5.3 Effects of 1 Hz rLSMS, 10 Hz rTMS and sham on rectal pain threshold. Data are represented as group % change following different interventions.
5.5.2 Effects of peripheral and cortical stimulation on anal sphincter sensory function

One Hz rLSMS: 1 Hz rLSMS had no effect on anal sphincter sensation and pain (Table 5.4 and figures 5.4 and 5.5).

Ten Hz rTMS: 10 Hz rTMS also showed no effect on anal sphincter sensation but significantly increased pain thresholds immediately and at 30 and 60 min following its application (p=0.032, 0.004 and 0.001, respectively) as illustrated in table 5.4 and figures 5.4 and 5.5.

Sham: Sham had no effects on sensory and pain thresholds (Table 5.4 and figures 5.4 and 5.5).

There was no significant change in pain characteristics as measured by visual analogue scores and the short-form McGill Pain Questionnaire following all interventions as shown in table 5.5.

Table 5.4 Sensory stimulation of the anal sphincter values (mA) across each time point for 1 Hz rLSMS, 10 Hz rTMS and sham stimulation.

<table>
<thead>
<tr>
<th>Sphincter</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>rLSMS 1 Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>6.3 ± 0.7</td>
<td>7.3 ± 1.2</td>
<td>7.1 ± 1.1</td>
<td>6.7 ± 1</td>
</tr>
<tr>
<td>P</td>
<td>24.3 ± 5.2</td>
<td>23.6 ± 5.1</td>
<td>25.2 ± 4.9</td>
<td>25.6 ± 4.7</td>
</tr>
<tr>
<td>rTMS 10 Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>5.3 ± 0.6</td>
<td>5.5 ± 0.7</td>
<td>5.4 ± 0.8</td>
<td>6 ± 0.7</td>
</tr>
<tr>
<td>P</td>
<td>23.3 ± 5.3</td>
<td>25.9 ± 6*</td>
<td>27 ± 5.4*</td>
<td>29.4 ± 5.9*</td>
</tr>
<tr>
<td>sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>5.8 ± 0.7</td>
<td>5.8 ± 0.6</td>
<td>6.4 ± 0.7</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td>P</td>
<td>22 ± 3.3</td>
<td>22.3 ± 3.1</td>
<td>24 ± 3.7</td>
<td>23.7 ± 3.8</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 10 IBS patients, sensory threshold (ST) and pain (P). Significant changes are shown in bold. * p<0.05.
Table 5.5 Questionnaire and VAS based anal sphincter pain characteristics at baseline and immediately, 30 min and 60 min following 1 Hz rLSMS, 10 Hz rTMS and sham.

<table>
<thead>
<tr>
<th>Anal sphincter</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rLSMS 1 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>4 ± 0.4</td>
<td>4.5 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>Intensity</td>
<td>4 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>1.9 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>5.3 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>1.6 ± 0.6</td>
<td>1.6 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td><strong>rTMS 10 Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.9 ± 0.2</td>
<td>3.9 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>5.5 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Urge</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.6</td>
<td>1.5 ± 0.6</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td><strong>Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain type</td>
<td>3.7 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Intensity</td>
<td>4.1 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>4 ± 0.3</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Unpleasantness</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Score out of 10</td>
<td>5.6 ± 0.4</td>
<td>5.4 ± 0.4</td>
<td>5.5 ± 0.3</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>Urge</td>
<td>1.1 ± 0.4</td>
<td>1.5 ± 0.5</td>
<td>1.6 ± 0.5</td>
<td>1.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 10 IBS patients.

Fig 5.4 Effects of 1 Hz rLSMS, 10 Hz rTMS and sham on anal sphincter sensory threshold. Data are represented as group % change following different interventions.
Fig 5.5 Effects of 1 Hz rLSMS, 10 Hz rTMS and sham on anal sphincter pain. Data are represented as group % change following different interventions.

5.6 Discussion

In summary, these experiments studied the effects of peripheral (1 Hz rLSMS) and cortical (10 Hz rTMS) paradigms and sham stimulation on sensory and pain thresholds of the anal sphincter and rectum recorded immediately, 30 and 60 min after each intervention in IBS patients. Electrical stimulation was used to measure sensation (sensory threshold and pain) arising from the anorectal area.

These data show that 1 Hz rLSMS and 10 Hz rTMS cause an increase in rectal pain thresholds significantly for up to 60 min after the intervention. On the other hand, only 10 Hz rTMS led to a significant rise in anal sphincter pain immediately and at 30 and 60 min after its application. Moreover, there was a significant increase in rectal sensory threshold following 1 Hz rLSMS and 10 Hz rTMS immediately, 30 and 60 min post intervention. There was no significant change in anal sphincter sensory threshold following 1 Hz rLSMS and 10 Hz rTMS. Sham rLSMS did not demonstrate any change in sensory and pain thresholds of anus and rectum.

This study demonstrated that short-term repetitive MS delivered over the lumbosacrum and anorectal motor cortex induces a post stimulus effect on the
pain and sensory pathways innervating the anorectum that is both frequency and time dependent in IBS patients.

The use of high frequency rTMS over the primary motor cortex is well known to reduce pain in patients suffering from chronic somatic pain syndromes [136-137, 140-142, 145]. In addition, there have been encouraging results with the use of low frequency (1 Hz) rTMS in visceral pain relief by stimulating right secondary somatosensory region in patients suffering from chronic pancreatitis [193].

