Central Modulation of Visceral Pain Hypersensitivity

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I, Ahmed Albu-soda, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

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Oral presentations

1. Digestive Week 2017

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2. Meeting of the Brtish Society of Gastroenterology 2018

- Systematic Review and Meta-Analysis: Conditioned Pain Modulation in Functional GI disorders

Ahmed Albusoda, James K Ruffle, Kathrine A Friss, Maximilian R Gysan, Asbjørn M Drewes, Qasim Aziz & Adam D Farmer

3. United European of Gastroenterology Week 2018

- Randomized Controlled Trial: The Effect of Transcutaneous Vagal Nerve Stimulation on Reversing Acid-Induced Oesophageal Hypersensitivity in Humans

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Abstract

Background: visceral pain hypersensitivity is a key feature in functional gastrointestinal conditions. This condition leads to an exaggerated response to known painful stimuli, or chronic pain with no apparent trigger. There is an important paucity of effective clinical interventions for visceral pain hypersensitivity.

Aim: To understand the central nervous system (CNS) control of visceral pain hypersensitivity via descending pain pathways to the spinal cord. Additionally, I aim to test the feasibility of a non-pharmacological intervention such as non-invasive vagal nerve stimulation to reduce this condition in healthy humans.

Methods: I used PRISMA guidelines for systemtic review and meta-analysis to investigate: i) decending pain control in visvceral pain, ii) The antinociceptive effect of vagal nerve stimulation. To investigate the descending pain control, I used a Conditioned Pain Modulation Paradigm where applying a second painful sitmuls inhibits the initial pain by triggering descending inhibiton. To test the effect of autonomic modulation on oesophageal pain hypersensitivity, I used a previously approved noninvasive transcutaneous vagal nerve stimulation device in a human model of experimentally induced pain hypersensitivity by slow infusion of hydrochloric acid in the distal oesophagus.

Results: My systematic review and meta-analysis demonstrated that Conditioned Pain Modulation is significantly inhibited in visceral pain hypersensitivity. I also showed that a reduced Conditioned Pain Modulation at baseline is a strong predictive factor of developing pain hypersensitivity in healthy humans. I also demonstrated that vagal nerve

stimulation is effective in various pain conditions in a meta-analysis, I then demonstrated in an experimental study that vagal nerve stimulation can reverse acid-induced oesophageal pain hypersensitivity.

Conclusions: there is a marked reduction in descending pain inhibition in visceral pain hypersensitivity. Poor descending pain inhibition is associated with developing experimental pain hypersensitivity. Vagal nerve stimulation can reverse experimental pain hypersensitivity, likely by a central mechanism.

Contents

Introduction

Pain is a defining symptom in many functional gastrointestinal disorders. Those disorders are very common. For example, Gastroesophageal reflux disease has a prevalence in Europe of 8.8-25.9%, with 10-40% of patients complain of persistent pain or discomfort despite adequate acid suppression, indicating important functional component (9).

The main aim of this thesis is to understand clinically relevant aspects of visceral pain hypersensitivity (exaggerated pain responses) with a special focus on the central aspect of hypersensitivity, such as the top-down control of pain. I also attempted to understand the rationale and the feasibility of using the autonomic nervous system as a portal to modulate visceral pain hypersensitivity.

I take a special interest in oesophageal pain hypersensitivity and use this as a model whenever it is possible. However, being a less studied subject, I used other functional gastrointestinal disorders with pain hypersensitivity to infer relevant conclusions on pain processing.

In the 1st chapter, I introduce relevant notions of the pain (nociceptive) system, the autonomic nervous system and the interaction between the two systems which are important for the understanding of the thesis.

In chapter 2, I present my 1st experimental study. In this study, I studied the autonomic signature of two interventions, previously known to have an analgesic effect on experimental visceral pain. These interventions include slow deep breathing and

modulation of attention. In this study, I aimed to determine whether a shared autonomic mechanism may explain the analgesic effect of interventions.

In chapter 3, I explore the top-down (brain-gut) modulation of pain control. This chapter is a meta-analysis to investigate if the top-down inhibition of pain is affected in visceral pain conditions.

Chapter 5 is an experimental study to understand the relationship between experimental pain hypersensitivity and the descending pain modulation (top-down control) of pain in a human model of oesophageal pain hypersensitivity.

Chapter 5 is a systematic literature review and meta-analysis of the effect of vagal nerve stimulation on pain in general. In this chapter, I show that vagal nerve stimulation is an effective treatment in various pain conditions. I then, in chapter 6, use vagal nerve stimulation in an experimental study to influence oesophageal pain hypersensitivity in humans.

In chapter 7, I summarised my findings and lay down future plans for research.

Chapter 1

The nociceptive and the autonomic nervous system

Introduction

This chapter aims to introduce topics relevant to this thesis such as pain transduction, transmission and perception. This chapter is not a comprehensive literature review of the physio-pathology of pain in general but a more focused overview of nociceptive pain which is relevant to this thesis.

The nociceptive system

Pain is defined by the International Association for Study of Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage(10). It is an undesirable, disagreeable sensation (11). It serves to protect the body from potentially harmful events (12, 13). The main survival aim of pain is to remove the body away from the noxious stimulus voluntarily.

Types of pain

Pain is classified in a variety of ways. In reality, these categories often overlap. Some classifications are more useful than others depending on the focus of the subject.

Acute pain and chronic pain

Acute pain is of short duration, has an apparent underlying causative event and may signify healing or a damage control process following that event (14, 15). Chronic pain may not have an obvious underlying cause. It usually extends beyond the reasonably expected time for tissue healing (16). This distinction is useful in a clinical setting; acute

pain requires more urgent management since its cause may be fatal or the cause of long-lasting disability (15). Chronic pain may be defined as pain lasting more than three months, according to the International Association for the Study of Pain (IASP) (16). However, somatic, acute and chronic pain differ in nature as well; acute pain may be described as sharp, pricking or stabbing sensation, while chronic pain is often said to be slow, aching, or burning (13, 15, 16).

Nociceptive pain

Nociceptive pain is experienced when sensory pain receptors (nociceptors) in tissues are stimulated. It is further classified into fast or first physiological pain and slow or second pathophysiological pain (15). Fast pain is felt in healthy tissue within a tenth of a second upon an acute painful stimulus, such as the application of an electric shock or sharp object (14, 15). Slow pathophysiological pain corresponds to tissue damage and may occur following the stimulus, with a milder or different type of stimulus, or even without a stimulus, and this is probably due to sensitisation(13). Fast pain is usually only felt in superficial tissues, whereas slow pain is also experienced in deep tissues. The character of slow pain is usually similar to that of chronic pain (13, 15, 17).

Nociceptive pain can also be described as either somatic or visceral pain (15). Somatic pain is easily localised, while visceral pain is difficult to describe as restricted to a specific body part, and often said to be “generalised” (18). The reason for this may be that there are fewer visceral sensory nerves than somatic sensory nerves and fewer visceral pain pathways in the central nervous system (18). The automatic reflexes elicited by visceral pain are often more prominent than those elicited by somatic pain (18). Inflammation plays an important role in the modulation of nociception. Release of inflammatory

mediators (ex: substance P, prostaglandins, serotonin, acetylcholine, bradykinin) sensitise the primary afferents resulting in a reduction if pain threshold (19).

Neuropathic pain

Neuropathic pain is now defined by the International Association for the Study of Pain (IASP) as 'pain caused by a lesion or disease of the somatosensory nervous system'(10). Neuropathic pain could affect both, the central or the peripheral part of the somatosensory system. Damage to the peripheral sensory nerves could result in chronic pain secondary to diabetic, or alcoholic neuropathy, radiculopathy, trigeminal neuralgia and other debilitating conditions. Neuropathic pain can also arise at the level of the central nervous system such as in multiple sclerosis, spinal injury or after stroke(20).

The nature of the sensation is often distinct from that of the nociceptive pain, it is usually described as "burning" or "electrical" (13).

Nociplastic pain

Nociplastic pain is a new category of pain added to the 2017 taxonomy of the International Association for the Study of Pain (IASP) (10). It refers to the activation of nociceptive receptors without clear evidence of tissue damage. It is most likely related to altered nociception. Examples include low back pain, complex regional pain syndrome and fibromyalgia.

Primary and Secondary Chronic Pain

This classification is on the latest International Classification of Diseases ICD11(21). Primary (idiopathic) chronic pain, overlaps with the nociplastic pain classification mentioned above. Primary chronic pain is recognised as a disease in itself such as low back pain, fibromyalgia and pain in functional gastrointestinal disorders. Whereas, secondary chronic pain is a symptom of an underlying condition such as cancer pain, neuropathic pain caused by multiple sclerosis and other chronic pain with a known aetiology (22, 23).

Pain pathways and systems

Overview

Nociception involves the signalling of pain (12). The nociceptive system refers to the entire system responsible for collection, transmission and processing of pain signals (13). Traditionally, those pathways are investigated by electrophysiologic studies in animals such as single unit recording by invasive electrodes(24-28). However, other methods such as functional Magnetic Resonance Imaging (fMRI) and Electroencephalograph (EEG) have also been used to map those pathways in humans (13, 29-31).

The first-order neurons are nociceptors in peripheral tissues, the axons of which synapse with neurons in the dorsal horn of the spinal cord (13, 14). These second-order neurons either directly ascend to higher centres in the brain or interact with spinal neurons (13).

Peripheral transmission of pain

Primary afferent neurons carrying pain signals are pseudo unipolar neurons with their cell bodies in the trigeminal or dorsal root ganglion (17).

Types of nociceptors

Nociceptors are sensory pain receptors in peripheral tissues(14). They consist of the free nerve endings of first-order neurons and are sensitive to a variety of stimuli including mechanical, thermal and chemical events (12, 14). We may classify nociceptors based on the type of stimulus they respond to, the type of nerve fibre (A or C), and whether they are silent or not(17). Silent ("wide-dynamic range" or "convergent") pain receptors are usually unresponsive to temperature and pressure, but become active to these stimuli if sensitised by molecules involved in inflammation (substance P, prostaglandins, serotonin, acetylcholine, bradykinin) (17, 32).

For example, nociceptors in the oesophagus respond to acid by activating two proton-gated channels: transient receptor potential vanilloid-1 (TRPV1- thermal) and acid-sensing ion channels (ASICs- chemical), while mechanical nociceptor such as TRPAI can detect distention (33).

Peripheral pain fibres

Fast and slow types of pain travel through different fibres to the spinal cord. Fast pain is transmitted through myelinated A fibres (the majority of which are A-δ fibres) at a velocity of 5m/s to 30m/s, which is faster than the transmission of slow or chronic pain through unmyelinated C fibres at 0.4m/s to 1.4m/s (17, 34). This transmission occurs simultaneously via both fibres when the same acutely painful stimulus is applied, resulting in a dual sensation of nociceptive pain; a sharp pain, followed by a lingering dull pain (15, 17).

A-fibres are clustered in groups, with each group serving a small location, and distributed less widely than C fibres, making fast pain localisation more precise than that of slow pain (17).

Aδ and C fibres travel to superficial and deep spinal cord laminae (Rexed's laminae I, II and V, VI; circumcanular lamina X) while Aβ fibres (the largest in diameter of these three types of fibres) mainly travel to the deep Rexed's laminae (III to VI) (32).

Central transmission of pain

When they reach the dorsal horn of the spinal cord, primary afferent neurons may synapse directly with neurons projecting to higher centres, or with interconnecting neurons in the spinal cord (11). These interneurons may be excitatory or inhibitory. Inhibitory interneurons can exert their effects on projection neurons (PNs), excitatory interneurons or the first-order neurons to dampen their actions (15, 32). The main neurotransmitters in descending inhibition are opioids and noradrenaline, while the main excitatory neurotransmitter is Serotonin or 5-hydroxytryptamine (5-HT). First-order neurones may also send ascending and descending collaterals, and these branches form the dorsolateral tract of Lissauer (35). After entering the dorsal grey matter, second-order neurons arise from Rexed's laminae, decussate (crossover) and ascend in the anterolateral spinal cord (35). Two main tracts carry pain signals from the spinal cord to the higher centres, called the neospinothalamic and palaeospinothalamic tracts (35). Interestingly, these tracts are named according to their evolutionary origin; the palaeospinothalamic tract has a more primitive origin, and perhaps this reflects the types of sensations this tract carries; slow poorly localised pain (36). The spinoreticular,

spinomesencephalic and spinohypothalamic pathways also carry pain signals to the brain (11, 37).

Neospinothalamic tract

Fibres from the periphery synapse in laminae of the dorsal horn, from where central neurons arise to form the neospinothalamic tract (15). The projection neurons promptly decussate and ascend in the anterolateral column of the spinal cord to the brain (15). A few fibres terminate in the reticular formation of the brain stem, and some other neurons wind up in the posterior nuclear group of the thalamus, but the majority terminate in the ventrobasal thalamus (15).

Palaeospinothalamic tract

The spinoreticulothalamic or palaeospinothalamic tract is formed from the deeper laminae of the spinal cord (15, 36). Pain signals follow a path similar to that for fast pain, as projections first decussate, then ascend in the anterolateral column of the spinal cord (15). Unlike the neospinothalamic pathway, however, at least three-fourths of the fibres terminate in the reticular formation, tectal area or the periaqueductal grey area of the brainstem with the remainder ending up in the thalamus (15). Pain is then further transmitted to basal portions of the brain through several neurons; however, chronic pain is still felt when these higher centres are sectioned in animals, underlining the importance of the basal areas in interpreting chronic pain (15). Please notice that these basal structures play an essential role in the autonomic refluxes as I will elaborate later in this chapter.

Pain modulation

Pain is modulated in a variety of ways, both centrally (such as via descending facilitatory and inhibitory pathways from higher centres-explained later) and peripherally (by the sensitisation of receptors) (11, 13, 32). Thus, the intensity of pain experienced by an individual can vary even with an identical set of stimuli.

Pain experience differs from individual to individual; however, depending on the circumstances, such experiences may also differ within the same individual. The difference is sometimes remarkable; for example, soldiers may report little pain to gun-shot wounds during the battle (38). Such variability in pain experience is possible due to a complex pain control system that intervenes at multiple levels; from pain conduction at the periphery, transmission to the central nervous system, to the processing of such stimuli at cortical levels.

Peripheral and central sensitisation

Central sensitisation is defined as an ''increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input''. While Peripheral sensitisation is defined as ''increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields''(10, 39). See figure 1.

Sensitisation of nociceptors

Most sensory receptors in the body adapt to their excitatory stimulus (15). However, nociceptors undergo little or no adaptation, allowing pain to be felt continuously as long as the precipitating condition is present (15). Paradoxically, in many cases of chronic pain, pain receptors undergo sensitisation as the stimulus persists (11).

There are various ways in which receptors can become more sensitive to pain. In some cases, the same receptor undergoes sensitisation (for example, silent receptors becoming active). This causes hyperalgesia (exaggerated response to a painful stimulus). Repetitive activation of nociceptors can cause central sensitisation at the level of the spinal cord leading to a progressive increase in pain intensity with the same stimulus. This central phenomenon is called temporal summation, which is one manifestation of hyperalgesia (11).

In other cases, surrounding receptors which may not even be "nociceptive" in nature (silent nociceptors) can begin to transmit pain, for example, mechanical allodynia (a painful response to non-painful stimuli, for example, painful touch)(11, 32). The activation of silent nociceptors is also one of the mechanisms for central sensitisation(11).

Allodynia can be explained by Aβ fibres which are responsible for a shift in the phenotype of first-order neurons so that they begin to produce excitatory molecules normally involved in pain signalling (11, 32). Besides, receptor neurons that undergo damage may be reorganised, placing primary sensory neurons in communication with ascending neurons and bypassing the normal dampening mechanisms of painful signals at the level of the spinal cord (32). This bypass mechanism might answer why traditional analgesics are not effective in relieving allodynia after nerve damage or neuropathic pain and suggests as a solution that we explore therapies targeting ascending neurons (32).

Descending tracts may cause sensitisation to pain in silent or wide-dynamic range receptors in several ways. This sensitisation occurs when a pain stimulus is repeatedly provided. One possibility of how this happens is unique communications between

descending pathways and primary afferent fibres (which may or may not involve inhibitory interneurons) (32). Another possibility is the inhibition of excitatory neurons that relay with projection neurons (32). Projection neurons may also be directly acted upon by descending tracts at specific loci physically shared with primary sensory neurons (32). Some believe that the ascending pain signals carried by projection neurons may be modified by intracellular signalling pathways that are acted upon by descending control (32). The clinical importance of studying these processes is that when looking at patients who exhibit this type of sensitisation, one needs to target therapies that will alleviate pain but not dampen the other senses which could prove challenging (32).

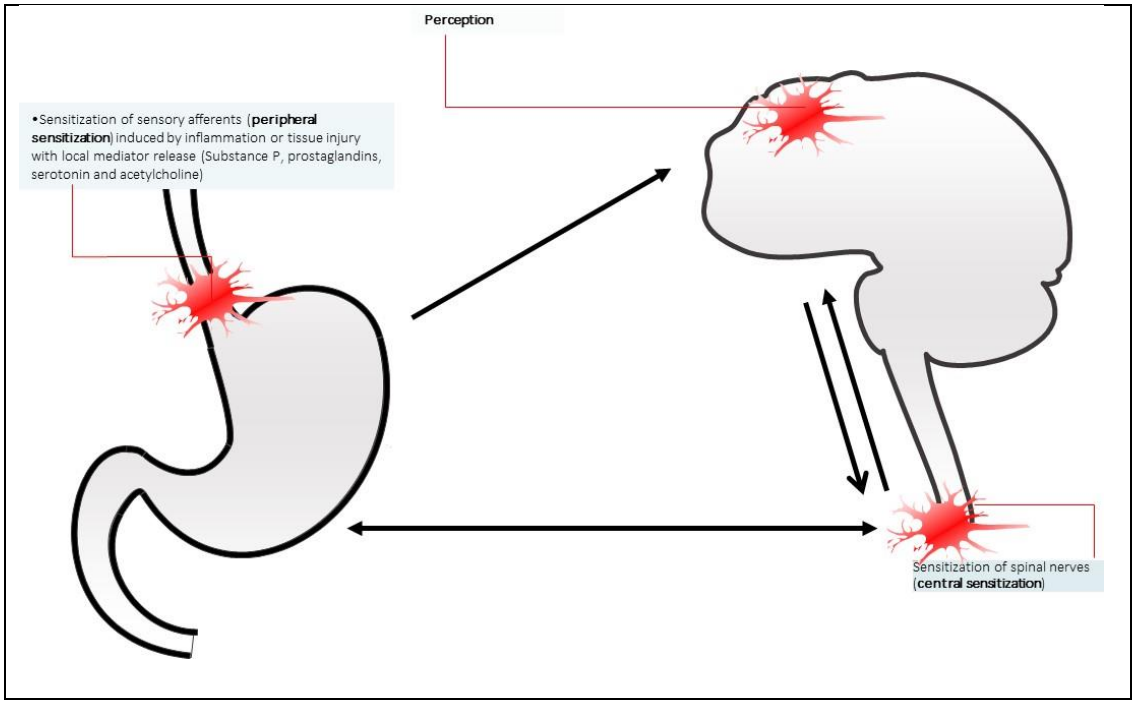


Figure 1: Peripheral and central sensitisation. Peripheral sensitisation refers to the increase in pain transduction at the level of the nociceptors, and this increase is usually triggered by tissue damage or inflammation with the release of local mediators such as Substance P, Prostaglandins, serotonin and acetylcholine. Central sensitisation refers to the increase in pain transmission at the level of the spinal cord. Perception of pain at the level of the spinal cord can, in turn, alter the overall experience of pain. Adapted from Aziz et al. 2000(40).

Gate control theory of pain

The gate control theory of pain was initially drawn up by Melzack and Wall (41) and proposed that peripheral pain signals to the central nervous system are "gated" in the spinal cord by various influences (42). These influences are provided by the cells in the substantia gelatinosa of the spinal cord (41). The non-painful sensory neurons projecting to the spinal cord can compete with painful stimuli to pass the "gate" and ascend to other structures (41). The gate control theory of pain is the main theory relied upon when explaining the antinociceptive effect of non-painful stimuli, such as massage and acupuncture. Since the proposal of this theory, our understanding of pain control has come a long way. Advances in the neurobiology of pain has implicated several synergic mechanisms that may determine the size of the ''gate opening''. One of the factors that determine the despatch of pain signal through the ''gate'' is the descending control of pain.

Descending pathways

Descending control refers to the ability of higher structures to inhibit or facilitate receptive pain fields at the spinal cord (32). See figure 2.

Descending pathways may interfere with primary sensory neurons ("pre-synaptic actions"), or spinal interneurons or projection neurons in the spinal cord ("post-synaptic actions") (32). When descending inhibition acts upon an interneuron that has an inhibitory nature in itself, either a different neurotransmitter is used, or the usual neurotransmitter acts via different second receptor and coupling mechanisms (32). Thus, it follows that the same neurotransmitter may have opposing functions; for instance,

serotonin (5H-T) may exacerbate or attenuate nociception in the spinal cord (32). It also follows, in theory, that drugs targeting specific receptor types or second messengers can provide pain relief with a high efficacy (32).

Apart from direct synapses between descending tract neurons and sensory neurons, “volume transmission” also takes place in the spinal cord (32). Volume transmission is a way of intracellular communication by means of diffuse neurotransmitter in the extracellular fluids, it is roughly analogous to injecting drugs into the spinal cord. Neurotransmitters such as dopamine and glutamate diffuse locally after their release, resulting in broad and lingering effects on nearby synapses or other cells in the vicinity, such as adjacent astroglia (32, 43). These effects are, to a good extent, dependent on the pharmacokinetic properties of the transmitter molecules (32).

Neurons are not the only spinal cord cells involved in descending control of pain. Descending tracts also affect spinal glial cells and invasive T cells, both of which produce substances that can modify pain transmission (32). For example, glial cells produce an acetylcholine-binding protein that can affect autonomic signalling (32).

Motor responses to pain, such as reflexes and the promotion of motionlessness to improve healing are also influenced by descending control on the ventral horn of the spinal cord (32).

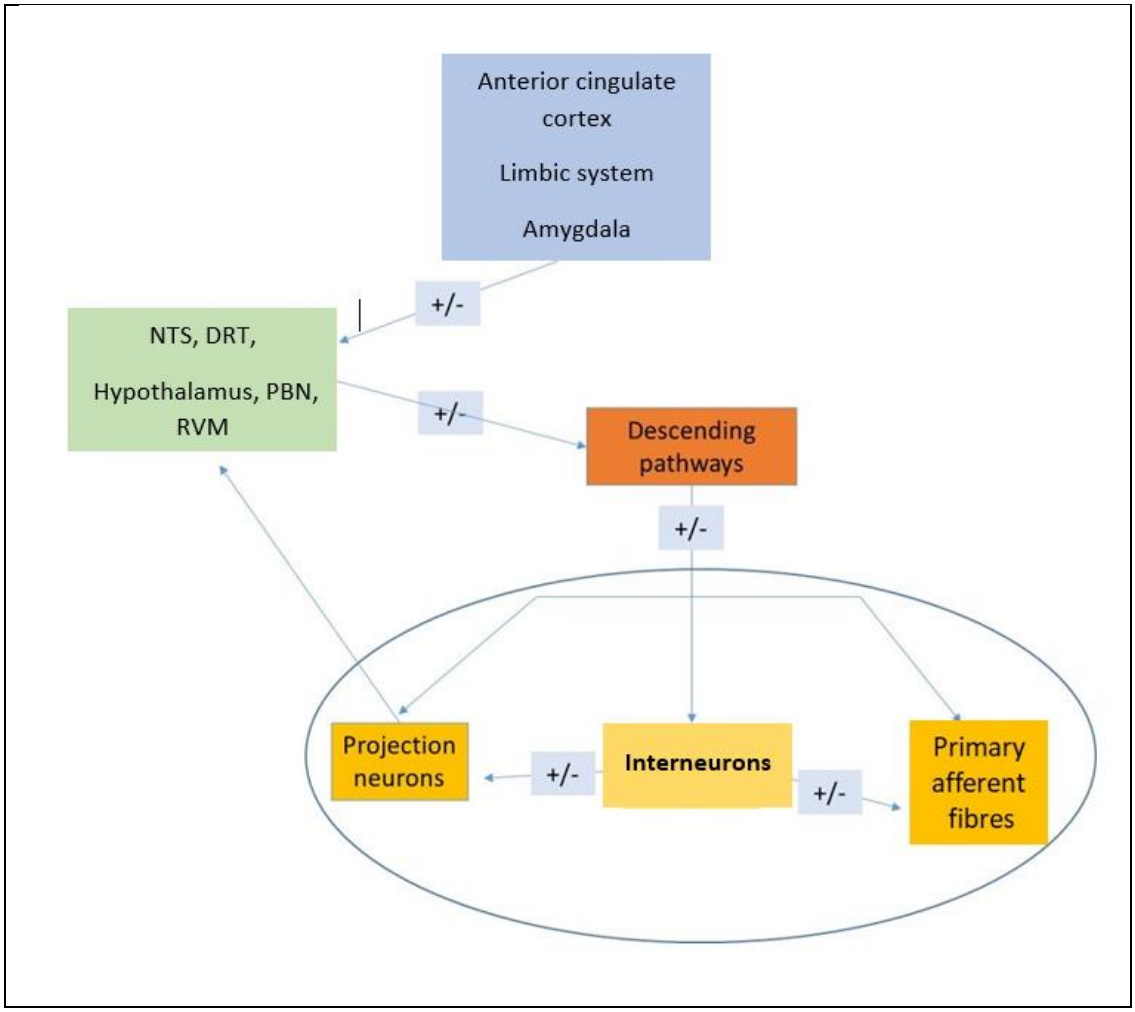


Figure 2: Schematic representation of the descending pathways. Several structures contribute to the origin of the descending pathways such as rostroventral medulla (RVM) and other brainstem nuclei, the nucleus tractus solitarius (NTS), the parabrachial nucleus (PBN), the dorsal reticular nucleus (DRT), the hypothalamus and the cortex. The separation of the descending pathways into facilitatory and inhibitory pathways is functional rather than anatomical. The descending pathway can alter pain transmission at the level of the primary afferent neuron, projection neuron or by activating interneurons (excitatory or inhibitory).

Types of descending control

Although, the same structures are involved in descending control, functionally there are two distinct pathways according to the type of influence they exert on pain processing in the spinal cord, descending facilitation and descending inhibition (32). As described above, the same neurotransmitter may modulate actions in both of these pathways simultaneously via different receptor neurons or different receptor types (32).

The rostroventromedial medulla has been extensively studied in this regard (32). There are two kinds of neurons recognised in the rostroventromedial medulla, “OFF” cells and “ON” cells (32). “OFF” neurons are involved in descending inhibition, are stimulated by opioid analgesics and upon receiving sensory pain input, pause their discharge before a pain reflex (32). “ON” cells behave oppositely and are implicated in descending facilitation (32).

Origin of the descending pathways

Central structures from which descending pathway neurons project to the dorsal horn are the hypothalamus, parabrachial nucleus, nucleus tractus solitarius, brainstem nuclei, cerebral cortex (frontal, parietal and anterior cingulate cortex) and periaqueductal grey matter (32). These pathways enter the spinal cord through the dorsolateral and ventrolateral funiculi (32).

Hypothalamus

The hypothalamus plays a significant role in organising sensory information and is well connected to the nucleus tractus solitarius, periaqueductal grey matter, rostroventromedial medulla and those parts of the corticolimbus that are associated with feeling pain and related emotions (32). Hyperalgesia can be elicited by damaging the medial hypothalamus and a few other hypothalamic nuclei (32). Several nuclei of the hypothalamus give rise to descending pathways which end in the spinal cord, for example, the paraventricular nucleus, the arcuate nucleus, the tuberomammillary nucleus and the posterior periventricular nucleus (32).

Parabrachial nucleus

Pathways originating in the parabrachial nucleus mainly influence the nerve cells of the superficial laminae of the dorsal grey matter of the spinal cord. Excitation of this area dulls the reaction of dorsal horn cells to all types of sensory signals, including pain (32).