There were few studies where rLSMS have been tried to modulate sensation and pain. Recently, Lo and his colleagues [194] have managed to reduce pain in patients suffering from chronic lumbosacral pain by applying non-invasive magnetic stimulation at 10 Hz over the sacral area. Similarly, back pain relief were observed in addition to improving urinary symptoms when patients with intractable neurogenic urinary bladder dysfunction secondary to lumbosacral nerve injuries were treated with 15 Hz rLSMS [161].

Furthermore, sacral nerve stimulation (SNS) application led to a significant pain reduction in patients with chronic functional anorectal pain disorders not responding to conventional treatments [167-168]. Temporary SNS also provided a significant reduction in diarrhoea-predominant irritable bowel symptoms including a substantial pain relief and improved quality of life [151].

**The differential effects of pain vs. sensation**

These results indicate that 10 Hz rTMS effects anorectal pain both visceral (rectal) and somatic (anal sphincter) in IBS patients while 1 Hz rLSMS is more specific to the visceral sensation and pain originating from rectal area. Again, it seems that pain might be a more useful and responsive perception to manipulate in IBS patients.

**The differential effects of healthy volunteers vs. IBS patients**

The effects of 1 Hz rLSMS and 10 Hz rTMS on increasing rectal pain thresholds were similar in healthy volunteers and IBS patients. However, there were some
differences regarding anorectal sensation as 10 Hz rTMS showed a significant increase in rectal sensory thresholds in IBS patients but not in healthy volunteers. Additionally, 1 Hz rLSMS showed no effect on anal sphincter sensation in IBS patients while its effect led to a significant increase in anal sphincter sensation in healthy volunteers. This may confirm previously known differences between IBS patients and healthy volunteers in response to electrical stimulation [201] and balloon distension [23].

**Mechanisms of Action of rTMS**

As discussed in chapter 4, the mechanisms underlying the rTMS modulatory effects on rectal pain remain poorly understood. It is thought to be related to the effect of rTMS on the superficial layers of the motor cortex which have a widespread spread connections to other areas of the brain including thalamo cortical, cortico cortical and local cortical projections which will result finally to a series of synaptic events leading to modulation in an widespread neural network that includes thalamic nuclei, limbic system, brainstem nuclei, and spinal cord [132, 195].

Electrophysiological and PET studies [154-155, 196] support this theory by showing that cerebral blood flow was found to increase mainly in thalamus following the stimulation of the motor cortex and in the orbitofrontal and anterior cingulate cortex, the anterior insula and upper brainstem adjacent to the periaquiductal grey matter.

Furthermore, there is growing evidence that motor cortex stimulation using rTMS [198] might implicate endogenous opioids systems in the pain reduction effects. Additionally, it was demonstrated that naloxone reverses the pain relief effects of epidural motor cortex stimulation in the rat [199] and significantly reduced the antinociceptive effects of rTMS of motor cortex stimulation [198].

**Mechanisms of Action of rLSMS**

As mentioned in chapter 4, the mechanisms underlying the effects of rLSMS on anorectal pain remain unknown although these effects may occur at both spinal and/or supraspinal levels. Stimulation of lumbosacral region could lead to direct
activation of the peripheral afferent fibres and induce sensory input to spinal cord as it was shown that the local application of repetitive magnetic stimulation successfully led to a decrease in musculoskeletal pain for several days [121].

The sensory inputs could also convey to the supraspinal level, where it might affect the excitability of cortico-rectal pathways and induce compensatory changes within the cerebral cortex as it is known that a long period of peripheral magnetic stimulation application resulted in modulation of the response of primary and secondary somatosensory cortices to afferent input [200].

The results of my study were limited to the parameters of rLSMS and rTMS and the duration of application. Moreover, although I used only 1 Hz rLSMS and 10 Hz rTMS because they showed greater effect on rectal pain and in order to make the study more convenient for IBS patients, the use of 10 Hz rLSMS and 1 Hz rTMS might have shown a better results in IBS patients compared with their modest effect in healthy volunteers.

In addition, measuring leg and hand sensory and pain thresholds as other controls was only omitted to reduce the time of experiments for IBS patients, even though, it could have illustrated how specific are rTMS and rLSMS sensory and pain modulating effects to anorectal region.

In conclusion, this study showed that 1 Hz rLSMS and 10 Hz rTMS led to changes in the anorectal sensation and pain in IBS patients which are frequency and time dependent. The anticipation is that these paradigms, once explored more formally in dose response studies of treatment scheduling and intervals between sessions could be used in IBS patients as a future therapeutic intervention.
CHAPTER 6

GENERAL DISCUSSION
6.1 Introduction

As each chapter contains its own discussion chapter 6 will summarise the most important aspects of the individual chapters and bring together the results from these section to provide an overview of the work contained within this thesis and its limitation. The discussion will conclude with suggestions for future research, build on the findings described here and explore their potential clinical application.

6.2 Summary of chapters

Chapter 1 discussed the background of IBS, its pathophysiology, and its current management modalities. I then provided an overview about the pain matrix. Physiological methods for evaluating anorectal sensation were discussed and techniques for modulating the nervous system were reviewed, including a detailed section on non-invasive magnetic stimulation and its role in pain management.

Chapter 2 described in detail the materials and methods which were used in this project.

Chapter 3 examined the effects of two repetitive modes of magnetic stimulation LSMS, TMS and sham on the anal sphincter contractility in healthy volunteers and showed that only 1 Hz rLSMS modulates lumbosacral-anal neural excitability at 30 min post intervention.

Chapter 4 comprises a series of studies investigating the effects of rLSMS, rTMS and sham on the anorectal sensation and pain in healthy volunteers. This showed that the optimal parameters for rectal visceral pain reduction were 1 Hz rLSMS and 10 Hz rTMS.

Chapter 5 explored the effects of 1 Hz rLSMS, 10 Hz rTMS and sham on the anorectal sensation and pain in IBS patients. This study demonstrated that 1 Hz rLSMS and 10 Hz rTMS were successfully able to modulate rectal visceral pain in IBS patients in a similar way as in healthy volunteers.
6.3 Overview of discussion points in thesis

6.3.1 Novel findings

Although rLSMS has been used before to try to modulate anal sphincter contractility [203], this is the first time to my knowledge that 1 Hz rLSMS has been reported to modulate lumbosacral-anal motor excitability/contractility as shown in chapter 3.

This is also the first work to examine the effects of peripheral and cortical application of rLSMS and rTMS on anorectal sensory and pain thresholds in healthy volunteers and demonstrates that 1 Hz rLSMS can modulate rectal sensation and pain. Moreover, 10 Hz rTMS also has effects on rectal and anal pain as reported in chapter 4.

Furthermore, in chapter 5, for the first time, I have shown that 1 Hz rLSMS and 10 Hz rTMS were both able to reduce rectal sensation and pain in IBS patients up to 60 min post intervention. In addition, 10 Hz rTMS also led to a reduction in anal sphincter pain.

6.3.2 General discussion

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder (FGID) which can be defined as chronic, relapsing visceral pain and bloating associated with changes in bowel habit. It affects up to 10-15% of adult population in the UK [1]. The cost of IBS in terms of health care utilisation is substantial, exceeding £45.6 million per year in the UK alone [4] and the pathophysiology incompletely understood. Although the exact aetiology remains speculative, and is likely to be multifactorial, the prevailing theory is that transient noxious or inflammatory events, such as viral gastroenteritis, lead to long lasting sensitisation of the gut [15]. This sensitisation occurs in the absence of detectable organic disease and results in normal physiological contractions in the gut being perceived as painful [16]. It has been postulated that the symptoms experienced, which can be long lasting, are due to either the afferent nerves in the gut becoming sensitised due to previous inflammation or injury and/or the brain processing of gut sensation being
altered. To achieve a better understanding of IBS and the care of patients with these problems further detailed research into anorectal sensory processing and how visceral pain can be modulated is required.

Visceral pain is the main and most difficult symptom to manage in IBS and many IBS female sufferers compare it to labour pain in its severity [1]. Recently in 2006, Rome III diagnostic criteria for IBS have been published and stated that recurrent abdominal pain or discomfort must be for at least 3 days a month in the past 3 months, associated with two or more of the following: 1) improvement with defecation, 2) onset associated with a change in frequency of stool, or 3) onset associated with a change in form (appearance) of stool, with the above symptoms begin at least 6 months before the diagnosis [9].

Modulating visceral pain in healthy volunteers and IBS patients is therefore an important clinical research priority. Non-invasive magnetic stimulation can play a crucial role in this respect. Single pulse transcranial magnetic stimulation (TMS), according to the literature, is a safe, and well tolerated technology in neurophysiological investigations in both healthy subjects and patients. Similarly, repetitive TMS is recognised to have an excellent safety profile in patients and is generally well tolerated. The long term risks of both single pulse and repetitive TMS in adults are not considered significant according to most of the available data [116].

It is recognised that nervous tissue can be activated or inhibited depending on the frequency, intensity, direction and the duration of the repetitive magnetic stimulation (MS). Fast repetitive MS (>1 Hz) is generally excitatory, and on the other hand, slow repetitive MS (≤1 Hz) is inhibitory [115]. Repetitive TMS (1 Hz) has shown an inhibitory effect on cortical excitation in both the pharynx and hand muscles in healthy control subjects [133]. In addition, peripheral short-term magnetic stimulation delivered over the lumbosacrum induces a post-stimulus effect on the excitability of the anal sphincter motor cortex that is both frequency and muscle specific [124]. More recently, repetitive magnetic stimulation to the cortex has been used as a method to alleviate visceral pain due to chronic pancreatitis [193], alter resistant neurogenic pain of the limbs [135] and help in the
management of intractable chronic pain syndromes [141]. Since it is recognised that sensory and motor cortical regions of the brain are activated during artificial stimulation of the anorectum [180], the application of repetitive magnetic stimulation to these cortical and lumbosacral areas might alter the perception of sensation following afferent stimulation of the anorectum.

The purpose of this project was to determine the best site (cortical or lumbosacral) and frequency of magnetic stimulation that will modulate artificially induced visceral pain in IBS patients. Ultimately, this may lead to a development of a non-invasive pain management method to treat IBS patients.

The first study in chapter 3 showed that peripheral low intensity (1 Hz) rather than high intensity (10 Hz) rLSMS or cortical stimulations (1 and 10 Hz rTMS) modulated lumbosacral-anal motor excitability at 30 min post stimulus in healthy volunteers. This result may be explained by effects of peripheral MS centrally at spinal and supraspinal levels. It is known that low frequency rTMS stimulation (1 Hz or below) to the human motor cortex has shown decreased excitability to both the pharyngeal [133] and hand motor cortical areas [186], while the 1 Hz rLSMS has shown an increase in lumbosacral-anal excitability. This can be explained by the suggestion that 1 Hz rLSMS might favour cortical inhibition over excitation when applied to pelvic nerves such that the cortex might be inhibited while the peripheral spinal system is excited, hence the lack of latency change in the cortico-anal MEPs.