Nucleus tractus solitarius

The nucleus tractus solitarius is important in managing input from the viscera. It receives a large amount of sensory information from the vagal nerve and neurons in the dorsal horn (32).

Brainstem centres

Monoaminergic pathways to the spinal cord arise from several groups of neurons in the brainstem (32). The rostroventromedial medulla receives mostly indirect sensory input, and each of its nuclei sends descending pathways to the superficial and deep spinal cord laminae; these pathways are considered to essentially cause continued pain in cases of inflammation and damage to the nociceptor cells (32).

The dorsal reticular nucleus of the medulla also has direct connections to superficial and deep Rexed's laminae, some of which are reciprocal, thus forming looped pathways as well (32). Hyperalgesia can be caused by dorsal reticular excitation, while damage to this structure causes numbing of the pain associated with inflammation (32). Further, it has also been suggested that the dorsal reticular nucleus is implicated in "Diffuse Noxious Inhibitory Controls" also called Conditioned Pain Modulation, which is analgesia in one body areas triggered by a painful stimulus to an anatomically distant area (32). Diffuse Noxious Inhibitory Control is discussed further later in this chapter. Unlike the

rostroventromedial medulla, the dorsal reticular nucleus is poorly studied concerning underlying mechanisms and connections (32).

Periaqueductal grey matter

Periaqueductal grey (PAG) is a critical structure in the descending pain control. It receives direct and indirect projections from higher structures such as the anterior cingulate cortex and amygdala(44, 45). PAG also receive ascending nociceptive inputs from the dorsal-horn via the Parabrachial nuclei. PAG, through its reciprocal connections with the RVM, plays a crucial role in the descending modulation. Activity in the PAG are mediated mainly by μ-opioid agonists but also GABAergic inhibitors, cannabinoid receptor agonists and results in monoaminergic descending inhibition control via RVM (32, 46).

Cerebral cortex

Nociception in the spinal cord can be modulated by even the highest levels in the central nervous system. Pain can be inhibited by excitation of the insular and ventro-orbital cortex via other areas of the central nervous system discussed above (32). However, excitation of other areas, such as the anterior cingulate cortex, has been shown to initiate descending facilitation pathways in the rat (32). Functional MRI studies have repeatedly implicated the anterior cingulate cortex in pain perception in humans(44, 45).

Fibres originating from the frontocortical, somatosensory and parietal cortex pass uninterrupted to the spinal cord where they act mainly on resident neurons of the dorsal horn; however, there are also many multi-neuronal pathways from the cortex to the spinal cord (32). Some of these pathways run to the dorsal column nucleus, which is known to play a part in both proprioception signalling and the initiation of visceral and neuropathic pain (32).

Conditioned pain modulation

One way of assessing descending inhibition is by measuring the conditioned pain inhibition. Conditioned pain modulation refers to the endogenous pain inhibition of a specific stimulus when a second pain stimulus is applied simultaneously in an anatomically distant part of the body. It is evaluated by assessing participant's pain threshold to a specific stimulus (test stimulus), then reassessing it after applying a second painful stimulus, also called conditioning stimulus. Following the principle that ''pain inhibits pain''. CPM was formally known as Diffuse Noxious Inhibitory Control described by Le Bars (47). Diffuse Noxious Inhibitory Control is a specific term that refers to a brainstem mediated mechanism. Thus, Conditioned Pain Modulation was adopted as an alternative term to incorporate the psychophysiological factors important in shaping this type of pain control in humans (48).

In healthy humans, there is a significant increase in pain threshold to the test stimulus after applying a second conditioning stimulus (49). Conditioned pain inhibition is thought to be mediated via Diffuse Noxious Inhibitory Control system. In rats, Diffuse Noxious Inhibitory Controls is thought to be mediated via neurones in the subnucleus reticularis dorsalis (SRD) (50). However, human studies suggest the involvement of other nuclei, such as the Periaqueductal grey and structures that allow for interactions with higher structures (51).

Conditioned pain modulation is reduced in a variety of chronic pain conditions such as Functional Abdominal Pain, Irritable Bowel Syndrome, Functional Dyspepsia, and other conditions such as osteoarthritis, diabetic neuropathy (52-54). This reduction in CPM has a large effect size, as confirmed by our meta-analysis in chapter 3 (55).

A reduction in the noradrenergic descending pain inhibition or activation of the serotonergic pain facilitation may play a key role in visceral pain hypersensitivity where there is an exaggerated response to a potentially painful stimulus (55). However, more work is needed to explain the sequence of events linking visceral hypersensitivity in chronic pain conditions, reduced conditioned pain modulation and descending pain control.

The autonomic nervous system

The autonomic nervous system (ANS) is a collection of sensory (afferent) and motor (efferent) neurons that link the central nervous system (CNS) with visceral effectors (56).

Newer definitions recognise the influence of the central nervous system in setting the baseline and modifying the activity of the autonomic nervous system (57).

Classifications

Langley classified the autonomic nervous system into the parasympathetic nervous system which is responsible for the body's rest and digest function and controls homeostasis; the sympathetic nervous system which responds to an emergency that causes stress or fear and requires a fight or take flight response (run away); and the enteric nervous system, which is also known as the second brain due to its independent reflex activity within the gastrointestinal tract (58). The autonomic nervous system is also called the automatic nervous system due to the involuntary nature of its responses.

Functions

Table 1 describes some of the innervation and functions of the effectors of the autonomic nervous system(59). The sympathetic nervous system also innervates the pineal gland and lymphatic tissues (60).

Table 1: Functions of the autonomic nervous system

		Sympathetic Nervous System	
Effector Organs	**Parasympathetic Nervous System**	**Receptor Type**	**Response**
Eyes			
Radial muscle of iris	—	α_1	Contraction (mydriasis)
Sphincter muscle of iris	Contraction (miosis)		—
Ciliary muscle	Contraction for near vision		—
Heart			
SA node	Decreased heart rate	β_1	Increased heart rate
Atria and ventricle	Decreased atrial contractility	β_1, β_2	Increased contractility
AV node and Purkinje fibers	Decreased conduction velocity	β_1	Increased conduction velocity
Arterioles			
Skin, splanchnic vessels	—	α_1	Constriction
Skeletal muscle	—	α_1/ β_2, M	Constriction/Dilation
Systemic veins	—	α_1, α_2/ β_2	Constriction/Dilation
Bronchial smooth muscle	Contraction	β_2	Relaxation
Stomach and Intestine			
Motility and tone	Increased	α_1, α_2, β_2	Decreased
Sphincters	Relaxation	α_1	Contraction
Secretion	Stimulation	—	
Gallbladder	Contraction	β_2	Relaxation
Urinary bladder			
Detrusor	Contraction	β_2	Relaxation
Sphincter	Relaxation	α_1	Contraction
Uterus (pregnant)	—	α_1/ β_2	Contraction/Relaxation
Male sex organs	Erection	α_1	Ejaculation
Skin			
Pilomotor muscles	—	α_1	Contraction
Sweat glands	—	M	Secretion
Liver	—	α_1, β_2	Glycogenolysis
Pancreas			
Acini	Increased secretion	α	Decreased secretion
Islet cells	—	α_2/β_2	Decreased/Increased secretion
Salivary glands	Profuse, watery secretion	α_1/β	Thick, viscous secretion/Amylase secretion
Lacrimal glands	Secretion		—
Adipose tissue	—	β_3	Lipolysis

A dash means the target tissue is not innervated by this division of the autonomic nervous system. Adopted from Gliman's The Pharmacological Basis of Therapeutics, 12 ed. NewYork(57)

Relevant anatomy

The neurons of the autonomic nervous system synapse at autonomic ganglia; thus, presynaptic and postsynaptic neurons are termed preganglionic and postganglionic neurons, respectively (57, 60).

Descending outputs from the CNS to the periphery make up the preganglionic neurons; the craniosacral (parasympathetic) outflow and the thoracolumbar (sympathetic) outflow (60). The neurotransmitter of presynaptic neurons in both sympathetic and parasympathetic is acetylcholine. Norepinephrine is the main postsynaptic transmitter in the sympathetic and acetylcholine is released by the postsynaptic parasympathetic neurons.

The vagus nerve is the longest of the cranial nerves. It carries afferent and efferent parasympathetic fibers. The main sensory nucleus of the vagal nerve is the nucleus tractus solitarius (NTS). NTS receives sensory input from the viscera (32).

The diagram (Figure3) helps describe the neuroanatomical arrangement of the autonomic nervous system(61).

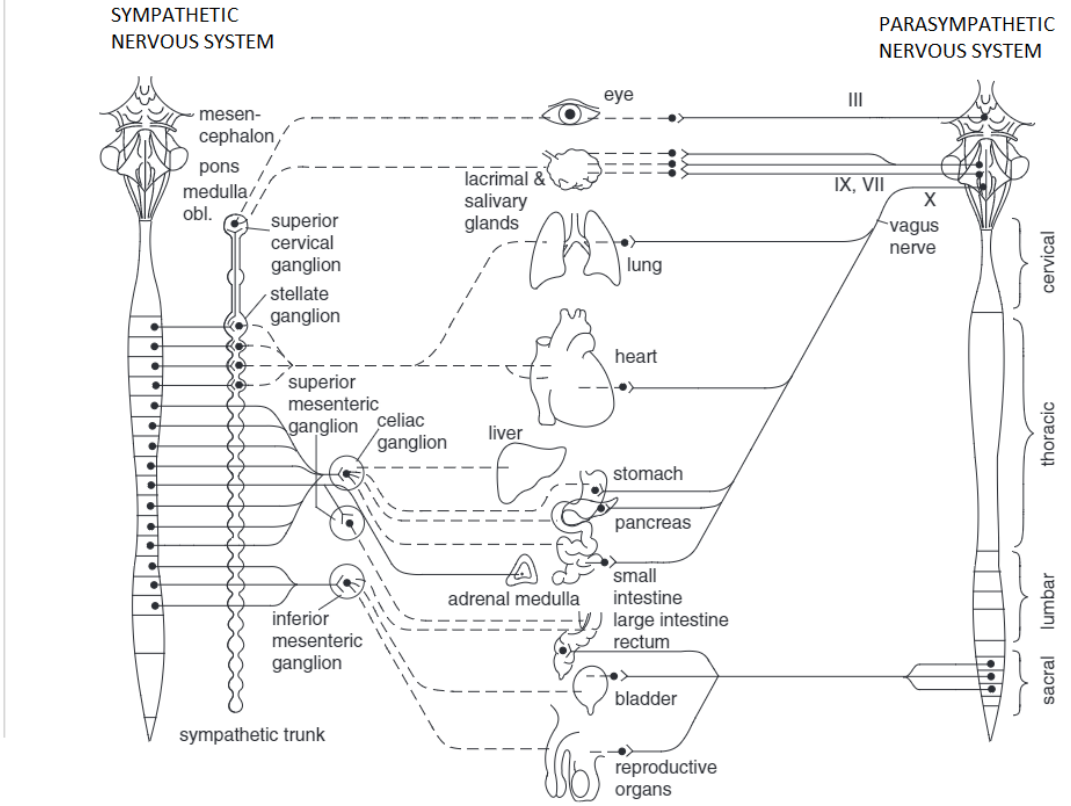


Figure 3: the neuroanatomical arrangement of the autonomic nervous system

Functional anatomy

Sympathetic Nervous System

Preganglionic neurons

The cell bodies of the preganglionic sympathetic neurons lie in the intermediolateral column of the spinal cord from the first thoracic to the upper third or fourth lumbar segments. These neurons exit the spinal cord in the ventral roots and white rami terminate in the sympathetic ganglia as described below, on the enteric nervous system, and some of the adrenal medulla. (57, 60)

Postganglionic neurons

The cell bodies of sympathetic postganglionic neurons are found in the paravertebral ganglia, the prevertebral ganglia or the pelvic splanchnic ganglia. The paravertebral ganglia form a chain (sympathetic trunks or chains) parallel to the vertebral column, which extends on each side to the sacrum. White and grey rami connect them to spinal nerves. A majority of the postganglionic neurons travel via the spinal nerves, and the rest through splanchnic nerves to supply viscera (60).

The prevertebral ganglia lie in front of the vertebral column, are unpaired and mostly located around the origin of the major branches of the abdominal aorta. The axons of the ganglia cells are long and mostly unmyelinated (60).

The pelvic splanchnic ganglia are located in the pelvic plexus. The preganglionic sympathetic neurons that terminate in these ganglia come through via the hypogastric nerves (or plexuses) (60).

Parasympathetic Nervous System

Preganglionic neurons

The cell bodies of preganglionic parasympathetic neurons are situated in the mesencephalon and the medulla oblongata (tectal and bulbar system) and the intermediate zone of the sacral spinal cord (sacral system). The third, seventh and ninth cranial nerves deliver them to the parasympathetic ganglia of the head; the tenth cranial nerve (vagus nerve) to the ganglia of viscera in the thorax and abdomen; and the pelvic splanchnic nerves to the pelvic ganglia. The sacral outflow also consists of preganglionic neurons to the enteric nervous system.(60)

Postganglionic neurons

The cell bodies of postganglionic neurons are situated in parasympathetic ganglia in the head, in or close to the walls of the target viscera, and in the pelvic plexus (60). Their axons are thus often short.

The interaction between the nociceptive and autonomic nervous systems

The nociceptive system and the autonomic nervous system are intertwined and exert effects on each other. See Figure 4 for some of the shared structures.

Descending control and higher autonomic centres

Several parts of the hypothalamus process sensory and autonomic information. Pathways (that likely use glutamine) connect the medial preoptic nucleus (MPN) of the hypothalamus to the PAG and the RVM.(32). The MPN and anterior and lateral hypothalamus all have an inhibitory effect on pain via descending parasympathetic pathways. The PBN and the NTS play similar roles as the hypothalamus with regards to the processing and inhibiting pain. The PBN has connections with the NTS, RVM, spinal DH and the trigeminal nucleus of the medulla. Although the NTS is usually implicated in the inhibition of pain, it has been observed in several studies that vagal input to the NTS involving the RVM can cause descending facilitation of pain (32)

Descending control and spinal autonomic centres

Certain descending control tracts greatly influence the sacral and thoracolumbar preganglionic autonomic ganglia, in particular, those that use 5-HT, noradrenaline,

Substance P and thyrotropin-releasing hormone (TRH). This influence must be considered when studying these mechanisms, so as not to be confused by results altered by cardiovascular (CVS) changes. Autonomic CVS changes, for example, mean arterial pressure changes, can alter pain signalling in the spinal cord. Conversely, this can also mean that spinally administered analgesics affect cardiovascular functions. Furthermore, ascending vagal fibres to the NTS can exert control over descending tracts via a looped pathway (32).