The second study in chapter 4 demonstrated that peripheral low intensity (1 Hz) rLSMS and cortical high intensity (10 Hz) rTMS were able to modulate rectal pain in healthy volunteers. The use of high frequency rTMS over the primary motor cortex (especially 10 Hz and above) to reduce chronic pain is well documented in the literature [136-137, 140-142, 145].

The pain reduction effects of rTMS over the motor cortical area was also noted to be enhanced and last longer when the frequency and intensity of the simulation was increased, with longer durations and more frequent therapeutic sessions [132].
Therefore, the third study in chapter 5 used 1 Hz rLSMS and 10 Hz rTMS to try to modulate artificially induced visceral pain in IBS patients. One Hz rLSMS and 10 Hz rTMS showed similar results and significantly reduced rectal pain in IBS patients. It seems that pain might be a more useful and responsive perception to manipulate in IBS patients.

In chapter 4 and 5, there was a significant difference between the baseline of anorectal sensation and pain in healthy volunteers and IBS patients. IBS patients showed higher anorectal pain thresholds compared with healthy volunteers. There is also a similar trend with anorectal pain intensity from VAS and scores out of 10 from short-form McGill pain questionnaire (table 1).

Harris and colleagues [101] assessed the baseline anorectal sensation and pain by electrical stimulation in eight healthy volunteers and their average first visit recordings were: anal sensory thresholds=3.7±1.2, anal pain thresholds=17.5±4.4, rectal sensory thresholds=13.1±2.7 and rectal pain thresholds=59.4±10.3. The only clear difference between my results and Harris et al is in higher rectal pain thresholds compared to my results.

Furthermore, Arebi and colleagues [201] have compared the rectal sensory and pain thresholds in 21 healthy volunteers and 33 IBS patients in three visits and showed that there were no significant difference of rectal sensory and pain thresholds in healthy volunteers and IBS patients in the first visit with average recordings as the following: ((healthy volunteers: rectal sensory thresholds=18.1 (15.2–20.9) and rectal pain thresholds=70.2 (60.2–80.2)) vs. (IBS patients: rectal sensory thresholds=18.7 (14.1–23.3) and rectal pain thresholds=57.7 (48.5–66.9)). They noticed a significant increase in IBS rectal sensory threshold in the second visit only and a significant decrease in IBS rectal pain thresholds in the third visit only than in healthy volunteers.

By comparing my results with Arebi et al baseline rectal measurements, the rectal pain is almost the same in my IBS patients and their IBS patients, while it is much lower in my group of healthy volunteers compared with their healthy volunteers. This difference in anorectal sensory and pain thresholds between healthy volunteers and IBS patients could be explained by the fact that seven out of 10 of
the IBS patients are predominantly constipated and may be already hyposensitive to stimuli (only six out of 33 IBS patients in Arebi et al study were constipation predominant).

Additionally, the low rectal pain thresholds in my group of healthy volunteers compared with other studies [101, 201] might be due to the subjectivity of perception of pain and a tendency of my particular group of healthy subjects to indicate discomfort at a lower level and anticipated. For example the VAS score for “pain” was 3.4 which is within the mild type of pain versus the average pain intensity in Harris et al [101] which was about 4.5 which is between moderate and severe intensity of pain on VAS. Why the group reported pain at a lower level is not clear, but possibly reflects the naivety of the group, and the open instructions for indicating discomfort given to the subjects.

However, although these differences in baseline measurements are of interest they do not alter my results as methodologically, I compared the effects of rTMS and rLSMS on anorectal sensation and pain of healthy subjects and IBS patients against their own baseline measurements. Moreover, if I had encouraged my healthy volunteers to go further with regards to their pain threshold perception to match the values obtained by Harris et al, presumably a similar percentage change would have occurred as I would also have encouraged them further post intervention.

By comparing the effects of 1 Hz rLSMS and 10 Hz rTMS on the rectal pain thresholds changes from the baseline, there were no significant differences between healthy volunteers and IBS patients as shown in table 6.2.
Table 6.1 Basal anorectal sensory and pain thresholds in mA and questionnaire and VAS based anorectal pain characteristics in my healthy volunteers and IBS patients.