Descending control and the sympathetic nervous system

The direction of sympathetic fibres into the spinal cord following sensory neuronal damage can cause increased neuropathic pain. Sympathetic changes can also have an effect on inflammation and pain in the periphery. Some spinal analgesic drugs, for instance, α2-AR agonists, dampen sympathetic influence (32).

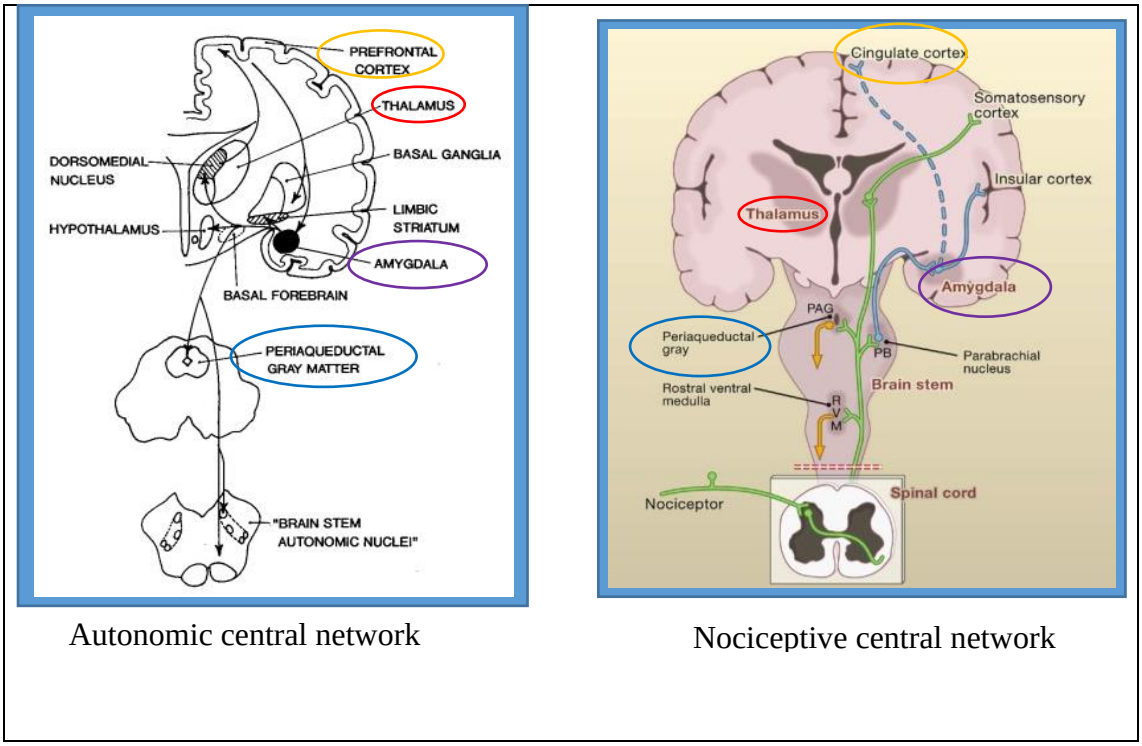


Figure 4: The autonomic and nociceptive networks. In this figure, we notice that the autonomic and the pain network share fundamental structures. For example, structures such as Cingulate cortex, thalamus, amygdala, periaqueductal grey are shared between the two functional networks.

There are other indirect connections between the pain system and the autonomic nervous system; one of those interesting connections is the three-way relationship between the autonomic nervous system emotions and pain. One of those theories that may link the autonomic nervous system with emotions and potentially pain is the James-Lange theory of emotions briefly explained below.

The James Lange Theory of Emotion

Emotions are known to alter the pain threshold. Likely by complex top-down control of nociception and by alteration to the cortical processing. Studies suggest a shared neuronal network between emotions and the nociceptive system (62).

The theory of the origin of emotion was first proposed by American psychologist William James (1884) and Danish physiologist Carl Lange (1887) independently. In essence, it suggests that emotions are produced by physiological changes such as autonomic changes(63). For example, if a person is in a dangerous environment, such as being attacked by a predator, subconsciously, that person will recognise the situation as a dangerous one, his or her heart will race, breathing will be shallow and rapid. Those physiological changes will trigger the emotion of fear. They argue that this is a more logical sequence of events(64). Psychology literature is rich in arguments and contra arguments for this theory. The theory has important limitations that are out of the scope of this theses. However, it emphasises the important observation that there are near-universal autonomic responses associated with specific types of emotions, such as fear and anxiety.

Another alternative interpretation of how autonomic responses can, by itself, trigger emotions is by pavlovian conditioning. Every time a person is in a stressful situation, the heart starts to race, the breathing frequency will increase, start sweating, and so on. Conversely, whenever a person is relaxed, the opposite of those physiological responses take place. Those situations repeat for a staggering number of times throughout our lives. One plausible hypothesis is that if we would design an intervention that mimics the autonomic responses of emotion, then we may be able to trigger that emotional

status. For example, slow deep breathing, both slows heartbeats and respiration rate, leading to a relaxed status that can trigger emotions that are more likely to increase pain thresholds and promote analgesia.

In summary, several classifications are used to describe pain, such as acute, chronic, somatic, visceral nociceptive, neuropathic and nociplastic pain. Those may overlap. Generally, there are three stages in pain-sensing; transduction, transmission and processing. Pain regulation depends on several intertwining systems. Higher structures send descending pathways to control pain transmission are the level of the spinal cord. Cortical, emotional, hormonal and autonomic factors can influence descending pathways.

In conclusion, the regulation of pain is complex and involves several interconnected pathways. The autonomic and the pain system are structurally and functionally intertwined. This relationship may allow for using the autonomic nervous system to influence the pain system.

Aims and Hypothesis

Aims

In this thesis, I aim to understand the central nervous system (CNS) control of visceral pain hypersensitivity via descending pain pathways to the spinal cord. Additionally, I aim to test the feasibility of a non-pharmacological intervention such as non-invasive vagal nerve stimulation to reduce this condition in healthy humans.

Hypotheses

I hypothesise that dysregulation in the descending pain modulation is a key contributor to visceral pain hypersensitivity. Moreover, I hypothesis that using electrical stimulation of the vagal nerve can modulate the nociceptive system and revere experimentally induced visceral hypersensitivity.

Chapter 2

Effect of slow deep breathing and attention on the autonomic nervous system

Introduction

The autonomic nervous system (ANS) is a complex bodily system, in addition to the enteric nervous system, it is comprised of two distinct yet intertwined entities. These are the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). The vagal nerve acts as a key aspect of the PNS, implicated in the homeostatic regulation of numerous internal bodily organ systems including the heart, lungs and gastrointestinal tract. Furthermore, the vagus nerve is thought to correspond to the functional state of the PNS, and consequently, over the past decades, the degree of vagal nerve activity has been quantified in research as differences in interbeat intervals (the time difference between two successive heartbeats. One measure of interbeat intervals that reflects parasympathetic tone is termed Cardiac Vagal Tone(65).

The PNS has previously been suggested to hold a critical role in the modulation of visceral pain, a complex phenomenon that is highly variable and influenced by a multitude of inter-individual factors (66, 67). In particular, a link between an individual's CVT and visceral pain perception has been investigated in recent years, leading to the suggestion

that an increase in CVT may correspond to a decrease in pain arising from the gastrointestinal (GI) tract(68). The proposed anti-hyperalgesic effect of the PNS is suggested to mediate through the efferent cholinergic pathway (69).

Distraction has been used successfully to control pain(70-72). The attention task used in this study has an antinociceptive effect at the level of the oesophagus. (30). Coen et al, used this task to study visceral pain provoked by balloon distention in the oesophagus. The mechanism was suggested to be central, mainly due to distraction.

Aims

In this study, we try to determine if there is a shared autonomic response between the two analgesic interventions (slow deep breathing and attention) that can contribute to the analgesic effect.

Methods

Ethical approval

The study protocol was reviewed and approved by Queen Mary, University of London, UK (reference QMERC2015/55). Besides, both written and informed consent was obtained from all study participants before reading the participant information sheet.

Subjects

Twenty-one volunteers participated in the study, recruited by local advertisement from staff and students of Queen Mary, University of London, UK. The study inclusion criteria were that of healthy individuals aged 18-60, with a gender distribution ratio not greater than 60:40. Women were studied during their follicular phase only to limit the effect of endogenous hormones upon autonomic parameters. Furthermore, participants were excluded if there was a positive history of anxiety or depression, drug abuse, cardiovascular conduction pathologies, or if women were either pregnant or breastfeeding.

Twenty-one participants expressed their interest and were subsequently recruited. One subject was excluded from all subsequent analysis due to poor data acquisition with regard to the autonomic measurements made, leaving a total cohort of twenty healthy volunteers (11 male; mean age 24 years, range 20 – 30) was utilised. Participant weight and height were recorded, along with the subsequent calculation of body mass index (BMI), in order to control for any influence of these parameters upon patient autonomic physiology. Mean ± standard deviation (SD) height was 1.70m ± 0.10, mean weight was

65.00 ± 13.30 kg and mean BMI was 22.4 kg/m^2 ± 3.50 kg/m^2, thus it was interpreted the cohort similar enough for inclusion and statistical comparison of autonomic physiological data.

Psychophysiological measurements

For all participants, personality traits and degree of anxiety were quantified using validated questionnaires (73, 74). The Big Five Inventory (BFI) was utilised to measure the numerical degree of personality traits extraversion, agreeableness, conscientiousness, neuroticism and openness, whereby a higher figure represents a greater degree of a given trait (73). BFI is scored using a percent of the maximum possible score (POMP) system(75). In addition, state and trait anxiety was quantified using the Spielberger State-Trait Anxiety Inventory (STAI) questionnaire (range 20–80, whereby a higher score equates to higher anxiety) assessing degree of anxiety on the day of the experiment (state (STAI-S)) and general anxiety (trait (STAI-T)) (74).

Autonomic neurophysiology

Throughout all experiments, ANS data were acquired through a multitude of parameters. Some were more specific to the parasympathetic nervous system, such as CVT, some were more specific to the sympathetic nervous system, such as cardiac sympathetic index (CSI) and, lastly, mixed measures of both arms of the autonomic system were also quantified, such as heart rate and blood pressure. These will be described below. Importantly, all ANS measurements were recorded in accordance with internationally agreed recommendations (76).

Mixed Measures

Using the previously validated photoplethysmographic technique (Portapres, Amsterdam, Netherlands) (77, 78), real-time arterial blood pressure was measured non-invasively in the ring finger of the left hand in all subjects, with the arm positioned at the level of the heart. Heart rate was also measured continuously, whereby electrocardiogram (ECG) electrodes (Ambu Blue Sensor P, Ballerup, Denmark) were placed in the right sub-clavicular area, the cardiac apex and left ankle, so as to correspond to an axis consistent with Eindhoven's Lead II. The ECG signal was acquired at 5 kHz, by means of a biosignals acquisition system (Neuroscope, Medifit Instruments, Enfield, UK), whereby real-time heart rate (HR, beats/min) was quantified by the interval between consecutive R waves (R-R intervals, in ms). In addition, raw data was fed into the NeuroScope for further processing and real-time derivation of the autonomic indices using VaguSoft (Medifit Instruments, London, UK).

Parasympathetic Nervous System Measurements: Cardiac Vagal Tone

Using the NeuroScope, CVT was measured continuously in real-time, as an index of brainstem parasympathetic nervous system efferent activity. A non-invasive continuous index of CVT described as pulse synchronised phase shifts in consecutive cardiac cycles is a form of pulse interval variability or jitter, was performed in real-time as previously described (79, 80). This also facilitates measurements of latencies of responses. The CVT is quantified in clinically validated units of a linear vagal scale (LVS) with zero reference point, equivalent to full atropinisation in healthy male human volunteers (65).

Contrasted to power spectral analysis of HR variability, CVT is validated for time epochs of less than 1 minute.

Sympathetic Nervous System Measurements: Cardiac Sympathetic Index

By calculating the cardiac sympathetic index (CSI), SNS activity was also quantified. CSI is derived from a modified Lorez plot of interbeat intervals(81).

Following the initial acquisition of R-R interval data, as described above, data was reformatted and entered into the Cardiac Metric program (CMetx, University of Arizona, AZ, USA). This permits the calculation of the validated Toichi's CSI, expressed as a ratio of R-R intervals, and hence has no units (81).

Study design

The experimental time course consisted of two separate visits in a crossover design, separated by at least one week to permit a 'wash out' period for the intervention subjects were exposed to. Before patient inclusion in the study, an initial telephone screening consultation was performed, to ensure all inclusion and exclusion criteria were met (see above). At the beginning of the first visit for any given subject, a 12 lead ECG was obtained to screen for any cardiovascular conduction pathologies that may otherwise confound results. Furthermore, participants would then complete the BFI questionnaire, along with the STAI-T (73, 74). Participants were studied between 0900-1200 only and were informed to refrain from alcohol and caffeine in the preceding 24 hours, smoking in the preceding 2 hours, and fast for 6 hours before the study, all of

which were undertaken to control for influential variables of autonomic activity. Furthermore, all experiments were undertaken in a quiet temperature-controlled environment (20 - 22°C).

Experimental time course

Following the screening consult, subjects were pseudo-randomised to receive one of two interventions during their first experimental visit, to which the other intervention would be employed during the second visit. The experimental intervention encompassed either a paced deep breathing exercise or a distraction task, both of which were 30 minutes in duration. Participants were positioned in a chair at 45 degrees, and when all real-time data acquisition devices were adequately recording (i.e. with minimal signal interference), the three-part experimental design was commenced. All autonomic data were acquired continuously throughout. The experimental paradigm was divided into the following epochs. Firstly, a 10-minute baseline reading was performed, whereby participants were simply told to relax. Following this, the 30-minute intervention would commence, either the paced deep breathing or distraction task. Finally, a further 5-minute baseline period was undertaken. Following this, participants would complete the STAI-S, thus concluding the experimental visit.

Deep Breathing Intervention

For the paced deep breathing intervention, participants would watch and listen to a 30-minute video whereby they were instructed to mimic the breathing patterns of the demonstrator. During the video, 1-minute periods of paced deep breathing would cycle

with a 5-minute rest period, when participants were told to breathe normally and relax. During the 1-minute deep breathing exercise, the full inspiratory capacity lasted 4 seconds and was followed by exhalation to forced expiratory vital capacity in 6 seconds, repeated at a frequency of 0.1 Hz, thus achieving a rate of 6 breaths per minute. In a subsequent analysis of the deep breathing intervention, the exact time course of the deep breathing activity was mapped to the beat numbers acquired from the Neuroscope, thus permitting data analysis of both the whole 30-minute period (encompassing both deep breathing and rest periods), but also during the deep breathing exercise only. The utility of paced deep breathing is comparable and validated by previously published studies (69, 82).

Distraction Task Intervention

The 30-minute distraction intervention employed was that of the validated 1-back task (Cogstate Ltd, USA). This task involves the presentation of a series of playing cards on a computer screen, whereby participants would need to continuously identify whether a current and sequential card were the same, or indeed different. Depending on if the cards were the same or different, participants were asked to use the computer mouse and identify this by clicking either the 'left' or 'right' button. The card presentation was pseudo-randomised and serves as a validated method to 'distract' study participants; this is a computerised version of the N-back Task proposed by Kirchner in 1958 (30, 83).

Statistical analysis

Data distribution was analysed using the Kolmogorov-Smirnov test. Quantitative data are herein presented either as mean ± SD, for parametric data, or median with interquartile ranges (IQRs) for parametric and non-parametric data respectively. Of note, CVT data values were not normally distributed. For non-parametric data, Wilcoxon signed-rank tests were utilised to compare CVT during experimental conditions. Furthermore, to assess the intervention effects on SNS and mixed measure parameters, repeated measures ANOVA were used, with posthoc correction using the Bonferroni method. Sphericity was confirmed by Mauchly's test for all ANOVA reports. For CVT and CSI, change from baseline was used rather than absolute values (delta-CVT, delta-CSI), this was chosen to reduce inter-individual variability. All statistical tests performed were two-tailed and statistical significance was thresholded to a criterion of $p<0.05$. All statistical analyses of both psychophysiological and autonomic neurophysiological data were performed using proprietary software (SPSS version 20 IBM, New York, USA).

Results

Table 2 below summarises the measurements of the autonomic variables during several epochs of each intervention.

Table 2 : absolute values of autonomic measurements during each intervention and demographic data.

Variable	Attention baseline	Attention	Post attention	Breathing baseline	Breathing	Post breathing
Cardiac vagal tone Median [IQR]	9.50 [5.2-11.7]	9 [6.2,11.7]	11 [8.2,12]	8.5 [5.2,13.2]	9 [7.25, 15.5]	8 [7, 13]
Cardiac sympathetic index Mean [SD]	2.50 [0.85]	2.67 [0.93]	2.66 [0.91]	2.60 [0.88]	3.18 [0.85]	3.24 [1.26]
Heart rate Mean [SD] beat/min	69 [14]	72 [13]	65 [11]	68 [11]	69 [10]	66 [10]

Systolic blood pressure Mean [SD] mmHg	115.9 [17.9]	127.6 [17.6]	127.6 [16.8]	116 [13.5]	120.5 [12.5]	124 [17.1]
Baroreceptor sensitivity Mean [SD]	8.2 [3.8]	7.7 [3.5]	8.5 [3.7]	8.6 [5]	8.3 [4]	8.7 [6]
Demographics	Age [years]	BMI		Weight	Height	Smoking
Mean [SD]	23.9 [2.57] years	22.4 [3.5] Kg/m2		65 [13.7] kg	1.69 [0.1] meter	0 %

Parasympathetic effects

Parasympathetic effects were measured by cardiac vagal tone. Using Wilcoxon test, SDB increased CVT (*p= 0.01*), Attention task did not affect CVT. However, post-Attention CVT was increased (*p=0.03*).

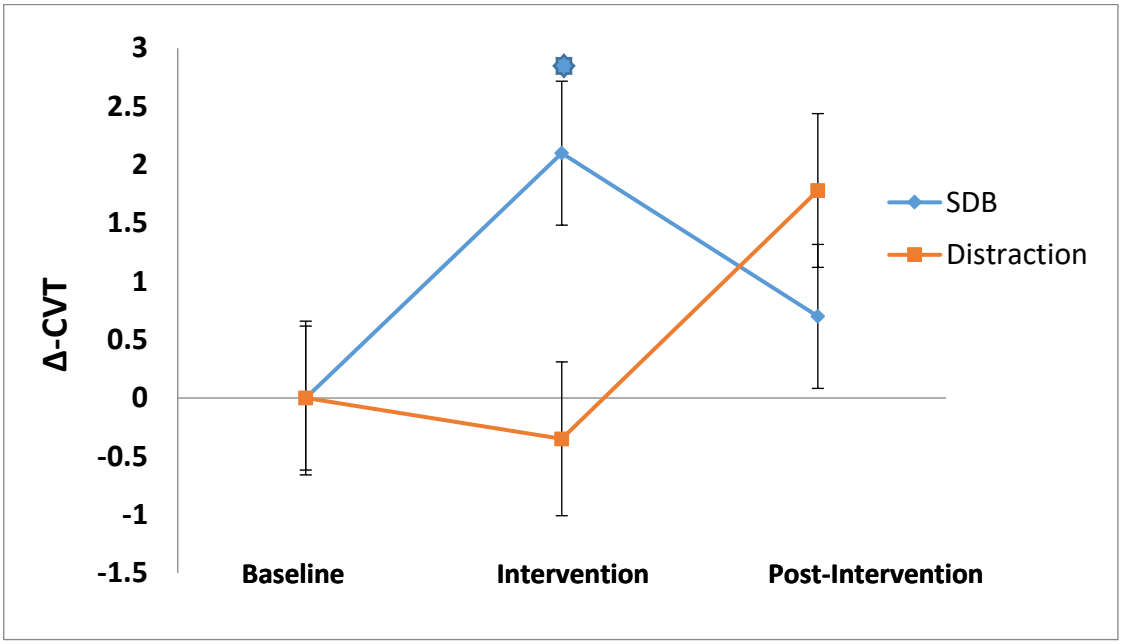


Figure 5: Delta Cardiac Vagal Tone (CVT) during slow deep breathing (SDB) and Attention.

Table 3: Parasympathetic effects. Using Wilcoxon test, SDB increased CVT (p= 0.01), Attention task did not affect CVT. However, post-Attention CVT was increased (p=0.03).

Variable	Attention baseline	Attention	Post attention	Breathing baseline	Breathing	Post breathing
Cardiac vagal tone Median [IQR]	9.50 [5.25-11.75]	9 [6.25, 11.75]	11 [8.25, 12]	8.5[5.25, 13.25]	9 [7.25, 15.5]	8 [7, 13]

Sympathetic effects

Sympathetic effects were measured by cardiac sympathetic index. ANOVA tests showed a significant effect on Cardiac Sympathetic Index (CSI) between interventions, F (2, 38) = 4.73, *p=0.015*. Follow-up pairwise comparisons reviled that SDB significantly increased CSI (Δ= 0.58, CI 0.56-1.1, *p= 0.027*)

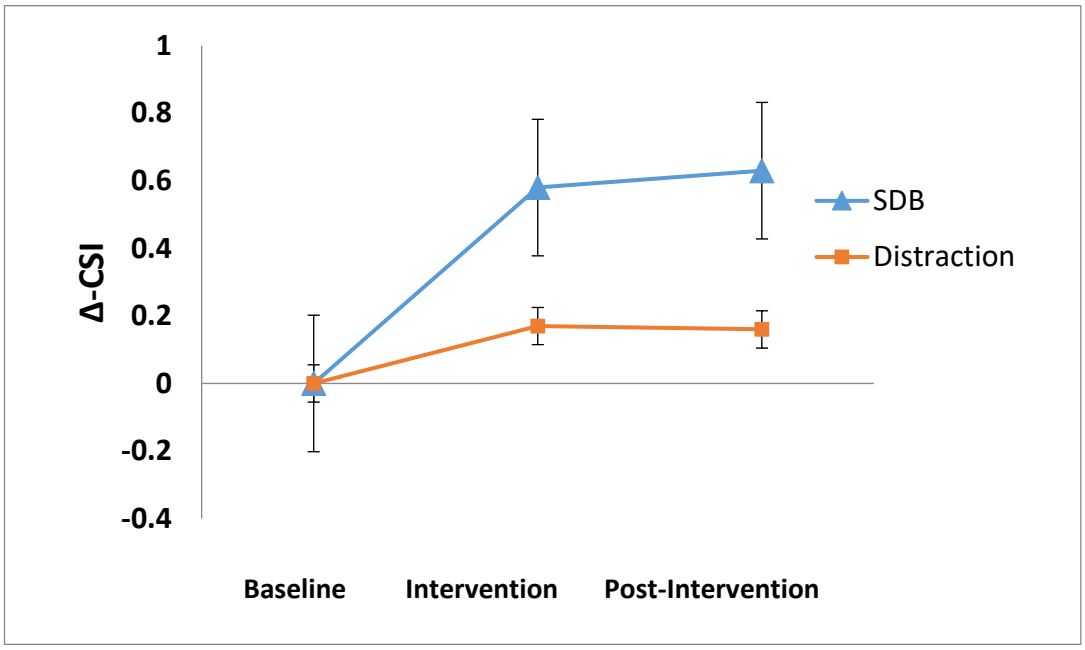


Figure 6: Delta Cardiac Sympathetic Index (CSI) during slow deep breathing (SDB) and Attention

Table 4: Sympathetic effects. ANOVA tests showed a significant effect on Cardiac Sympathetic Index (CSI) between interventions, F (2, 38) = 4.73, p=0.015. Follow-up pairwise comparisons reviled that SDB significantly increased CSI (∆= 0.58, CI 0.56-1.1, p= 0.027)

Variable	Attention baseline	Attention	Post attention	Breathing baseline	Breathing	Post breathing
Cardiac sympathetic index Mean [SD]	2.50 [0.85]	2.67 [0.93]	2.66 [0.91]	2.60 [0.88]	3.18 [0.85]	3.24 [1.26]

Mixed Effects

Mixed effects refer to measurements that are influenced by both sympathetic and parasympathetic systems. Those are heart rate and blood pressure. Attention increased both HR and SBP, (Δ= 2.9, CI 0.35-5.3, p<0.02), (Δ= 10.7, CI 5.2-16.2, p, 0.001) respectively.

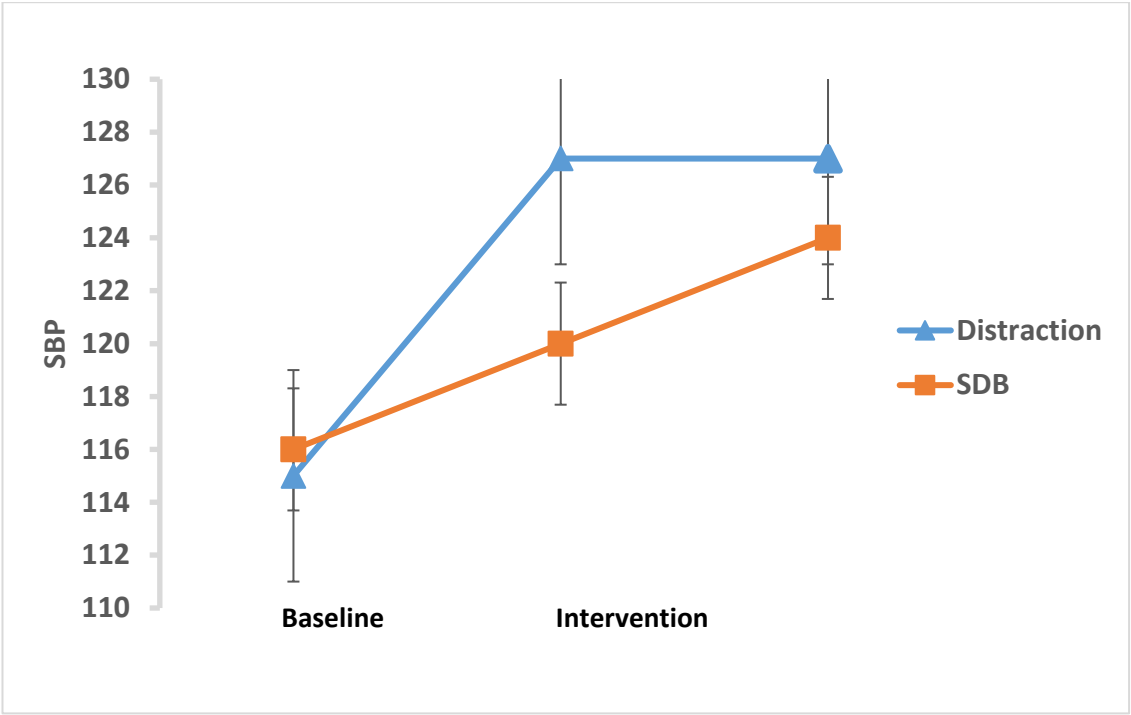


Figure 7: Systolic blood pressure (SBP) during slow deep breathing (SDB) and attention. Attention increased both SBP, (Δ= 10.7, CI 5.2-16.2, p, 0.001) using a Wilcoxon test.