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers</th>
<th>IBS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anal sensory thresholds</td>
<td>3.8±0.2</td>
<td>5.8±0.4*</td>
</tr>
<tr>
<td>Anal pain thresholds</td>
<td>10.4±0.5</td>
<td>23.2±2.6*</td>
</tr>
<tr>
<td>Anal pain type</td>
<td>3.3±0.1</td>
<td>3.9±0.2*</td>
</tr>
<tr>
<td>Anal pain intensity</td>
<td>3.3±0.1</td>
<td>4±0.1*</td>
</tr>
<tr>
<td>Anal unpleasantness</td>
<td>2±0.1</td>
<td>2±0.1</td>
</tr>
<tr>
<td>Anal urge</td>
<td>0.5±0.1</td>
<td>1.3±0.3*</td>
</tr>
<tr>
<td>Anal pain score out of 10</td>
<td>4.4±0.2</td>
<td>5.5±0.2*</td>
</tr>
<tr>
<td>Rectal sensory thresholds</td>
<td>16.4±1</td>
<td>22.8±1.6*</td>
</tr>
<tr>
<td>Rectal pain thresholds</td>
<td>27±1.1</td>
<td>42.9±3*</td>
</tr>
<tr>
<td>Rectal pain type</td>
<td>4.5±0.3</td>
<td>2.9±0.5*</td>
</tr>
<tr>
<td>Rectal pain intensity</td>
<td>3.4±0.1</td>
<td>4±0.1*</td>
</tr>
<tr>
<td>Rectal unpleasantness</td>
<td>2.1±0.1</td>
<td>2±0.1</td>
</tr>
<tr>
<td>Rectal urge</td>
<td>1.5±0.2</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>Rectal pain score out of 10</td>
<td>4.5±0.2</td>
<td>5.7±0.2*</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 healthy subjects and 10 IBS patients. Significant changes are shown in bold. * p<0.05.

Table 6.2 Percentage of change from the baseline of rectal pain thresholds following 1 Hz rLSMS and 10 rTMS in healthy volunteers and IBS patients.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Measurement</th>
<th>Healthy volunteers</th>
<th>IBS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hz rLSMS</td>
<td>Immediately</td>
<td>16.2±5.5</td>
<td>19.9±5.9</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>19.1±7.8</td>
<td>19±7</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>29.1±9.2</td>
<td>23±7.5</td>
</tr>
<tr>
<td>10 Hz rTMS</td>
<td>Immediately</td>
<td>14±4.3</td>
<td>15.4±7.2</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>15.7±4</td>
<td>21.7±8.9</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>17.1±4.5</td>
<td>29.9±9.4</td>
</tr>
</tbody>
</table>

Values reported are the mean (SEM) of 16 healthy subjects and 10 IBS patients.
Mechanisms of action of repetitive MS
The exact mechanism behind the pain relief effect of rTMS on motor cortical area is still unclear but it is thought to be related to the effect of rTMS on the superficial layers of the motor cortex which have a wide spread connections to other areas of the brain including thalamo cortical, cortico cortical and local cortical projections which will result finally to a series of synaptic events leading to modulation in an widespread neural network that includes thalamic nuclei, limbic system, brainstem nuclei, and spinal cord [132, 195].

There is a small volume of published studies on the use of rLSMS to modulate sensation and pain. In one animal study [160], high intensity magnetic stimulation (20 Hz) over the lumbosacral area of rats resulted in reduction in pain induced by both mechanical and heat stimuli. These pain relief effects required an intact supraspinal pathway and were opioidergic mediated as these effects were abolished by the use of naloxone (a well-known opiate antagonist) [160]. Likewise in one human study [194], 10 Hz non-invasive MS over the sacral region resulted in a significant pain improvement in 10 lumbosacral spindylotic patients up to 4 days after its use compared with sham.

Mechanisms of action of rTMS
Although there is a limited literature about the use of repetitive MS to modulate visceral pain in IBS patients, recently low frequency (1 Hz) rTMS has been applied over the right secondary somatosensory region has resulted in significant visceral pain relief in patients suffering from chronic pancreatitis [193]. In addition, the application of 10 Hz repetitive MS over sacral area managed to reduce pain in patients suffering from chronic lumbosacral pain [194]. Similarly, back pain relief were observed when patients with intractable neurogenic urinary bladder dysfunction secondary to lumbosacral nerve injuries were treated with 15 Hz rLSMS [161].

The mechanisms underlying the rTMS modulatory effects on rectal pain remain poorly understood. As mentioned before, It may be linked to the effect of rTMS on the superficial layers of the motor cortex with its wide spread connections to other areas of the brain including thalamic nuclei, limbic system, brainstem nuclei, and spinal cord [132, 195].
Electrophysiological and PET studies [154-155, 196] support this theory by showing that cerebral blood flow was found to increase mainly in thalamus following the stimulation of the motor cortex and in the orbitofrontal and anterior cingulate cortex, the anterior insula and upper brainstem adjacent to the periaqueductal gray matter.

There is growing evidence that rTMS over motor cortex [198] might involve endogenous opioids systems in the pain reduction effects. Additionally, it was demonstrated that naloxone significantly reduced the antinociceptive effects of rTMS of motor cortex stimulation [198].

**The mechanisms of action of rLSMS**

The mechanisms underlying the rLSMS rectal pain reduction effects remain unknown although these effects may occur at both spinal and/or supraspinal levels. Stimulation of lumbosacral region could lead to direct activation of the peripheral afferent fibres and induce sensory input to spinal cord as it was shown that the local application of repetitive magnetic stimulation successfully led to a decrease in musculoskeletal pain for several days [121].

The pain reduction effects of rLSMS could also be at the supraspinal level, where it might affect the excitability of cortico-rectal pathways and induce compensatory changes within the cerebral cortex as it is known that a long period of peripheral magnetic stimulation application resulted in modulation of the response of primary and secondary somatosensory cortices to afferent input [200] and repetitive MS over the lumbosacral area of rats resulted in pain relief effects which required an intact supraspinal pathway. These effects were also opioidergic mediated as they were abolished by the use of naloxone [160].