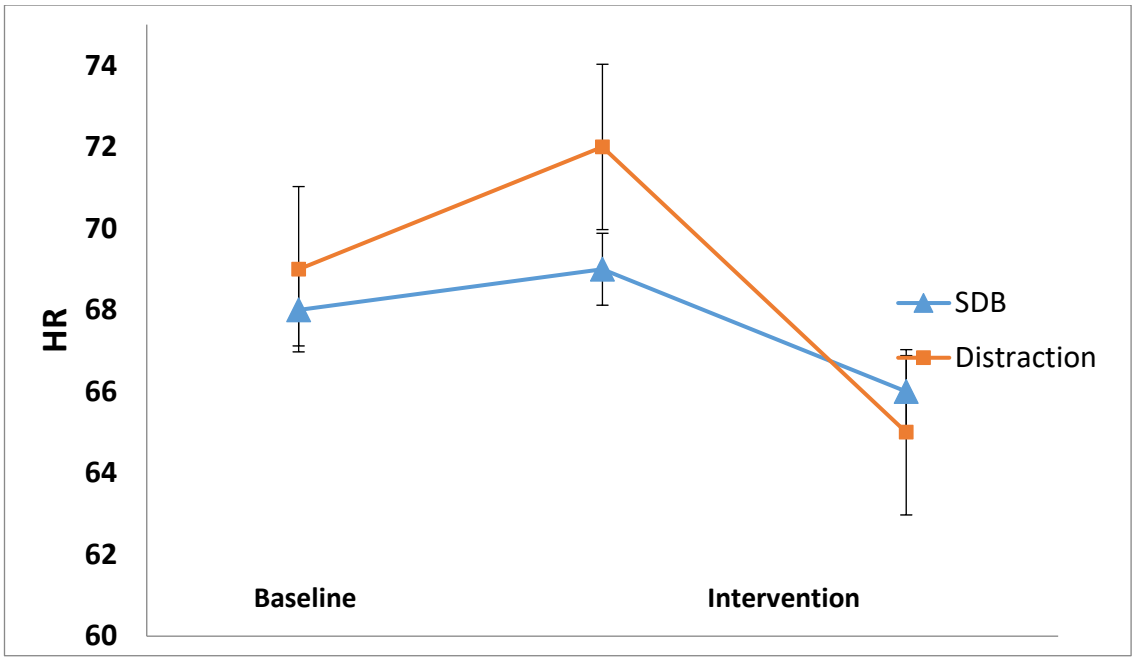


Figure 8: Heart rate (HR) during slow deep breathing (SDB) and attention. Attention increased both HR (Δ= 2.9, CI 0.35-5.3, p<0.02) using Wilcoxon test.

Table 5. Attention increased both HR and SBP, (∆= 2.9, CI 0.35-5.3, p<0.02), (∆= 10.7, CI 5.2-16.2, p, 0.001) respectively.

Variable	Attention basline	Attention	Post-attention	Breathing baseline	Breathing	Post-breathing
Heart rate Mean[SD] beat/min	69 [14]	72 [13]	65 [11]	68 [11]	69 [10]	66 [10]
Systolic blood pressure Mean [SD] mmHg	115.9 [17.9]	127.6 [17.6]	127.6 [16.8]	116 [13.5]	120.5 [12.5]	124 [17.1]

Psychophysiological data

All participants completed the BFI and STAI. Of the BFI score dimensions, mean extraversion was 28.2 ± 5.9, mean agreeableness was 38.3 ± 5.1, mean conscientiousness was 35.3 ± 5.4, mean neuroticism was 16.2 ± 5.3 and mean openness was 37.1 ± 4.4. Mean trait anxiety was 46.4 ± 3.6, mean state anxiety after the PDB task was 47.6 ± 3.7 and mean state anxiety following the distraction task was 48.2 ± 3.6. Anxiety states did not significantly differ following either intervention. After correcting for multiple testing, personality trait and anxiety inventory did not correlate with autonomic variables.

Discussion

Slow Deep Breathing and Attention had distinct patterns of autonomic responses. Slow deep breathing activated both the sympathetic and parasympathetic system while the significant effect of attention on Cardiac Vagal Tone was restricted to the post-intervention period; this effect is likely due to relaxation. Attention significantly increased HR and systolic blood pressure. During Attention, there was a trend towards increasing in Sympathetic Tone and a reduction in Parasympathetic Tone, without being statistically significant, likely to be a type 2 error.

Slow deep breathing caused a pronounced activation of the parasympathetic nervous system measured by CVT. This activation was instant, lasted throughout the intervention and returned to baseline immediately after the cessation of the task. Attention task did not change CVT significantly from baseline. However, there was an increase in CVT after the cessation of the task.

Parasympathetic activation of slow deep breathing is consistent with multiple studies that reported a correlation between slow breathing and increased HRV (69, 84-88).

The parasympathetic activation of slow deep breathing disappeared almost instantly after the cessation of the task, suggesting that if long term activation of the parasympathetic nervous system is needed, then, longer epochs of slow deep breathing might be necessary.

Several studies reported a correlation between parasympathetic activity and pain thresholds (69, 86, 87). Interestingly, Busch et al., studied the effect of two paradigms of slow deep breathing on pain; one task required constant attention while the other was aimed to be relaxing, only slow deep breathing with relaxation had an anti-nociceptive effect (89).

Attention, on the other hand, did not significantly alter the cardiac vagal tone. However, there was a pronounced post attention activation of the parasympathetic nervous system. This increase in cardiac vagal tone is most likely due to relaxation at the end of a stressful task. Although, in this study, we did not measure pain tolerance, the role of attention in pain modulation is well documented in the literature (30, 90). It is most likely related to distraction from the painful stimulus. This indicates that the antinociceptive effect is likely at the level of cortical processing of pain.

Attention in this study mainly refers to distraction from pain stimulus by shifting the attention to another task; it does not refer to attention to the painful stimulus.

There is some evidence to suggest that interventions combining both breathing techniques and distraction, such as mindfulness are superior to distraction alone(91).

Limitations

This study is not without limitations. One of those is the lack of breathing monitoring during the attention task. There is a possibility that the autonomic effect of attention was driven by changes in breathing pattern due to the difficulty of the task, this change in breathing could, in turn, be responsible for the autonomic signature. However, Chang et al. showed that attention did not change the breathing rate with mild, moderate and intense attention tasks (92).

The other limitation is the absence of a painful stimulus. The presence of a painful stimulus could change the autonomic response.

Conclusions

The two intervention mounted rather distinct autonomic responses. This finding, making it less likely that there is a fixed autonomic behaviour that can change the pain threshold.

The two interventions are likely to operate via separate mechanisms. Attention is likely due to a central mechanism mediated via cognitive structures, likely related to distraction. Slow deep breathing antinociceptive mechanism has been suggested to operate via the efferent vagal nerve. Botha et al. showed that cholinergic blockade diminished the analgesic effect suggesting and efferent mechanism (69). If the mechanism of action is distinct between the two interventions, then a synergic antinociceptive effect is plausible.

Chapter 3

Conditioned Pain Modulation in Irritable Bowel Syndrome; Systematic Review and Meta-Analysis:

Introduction

The central nervous system can profoundly influence the intensity and hence perception of ascending nociceptive sensory signalling. This 'descending modulation' is mediated through endogenous pain inhibitory or excitatory pathways (41, 93). A balance between those two opposing pathways will determine the nociceptive influx to higher brain structures. Inhibitory pathways are especially important for the scope of this review. They mediate a physiological phenomenon termed Conditioned Pain Modulation (CPM) where a painful stimulus can inhibit another existing pain(48).

Experimentally, conditioned pain modulation can be objectively quantified in three steps. Firstly, pain thresholds are measured after an initial test stimulus. Secondly, a separate, or what is referred to as a conditioning, a tonic stimulus is applied to an anatomically distant region. Finally, the initial test stimulus is reapplied with pain

threshold recorded a second time (94). Normally, the pain thresholds between the first and second test stimulus increase, when measured at the same time of applying a conditioning stimulus. However, in chronic pain disorders, such as migraine, fibromyalgia and temporomandibular disorder, pain thresholds to the test stimulus fail to increase in the presence of a conditioning stimulus, this, in turn, suggests a degree of deficiency in conditioned pain modulation (95-97). Considering that many of these pain disorders are frequently comorbid with IBS, it is plausible to suggest that deficient conditioned pain modulation may also be important, but under-recognised, a pathophysiological feature that contributes to visceral hypersensitivity. Thus, we aimed to address this knowledge gap in IBS by performing a systematic review with meta-analysis to assess whether conditioned pain modulation is deficient in IBS patients, compared to healthy subjects. Secondary aims included investigation of the influence of the diagnostic criteria used to define IBS, as well as the predominant bowel habit, on conditioned pain modulation.

We chose irritable bowel syndrome as a representative of visceral pain hypersensitivity because it is the most studied condition in the literature with good quality studies that can infer some useful information on the status of conditioned pain modulation in functional GI disorders with pain hypersensitivity as the main feature.

Irritable bowel syndrome (IBS) is a common disorder characterised by recurrent abdominal pain associated with a change in bowel habit (98). With a reported population prevalence of 11.2% (99), it is associated with a significant reduction in health-related quality of life and work productivity (100). Heightened sensitivity of the viscera to experimental stimulation, referred to as 'visceral hypersensitivity', is an important

independent contributor to the gastrointestinal (GI) symptom burden of IBS (101). The pathogenesis of abdominal pain and visceral hypersensitivity in IBS is complex, multidimensional, and incompletely understood (102). However, dysregulation within the 'brain-gut axis', a bidirectional interface between the brain and the viscera, has been implicated (103). Several alterations have been suggested such as peripheral sensitisation of nociceptors, low grade inflammation, impaired mucosal function, central sensitisation, dysregulated descending inhibition of pain, perception alterations and psychiatric predisposition(102).

Materials & methods

Search strategy

We performed a systematic review and meta-analysis following PRISMA recommendations (104). Firstly, we did a literature search using MEDLINE and Web of Science (1980 – 10th of May 2018). We searched for studies using the terms '*irritable bowel syndrome* and *functional bowel disorder* as a medical subject heading (MeSH) and free-text terms. Then, we combined those with the set operator "AND" with following terms: diffuse noxious inhibitory control, DNIC, conditioned pain modulation, conditioning pain modulation, CPM, heterotopic noxious conditioning stimulation, heterotopic noxious counter stimulation, heterotopic nociceptive conditioning stimulation, heterotopic nociceptive counter stimulation, descending pain modulation, descending pain inhibition, counter stimulation, counter-irritation as free text terms. Publications were restricted to those studying adult populations, defined as greater than 16 years old, with a documented diagnosis of IBS according to any internationally

accepted definition of IBS (Manning, Kruis or Rome criteria (i.e. Rome I, II, III or IV)) were included. Additional inclusion criteria were: i) use of a conditioned pain modulation model; ii) presence of a control group; iii) at least ten subjects in each group and iv) a clearly stated outcome measure to calculate conditioned pain modulation. We excluded studies if the subjects were taking opioid analgesics or had concomitant chronic pain conditions. No language restrictions were set. Relevant studies were independently reviewed in full by two investigators (KF and MG). Disagreements were resolved by consensus. Conference proceedings from 4 international meetings (Digestive Diseases Week, United European Gastroenterology Week, International Association for the Study of Pain World Congress and the Joint International Neurogastroenterology and Motility meeting) were also searched from 1997-2018.

Outcome assessment

The name of the first author, year of publication, number of subjects, diagnostic criteria used, IBS subtypes, study design and conditioned pain modulation paradigm and outcomes were recorded in a standardised fashion using an Excel spreadsheet (Excel for Mac 2011, Microsoft, Redmond, USA).

Study methodology quality assessment

The independent reviewers were blinded to each other's assessment. The studies were assessed for bias in 6 categories; the 1st four categories are adapted from a previous meta-analysis that looked at the effect of chronic pain on conditioned pain modulation (51). We added two extra criteria that we considered necessary for this type of study;

those are the assessment of outcomes and the possibility of co-existence of other painful conditions. Each category was numerically graded as 0, 1 or 2, which were considered as low, moderate and high risk of bias, respectively. Contingent on these six distinct parameters, each study received an overall bias score from 0-12. These categories were: 1) blinding of assessors (high-risk if un-blinded or not stated); 2) cases representative of the population by use of internationally accepted criteria to identify patients (high-risk if no criteria were mentioned, moderate risk if specified but not internationally validated); 3) comparability of cases and controls on age and gender (low risk if <10 %, moderate if between 10-20%, high-risk if >20%); 4) controlling for known confounders, including menstrual cycle phase; the time of day of assessment; caffeine or alcohol intake; presence of other types of pain during testing; attention to the test stimulus/distraction from the conditioned pain; medication that could alter the pain perception; psychological disorders. If a study controlled for three or more of the confounders then it was considered at low risk, if at least two confounders were controlled for in the study, then it was considered as moderate risk, and if the study was controlled for 1 or 0 confounders, then it was considered at high risk. 5) Assessment of outcomes (low risk if used a validated conditioned pain modulation paradigm with a painful test and conditioning stimulus, high risk if the painful nature of either stimulus is not clear; (6) other concomitant disorders of chronic pain (low risk if they were excluded or statistically accounted for, moderate risk if it was specified but not excluded and high risk if not mentioned in the study).

In this meta-analysis, we excluded subgroups with concomitant pain conditions or psychological factors that may influence conditioned pain modulation, such as in Heymen *et al*. we excluded patients with migraine and temporomandibular joint

disorders (105). We also excluded conditioned pain modulation measured inside the MRI machine in Wong *et al.* (106). Wilder-Smith *et al.* [2014] used two conditioned pain modulation paradigms; foot heat and capsaicin as test stimuli (54). However, enough data to calculate an effect size was only provided for capsaicin as test stimulus and foot heat stimulation as conditioning stimulus, and thus only this paradigm was used in this meta-analysis (54).

We excluded Wilder-Smith *et al*. [2004] from the meta-analysis because it did not provide means and SDs, but medians and interquartile ranges instead (53). Due to the small number of participants in this study (n=10 in each arm), it was not technically possible to convert this into means and SDs to calculate an effect size necessary for the meta-analysis or use other data to calculate a pooled odds ratio(107).

Statistical analysis

Data were pooled by a random-effects model using Der Simonian-Laird weights (108), as this was considered the most plausible methodology given the likely heterogeneity between studies and would provide a more conservative estimate of the effect and its confidence interval. Data such as group's means, group's means before and after interventions, standard deviations, *p*-values and number of participants, were used to calculate a standardised difference in means with 95% confidence interval. The standardised difference in means was then converted to the natural logarithm of the odds ratio using relevant formulas that can be found in this reference(109).

Outcomes are expressed as pooled odds ratio with 95% confidence intervals (CI). The odds ratio is a measure that explains the association between an exposure and an

outcome (110) — for example, exposure to disease (IBS) and outcome (reduced conditioned pain modulation). We also calculated Hedge's g effect size; this is a measure of the standardised mean difference between 2 groups(111). An effect size of > 0.8 is generally considered as a large effect size(112).

The I^2 statistic and Cochran's Q test were used to assess for study heterogeneity. The I^2 statistic describes the percentage of variation across studies that is due to a true heterogeneity, rather than chance, with values ranging from 0% to 100%. I2 values of 25%, 50%, and 75% were considered low, moderate, and high(113). Cochran's Q is distributed as per the chi-square statistic. We performed pre-specified subgroup analyses to ascertain whether there was effect modification by diagnostic criteria used or IBS sub-type. Tests were considered statistically significant if the *p*-value was < 0.05. We used a funnel plot to visually inspect for publication bias. Propriety software (Comprehensive Meta-Analysis, Biostat, New Jersey, USA, Version 2), was used to perform all the calculations for the meta-analysis and generate Forest plots for the stated outcomes.

Results

Search results

The search generated 645 citations, of which 13 were relevant, and 12 met the inclusion criteria, *see Figure 9.* Of the 12 studies included, there were 248 patients and 216 controls. All studies had a case-control design, *see Table 5.* 11 of the 12 studies were conducted in females only.

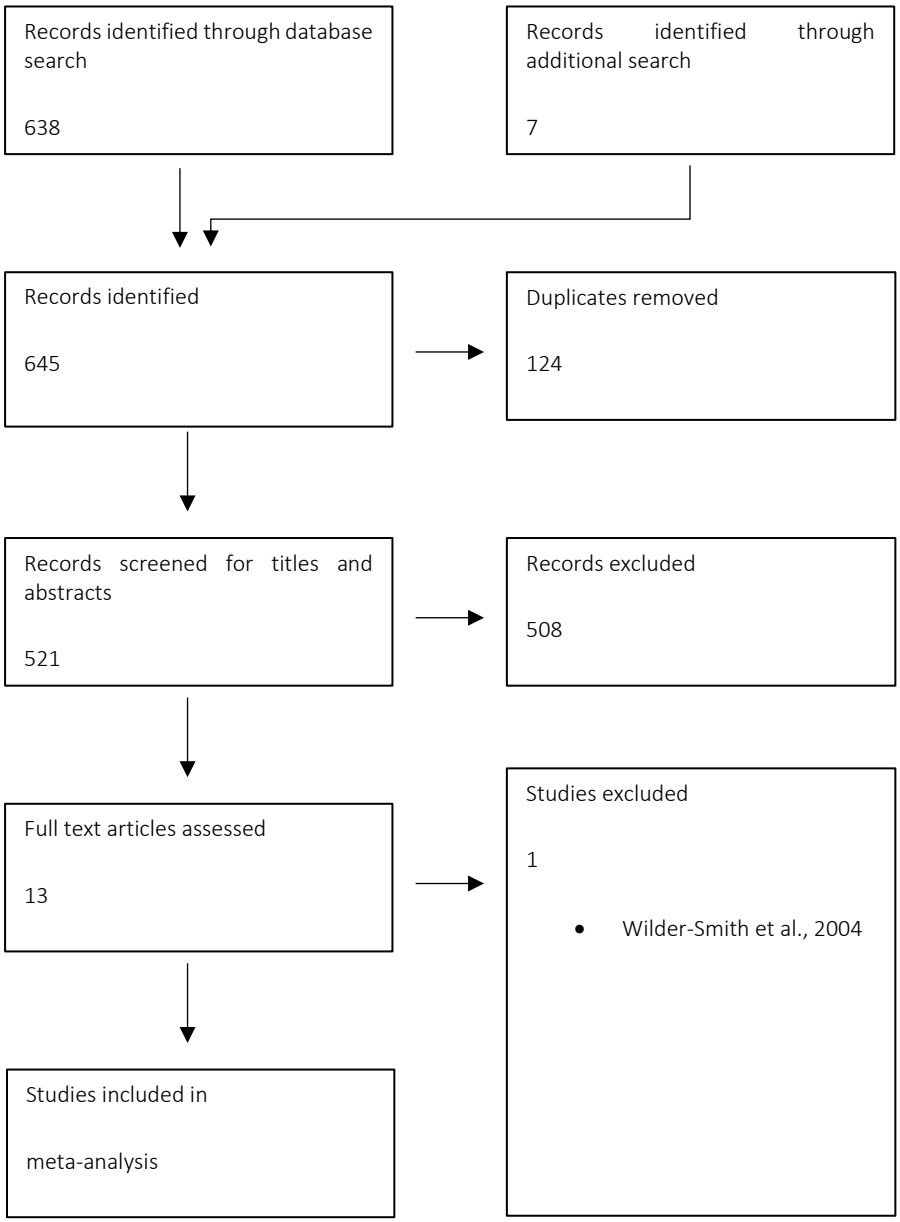


Figure 9: Flow diagram for the assessment of studies identified in the systematic review.

Table 6: Summary of included studies. Abbreviations: F, females; HC, healthy controls; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome-constipation predominant; IBS-D, irritable bowel syndrome-diarrhoea predominant; M, males; n, number of subjects.

Study	IBS sub-type	Cohort demographics	Test stimulus & location	Conditioning stimulus, location and duration	Outcome measure	CPM result
Bouhassira et al. (114, 115) (2013)	IBS-C	IBS, n = 20 HC, n = 11 Females only	Electrical stimulus Sural nerve	Cold pressor test (2-4°C) Contralateral hand 1 min.	Reflex (RIII)	HC>IBS
Heymen et al. (51, 105) (2010)	IBS (all subtypes)	IBS, n = 27 HC, n = 21 Females only	Heat stimulus Left hand	Cold pressor test (12°C) Right hand 44 sec.	Pain rating	HC>IBS
Jarrett et al. (2016) (116)	IBS (all subtypes)	IBS, n = 46 HC, n = 31 Females only	Heat stimulus Forearm	Hot pressor test (46.5°C) Contralateral hand 1 min.	Pain rating	HC=IBS
Jarrett et al. (2014) (117)	IBS (all subtypes)	IBS, n = 20 HC, n = 20 Females only	Heat stimulus Forearm	Cold pressor test (12°C) Contralateral hand 1 min.	Pain rating	HC=IBS
King et al. (2009) (118)	IBS (all subtypes)	IBS, n = 14 HC, n = 28 Females only	Heat stimulus Left hand	Cold pressor test (8-16°C ±0.1°C) Right foot 30 sec.	Pain rating	HC>IBS
Piché et al. (2013) (119)	IBS-D	IBS, n = 14 HC, n = 14 Females only	Electrical stimulus Retro-malleolar path of right sural nerve	Cold pain (Ice pack - 12°C) Left forearm 2 min.	Pain rating	HC=IBS
Piché et al. (2011) (120)	IBS-D	IBS, n = 14 HC, n = 14 Females only	Electrical stimulus	Cold pain (Ice pack - 12°C) Left forearm 2min.	Pain rating	HC>IBS

			Retro-malleolar path of right sural nerve			
Piché et al. (2010) (121)	IBS-D	IBS, n = 11 HC, n = 18 Females only	Electrical stimulus Retro-malleolar path of left sural nerve	Cold pressor test (4°C) Right hand 2 min.	Pain rating	HC>IBS
Song et al. (2006)(122)	IBS (all subtypes)	IBS, n = 12 HC, n = 12 Females only	Rectal distention	Cold pressor test (4°C) Left foot 30 sec.	Pain rating	HC>IBS
Wilder-Smith et al. (2007) (123)	IBS (all subtypes)	IBS, n = 40 HC, n = 20 Females only	Rectal distension	Cold pressor test (4°C) Left foot 2 min.	Pain rating	HC>IBS
Williams et al. (2013) (124)	IBS (all subtypes)	IBS, n = 22 HC, n = 16 Females only	Heat stimulus Right forearm	Cold pressor test (12°C ±1°C) Left hand 1 min.	Pain threshold	HC>IBS
Wong et al. (2016) (106)	IBS (all subtypes)	IBS, n = 13, M = 6, F = 7 HC, n = 11, M = 4, F= 7 Mixed gender	Rectal distension	Heat stimulus (≈44,5°C) Left foot 30 sec.	Pain threshold	HC=IBS

Diminished conditioned pain modulation in IBS

Conditioned pain modulation in IBS populations versus healthy controls was more likely to be diminished with an odds ratio of 4.84 (95% CI 2.18-10.71, p<0.0001). There was a large standardised difference in mean between IBS and healthy controls with a hedges' g effect size of 0.85 (95% CI 0.42 - 1.28, p<0.001) (125). Significant heterogeneity between studies was noted (Q-test χ^2 =52, p<0.001, I^2 =78.8). Visual inspection of the Funnel plot did not provide evidence of publication bias, see figure 10.

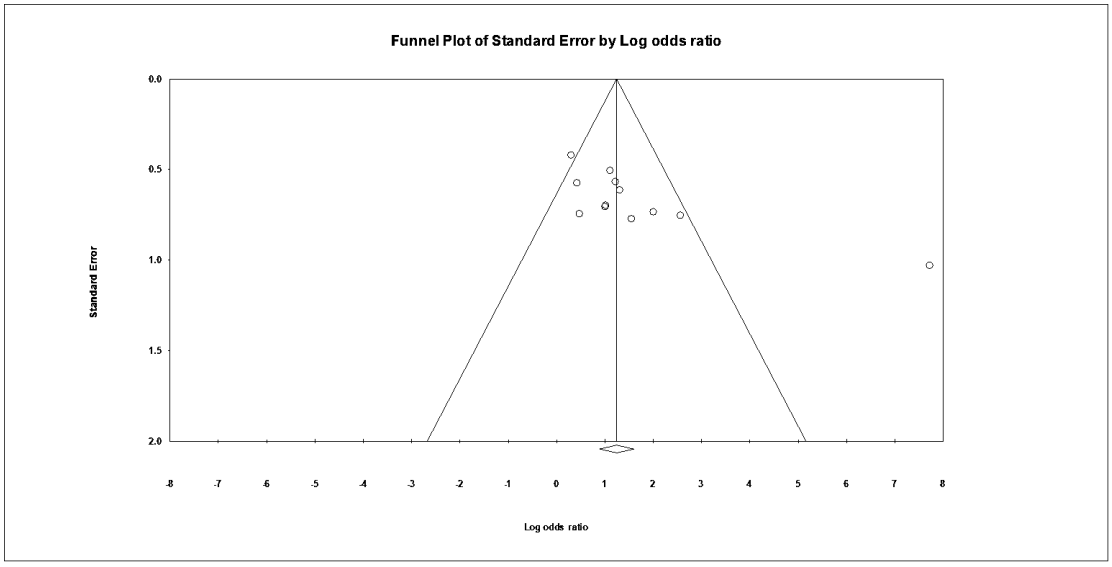
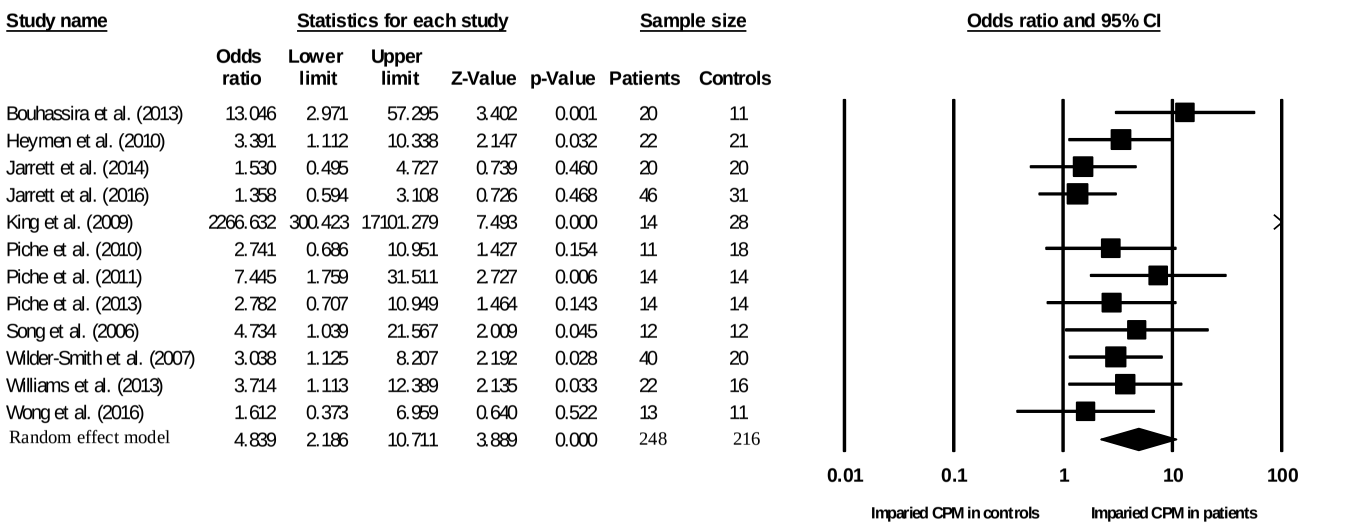


Figure 10: A Funnel plot of the included studies demonstrating symmetry which suggests that there is no significant publication bias.



Meta Analysis

Figure 11: A Forest plot of the odds ratio of deficient conditioned pain modulation in IBS vs. healthy subjects. The pooled odds ratio of deficient conditioned pain modulation in IBS patients was 4.84 (95% CI 2.19 – 10.71, p<0.0001).

Effect modification by diagnostic criteria used

Of the 12 studies that met the inclusion criteria, nine studies used the Rome III definition and three studies used Rome II. The odds ratio of impaired conditioned pain modulation using the Rome II and III criteria was 3.44 (95% CI 1.76 - 6.70, *p*<0.0001) and 5.65 (95% CI 1.87 - 17.04, *p=0.002*) respectively, *see figure 12.* However, between groups analysis did not reveal a statistically significant difference; Q= 0.54, df= 1, *p=0.46*.