**6.3.3 Limitations of the experimental protocols**

The results of my studies were limited to the parameters of rLSMS and rTMS and the duration of application and post-stimulus measurement intervals as until now there was no “golden rule” to choose the perfect intensity, number of pulses and the duration for optimal neuromodulation effects.
Sample size calculations in healthy volunteers studies were based on previous work performed within the department of GI Sciences using rTMS [133], rLSMS [124] and anorectal electrical stimulation measurements [101] on healthy subjects. However, there remains the possibility that there may be subtle differences in the methodology of my studies compared to the previous ones, affecting the accuracy of the results. Since my studies are novel and were designed to be proof of concept, further investigations will be required to validate these methods.

In the IBS study, we used the healthy volunteers’ data to calculate the sample size even though there are recognised differences between the two populations. However, as there is no previous work performed in investigating the changes in the anorectal sensitivity in IBS patients using electrical stimulation, it was deemed appropriate to adoptive this approach. Nonetheless, the results of this IBS pilot study could also be used to inform a larger definitive study, once the feasibility of the interventions (1 Hz rLSMS and 10 Hz rTMS) have been assessed.

The studies were double blinded; all analysis of primary outcome measures were performed by a colleague who received anonymised data. The investigator was blinded to the intervention delivered (however, due to circumstances beyond the control of the investigators, blinding was not possible on four of the 96 of interventions delivered on to healthy volunteers). All patients and healthy volunteers were blinded to the stimulation paradigm delivered on each occasion.

In chapters 3, 4 and 5, I only used electrical stimulation to measure the anorectal sensation and pain due to its high reproducibility and tolerability compared with other anorectal sensory measurement techniques [101], the utilization of rapid balloon dilatation and/or barostat might have given more data to compare between the effects of rTMS and rLSMS on sensation and pain measured by these different techniques in healthy volunteers and IBS. The barostat is a more established technique used in IBS patients [104] and may have provided us with additional data of interest.

In chapter 3, only 600 pulses of rTMS at 90% sub threshold intensity and rLSMS were used for safety and tolerability which is relatively low to modulate sphincter muscle as Lou et al [203] used a total of 2250 pulses rLSMS to show some
changes in cortico-anal excitability after only 15 Hz rLSMS. This may explain why there was no measurable rTMS effect on anal sphincter excitability and no cortico-anal excitability change after 1 Hz rLSMS compared to lumbosacral-anal excitability.

In addition, although I only measured EMG recording at 30 min after different rLSMS and rTMS interventions for tolerability and also based on previous studies when maximum response where measured at 30 min [185-186], however, I might have missed possible changes before or after 30 min from each intervention.

In chapter 4 and 5, I used anal sphincter as a control due to its mainly somatic sensory pathway compared with the rectal visceral sensation and pain and omitting the use of leg and/or hand as other controls to reduce the duration and discomfort associated with these studies in healthy volunteers and IBS patients. Nevertheless, the use of leg and hand sensory and pain thresholds as other controls could have shown more the specificity of rTMS and rLSMS sensory and pain modulating effects to anorectal region or beyond that to a generalised effect reaching leg and/or hand as well.

In chapter 5, only 1 Hz rLSMS and 10 Hz rTMS were applied due to their greater effect on rectal pain in healthy volunteers study compared with other paradigms and in order to make the study more convenient for IBS patients, but the use of 10 Hz rLSMS and 1 Hz rTMS might have shown different effects on rectal pain of IBS patients compared with their effects on healthy volunteers.

6.4 Directions for future research

This thesis has examined the effects of rLSMS and rTMS on anal sphincter excitability in healthy subjects and anorectal sensation and pain in healthy volunteers and IBS patients. Therefore there are a number of ways in which this research could be taken forward.

6.4.1 Reproducibility studies

In the studies with healthy volunteers, there were two sham and four active interventions applied with at least a week apart which reduced any chance of
making false positive results. Moreover, the positive results were verified later with the study in IBS patients. It should be emphasised that baseline data across arms of the study were similar and stable suggesting that baseline values were reproducible, so reducing the likelihood of methodological effects altering my results. However, the conduct of formal reproducibility studies on both healthy volunteers and IBS patients using 1 Hz rLSMS and 10 Hz rTMS will further strengthen the validity of these results.

6.4.2 Outcome measures

In my current studies I used single pulse MS over motor cortex and lumbosacrum and electrical stimulation to measure the changes in responses of anal sphincter excitability and anorectal sensation and pain to rTMS and rLSMS, respectively. Many studies have used functional imaging tests such as fMRI (functional Magnetic Resonance Imaging), PET (Positron Emission Tomography) and magnetoencephalography to assess the cortical processes involved in GI motor excitabilities, somatic and visceral pain and the physiological responses to cortical stimulation of primary motor and lumbosacral areas. Using such techniques would enable a better understanding of how the multiple cortical areas involved in the control of anorectal sensation and pain are modified by cortical and/or lumbosacral stimulation and perhaps provide an idea of the temporal sequencing of these events. Understanding of intracortical inhibition and facilitation in order to elucidate the mechanisms by which changes in cortical excitability occur is very important future research area; these findings could be studied by the use of magnetic resonance spectroscopy to determine the levels of neurotransmitters such as glutamate and GABA before and following stimulation.

6.4.3 Repetitive Magnetic Stimulation

In my studies, I chose to use 1 and 10 Hz repetitive MS over anorectal area of primary motor cortex hypothesising that this would be the closest model to modulate anorectal pain in the same way is presumed to have occurred in other studies where rTMS have been used to reduce chronic somatic pain syndromes. I also know that there are other multiple areas involved in the control of anorectal motor and sensory functions and there is evidence to suggest that stimulating
these cortical areas such as the secondary sensory area may also improve anorectal pain and with anatomical MR images and Brain Site it is possible to accurately stimulate these areas, therefore it would seem logical to attempt a similar studies in these cortical areas, comparing its effects on how efficient in reducing pain in healthy volunteers and IBS patients.

Similarly, I used 1 and 10 Hz peripherally at lumbosacral area to try to modulate anorectal sensory and motor function. Stimulating posterior tibial nerve is known to modulate other pelvic organs and shows promising results similar to other lumbosacral nerves stimulating techniques and it may has a role in modulating anorectal area. Future studies are warranted by using local repetitive MS or electrical stimulation to stimulate posterior tibial nerve to try to reduce visceral pain in healthy volunteers and IBS patients.

6.4.4 Transcranial Direct Current Stimulation

I have shown that increasing the amount of stimulation from 1 Hz to 10 Hz increases the pain modulation of cortical excitability, but greater responses may be seen if the duration or intensity of stimulation is further increased. Therefore exploration of the effects of TDCS on anorectal primary motor cortex should also be studied if it is to progress as a therapeutic technique as it is a simple and painless tool, ideal for clinical use and the next stage would be to investigate the effects of stimulation on IBS patients.

6.4.5 Treatment of visceral pain in IBS patients

From the results of the few IBS patients described in this thesis 1 Hz rLSMS and 10 rTMS shows promise as a therapeutic tool for the treatment of visceral pain in IBS patients. One Hz rLSMS, in my opinion, is preferable as its application is straightforward and does not cause any discomfort and no potential side effects and its use will not be affected by concurrent use of pharmacological medications like antidepressants which is commonly used by IBS patients. By contrast, 10 Hz rTMS can be used in a subgroup of IBS patients who suffer from visceral pain and anal discomfort as in my studies 10 Hz rTMS has been shown to reduce both
rectal and anal pain. Future studies should investigate the number and frequency of treatments required to maintain and promote visceral pain relief in IBS patients.

My IBS patients’ pilot study could be used accurately to measure a sample size for a larger randomised, double-blind placebo controlled trial involving IBS patients with moderate to severe visceral pain. On balance, in considering both pragmatism and size of effect, I believe the most practical treatment protocol to be the use of 1 Hz rLSMS in daily sessions for 10 days per IBS patient compared with sham as the application of repetitive MS protocols in daily sessions for about 10 days has been shown to be effective to produce long term pain reducing effects as demonstrated by Khedr et al [137] and Fregni et al [193].

Neurostimulation techniques keep improving in design, portability, efficiency and ease of use as clinical investigative as well as therapeutic tools and new techniques continue to emerge and although investigated individually they should also be directly compared to ascertain if there is a ‘gold standard’ treatment for visceral pain in IBS and other functional GI conditions.
REFERENCES


APPENDICES
8. The following questions refer to your general well being over the last few weeks. Please place a tick in the box appropriate to the answer which most accurately describes how you feel.

Tick only one box in each section

a) I feel tense or wound up:
   - Most of the time: 3
   - A lot of the time: 2
   - From time to time, occasionally: 1
   - Not at all: 0

b) I still enjoy the things I used to enjoy:
   - Definitely: 3
   - Not quite so much: 1
   - Only a little: 2
   - Hardly at all: 3

c) I get a sort of frightened feeling as if something awful is about to happen:
   - Very definitely and quite badly: 3
   - Yes but not too badly: 2
   - A little but it doesn't worry me: 1
   - Not at all: 0

d) I can laugh and see the funny side of things:
   - As much as I always could: 3
   - Not quite so much now: 1
   - Definitely not so much now: 2
   - Not at all: 3

e) Worrying thoughts go through my mind:
   - A great deal of the time: 3
   - A lot of the time: 2
   - From time to time, but not too often: 1
   - Only occasionally: 0

f) I feel cheerful:
   - Not at all: 3
   - Not often: 2
   - Sometimes: 1
   - Most of the time: 0

g) I can sit at ease and feel relaxed:
   - Definitely: 3
   - Usually: 2
   - Not often: 1
   - Not at all: 0

h) I feel as if I'm slowed down:
   - Nearly all the time: 3
   - Very often: 2
   - Sometimes: 1
   - Not at all: 0

i) I get a sort of frightened feeling like 'butterflies' in the stomach:
   - Not at all: 3
   - Occasionally: 2
   - Quite often: 1
   - Very often: 0

j) I have lost interest in my appearance:
   - Definitely: 3
   - I don't take so much care as I should: 2
   - I may not take quite as much care: 1
   - I take just as much care as ever: 0

k) I feel restless as if I have to be on the move:
   - Very much indeed: 3
   - Quite a lot: 2
   - Not very much: 1
   - Not at all: 0

l) I look forward with enjoyment to things:
   - As much as ever: 3
   - Rather less than I used to: 2
   - Definitely less than I used to: 1
   - Hardly at all: 0

m) I get sudden feelings of panic:
   - Very often indeed: 3
   - Quite often: 2
   - Not very often: 1
   - Not at all: 0

n) I can enjoy a good book or radio or TV programme:
   - Often: 3
   - Sometimes: 2
   - Not often: 1
   - Very seldom: 0

For office use only

A

TOTAL HAD SCORE: 173
IBS QUESTIONNAIRE

Name: ____________________________  G.P. Name: ____________________________

Address: ____________________________  Address: ____________________________

____________________________________  ________________________________

____________________________________  ________________________________

Telephone: ____________________________  Telephone: ____________________________

Date of birth: ____________________________

Marital status: Single / Married / Divorced / Widowed / Co-Habit

Occupation: ____________________________  Sex: [M] [F]

Ethnic background: Caucasian (white) / Afro-Caribbean / Asian / Oriental

Fathers Occupation (even if retired): ____________________________

INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your IBS. It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (ie over the last 10 days or so). All information will be kept in strict confidence.

1. For questions where a number of different responses are a possibility please circle the response appropriate to you.

2. Some questions will require you to write in an appropriate response.

3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem.

For example:

How severe was your pain?

0%  |  no pain  |  not very severe  |  quite severe  |  severe  |  very severe  |  100%

this answer would indicate that pain is approximately 80% severe
PART 1 : SEVERITY SCORE

1. a) Do you currently suffer from abdominal (tummy) pain?
   b) If yes, how severe is your abdominal (tummy) pain?

   0% no pain  |  not very severe  |  quite severe  |  severe  |  very severe  | 100%

   Circle appropriate box

   c) Please enter the number of days that you get the pain in every 10 days.
      For example if you enter 4 it means that you get pain 4 out of 10 days. If you get pain
      every day enter 10

   Number of days with pain x10

2. a) Do you currently suffer from abdominal distension* (bloating, swollen or tight tummy)
      (*women, please ignore distension related to your periods)
   b) If yes, how severe is your abdominal distension/tightness

   0% no distension  |  not very severe  |  quite severe  |  severe  |  very severe  | 100%

   Circle appropriate box

3. How satisfied are you with your bowel habit?

   0% very happy  |  quite happy  |  unhappy  |  very unhappy  | 100%

4. Please indicate with a cross on the line below how much your Irritable Bowel Syndrome is affecting or interfering with your life in general

   0% not at all  |  not much  |  quite a lot  |  completely  | 100%

   IBS SEVERITY SCORE:

175
PART 2: OTHER IBS DATA

BOWEL HABIT

5. a) What is the most number of times you open your bowels per day/week/month?

Number of times [ ] per day / week / month (Circle appropriate)

Note: For some people the answer to part a and b could be the same

b) What is the least number of times you open your bowels per day/week/month?

Number of times [ ] per day / week / month (Circle appropriate)

6. In the following questions you may circle more than one answer:

Are your motions ever:

a) normal often / occasionally / never (Circle appropriate)
b) hard often / occasionally / never (Circle appropriate)
c) very thin (like string) often / occasionally / never (Circle appropriate)
d) in small pieces (like rabbit pellets) often / occasionally / never (Circle appropriate)
e) mushy (like porridge) often / occasionally / never (Circle appropriate)
f) watery often / occasionally / never (Circle appropriate)

7. In the following questions you may circle more than one answer:

Do you ever:

a) pass mucus (or slime or jelly) with your motions

b) pass blood with your motions

c) have to hurry/rush to the toilet to open your bowels

d) strain to open your bowels

e) feel you haven't emptied your bowel completely after you have passed a motion

Circle appropriate box

YES NO

YES NO

YES NO

YES NO

YES NO

176
PART 2 : Continued

SITE OF PAIN

Please mark with a cross (x) on the diagram below where you get your pain (use more than one x if necessary)

8. Do you ever:

   a) notice your stools are more frequent or loose when you get pain
      
         ![Yes/No options]

   b) notice whether the pain is frequently eased by opening your bowels
      
         ![Yes/No options]

9. In the last year on approximately how many weeks were you:

   i) absent from work due to IBS
      (enter 52 if you have given up completely work because of IBS)
      
      — — — — — — —

   ii) at work suffering from IBS
      
      — — — — — —
PAIN INTENSITY

1. FAINT
2. WEAK
3. MILD
4. MODERATE
5. STRONG
6. INTENSE
UNPLEASANTNESS

1. MILD

2. DISCOMFORTING

3. DISTRESSING

4. HORRIBLE

5. EXCRUCIATING
URGE INTENSITY

1. FAINT
2. WEAK
3. MILD
4. MODERATE
5. STRONG
6. INTENSE
Appendix IV

SHORT FORM McGill Pain Questionnaire

Date: ________________________________
Name: ______________________________

Check the column to indicate the level of your pain for each word, or leave blank if it does not apply to you.

<table>
<thead>
<tr>
<th></th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Throbbing</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>2</td>
<td>Shooting</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>3</td>
<td>Stabbing</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>4</td>
<td>Sharp</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>5</td>
<td>Cramping</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>6</td>
<td>Gnawing</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>7</td>
<td>Hot-burning</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>8</td>
<td>Aching</td>
<td>______</td>
<td>______</td>
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<tr>
<td>9</td>
<td>Heavy</td>
<td>______</td>
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<tr>
<td>10</td>
<td>Tender</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>11</td>
<td>Splitting</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>12</td>
<td>Tiring-Exhausting</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>13</td>
<td>Sickening</td>
<td>______</td>
<td>______</td>
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<tr>
<td>14</td>
<td>Fearful</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>15</td>
<td>Cruel-Punishing</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

Indicate on this line how bad your pain is—at the left end of line means no pain at all, at right end means worst pain possible.

No ________________________________10  Worst Possible Pain
Pain