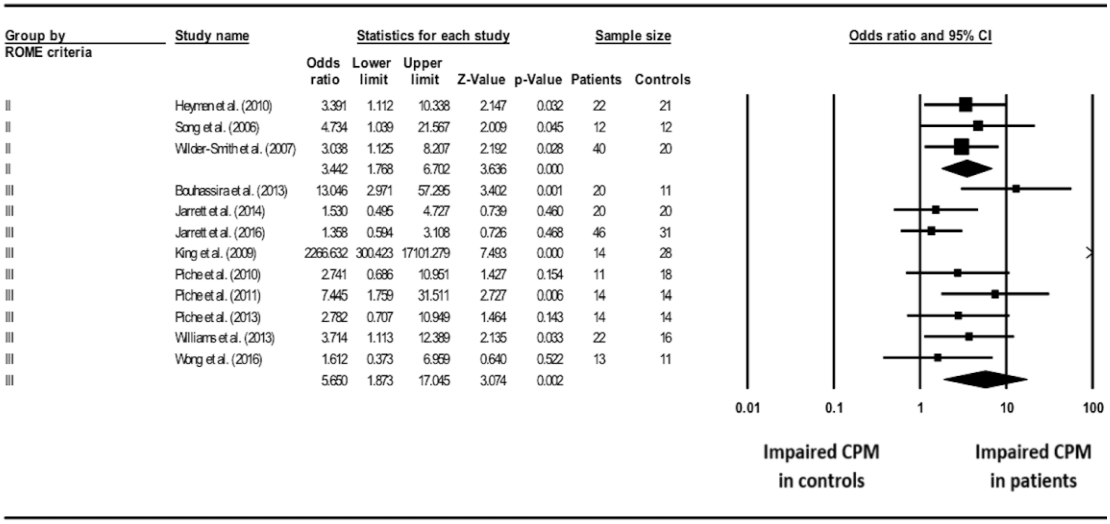


Figure 12: Forest plot of the odds ratio of impaired Conditioned pain modulation according to different Rome IBS definition. The pooled odds ratio for Rome II, Rome III was 3.44 (95% CI 1.76 - 6.70, p<0.0001) and 5.65 (95% CI 1.87 - 17.04, p-0.002) respectively. Abbreviations: CPM: Conditioned pain modulation, CI: confidence interval.

Effect modification by IBS SUBTYPE

Of the 12 studies that met the inclusion criteria, eight studies included pooled both subtypes of IBS patients into one group without reporting separate conditioned pain modulation outcome for each subtype, 1 study included only IBS-constipation (IBS-C) and three studies only IBS-diarrhoea (IBS-D) patients. The odds ratio of impaired conditioned pain modulation in IBS-D was 3.76 (95% CI 1.68 - 8.44, *p*=0.001), Q-test χ^2 = 1.25, df =2,

p=0.54, I^2 =0. Odds ratio based on the one study of IBS-C was 13.05 (95% CI 2.97 - 57.29, p=0.001), Q-test χ^2 = 0, df =0, p=1, I^2 =0. For the studies with mixed populations of IBS, χ^2 = 47.4, df =7, p<0.001, I^2 =85.23. There was no statistically significant difference when comparing the three groups of studies (IBS-D, IBS-C, and studies with mixed IBS populations), Q= 2.08, df=2, p=0.35. *See figure 13.*

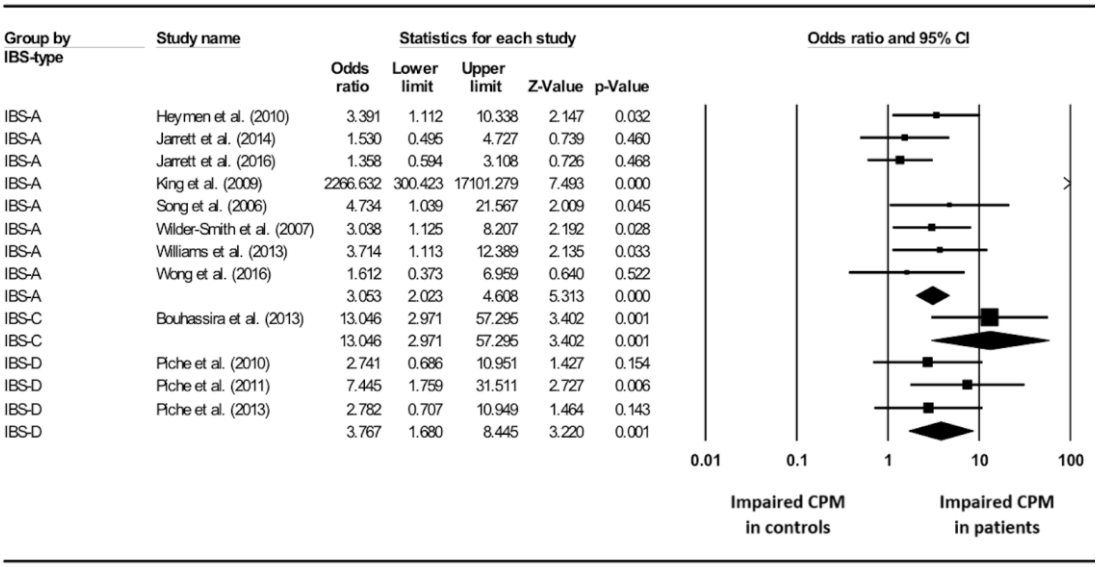


Figure 13: Forest plot of the odds ratio of Impaired Conditioned pain modulation in IBS subtypes. The pooled odds ratio for IBS-C was 13.04 (95% CI 2.97 - 57.29, p=0.001) and for IBS-D 3.76 (95% CI 1.68 - 8.44, p=0.001). Abbreviations: CPM: Conditioned pain modulation; CI: confidence interval; IBS-A, all mixed subtypes of IBS (i.e. not classified in the reporting paper); IBS-C, IBS with constipation predominance; IBS-D, IBS with diarrhoea predominance.

Study methodological quality assessment

The methodological quality of the included studies is summarised in *Table 6*. A total bias assessment scale was agreed to range between 0-12, depending on individual criteria, with 0 indicating no bias. All the included studies scored between 2 to 5. None of the studies was blinded; hence the lowest total score was 2.

Table 7: Study methodological scoring.

Study	Blinding of outcome assessors to participant group	Patients representative of the population	Control for known confounders	Comparability of cases and controls (gender and age)	Assessment of outcomes	Coexistence of other chronic pain condition	Total bias score
Bouhassira *et al.* (2013)(115)	2	0	2	0	0	1	5
Heymen *et al*. (2010) (105)	2	0	0	0	0	0	2
Jarrett *et al*. (2016) (116)	2	0	1	0	0	2	5
Jarrett *et al.* (2014) (117)	2	0	0	0	0	0	2
King *et al*. (2009)(118)	2	0	1	0	0	0	3
Piché *et al*. (2013) (119)	2	0	0	0	0	0	2
Piché *et al*. (2011)(120)	2	0	1	0	0	0	3
Piché *et al.* (2010)(121)	2	0	1	0	0	0	3

Song *et al*. (2006) (122)	2	0	1	0	0	2	5
Wilder-Smith *et al.* (121) (2007)(123)	2	0	1	0	0	0	3
Williams *et al.* (2013) (124)	2	0	0	0	0	0	2
Wong *et al*. (2016)(106)	2	0	0	1	0	0	3

Discussion

Our meta-analysis illustrates that IBS patients are nearly five times more likely to have diminished conditioned pain modulation when compare to healthy controls. The increased likelihood is also associated with an important standardised difference in mean reflected by a large Hedge's g effect size. Between groups comparisons, failed to show a significant difference between IBS subtypes or the Rome criteria used for diagnosis, although some interesting trends were noticed in this regard. Of note, King et al. yielded an odds ratio of impaired conditioned pain modulation that is many folds greater than any other study (118). However, this particular study was assigned the smallest relative weight in the random model used in this meta-analysis, making it unlikely that this has significantly skewed the overall results. Most studies (10/12) either used rigours criteria to exclude other painful conditions except IBS or reported separate data of patients with concomitant painful conditions; this enabled us to exclude such data from the meta-

analysis. The latter makes it likely that the effect seen in this meta-analysis is related to IBS.

These results have several important implications across the field, particularly concerning underlying pathophysiology of the disorder, as well as future clinical practice.

The central defining characteristic of IBS is chronic abdominal pain, with a percentage of patients displaying heightened pain sensitivity to visceral stimuli, termed visceral hypersensitivity (126). Visceral hypersensitivity may arise, and be maintained, due to abnormalities at any level of the brain-gut axis, such as sensitisation of peripheral and central neurons (127). Moreover, data from several functional brain imaging studies have also provided evidence for aberrant central pain processing in cortical and subcortical regions (102, 128, 129). Abnormal descending pain modulation is likely to adversely contribute to many of these mechanisms as it includes many of the constituent components of the brain-gut axis. Thus, there are three plausible explanations as to the deficiency in conditioned pain modulation that we have identified in IBS patients. Firstly, in this patient group, there is a true imbalance between descending inhibition and descending pain facilitation. Secondly, the 'normal' physiological descending inhibitory pain regulatory system is insufficient to dampen nociceptor recruitment at the level of the dorsal horn, where "gating" of visceral nociceptive afferent transmission occurs. Finally, a combination of dysregulation within descending pain modulatory pathways and established central sensitisation at the central nervous system may result in an overall impairment in conditioned pain modulation (130). This latter explanation is the most likely as the development of central sensitisation at the spinal dorsal-horn neurone level due to peripheral injury or inflammation may be a consequence of dysregulated

descending control from centres such as the rostral ventromedial medulla to the spinal dorsal horn (131).

Several central nervous system regions are involved in the descending pain modulation, for instance, the insula, prefrontal anterior cingulate cortex, amygdala, hypothalamus, rostral ventromedial medulla and dorsal pons (132-134). The major neurotransmitters within the descending pathways are serotonin (5-HT), norepinephrine, and dopamine which regulate the excitability of dorsal horn neurons (32, 135, 136)- see figure 14. Notably, the above-mentioned brain regions and neurotransmitter pathways have important interactions with the autonomic nervous system (137, 138). Interventions that could reduce the descending excitatory effect, or enhance the descending inhibitory effect, may, therefore, be theoretically useful in the management of pain in IBS and such intervention could be physiological, pharmacological, or even electrical.

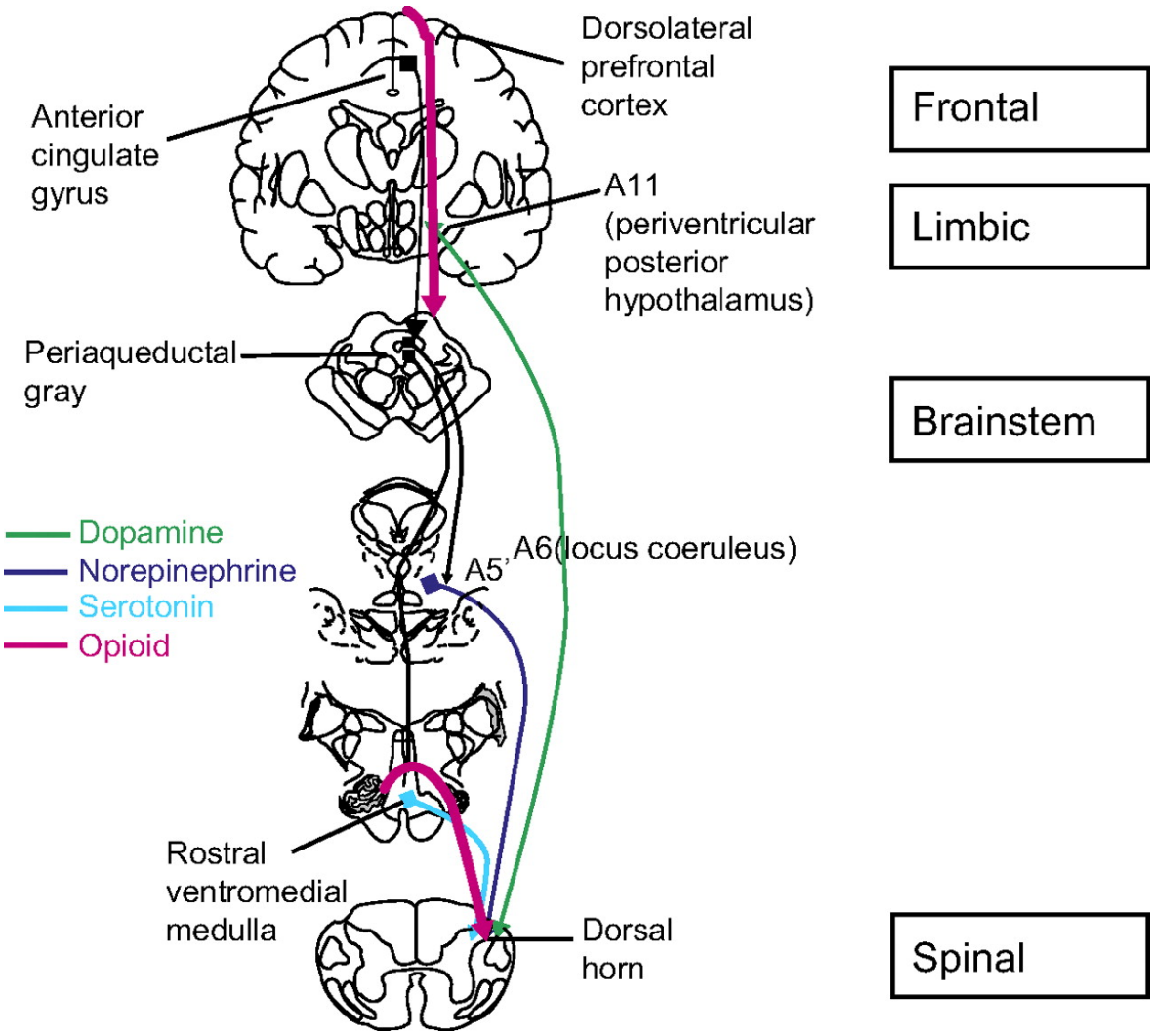


Figure 14: Descending pain modulation pathways identifying the main transmitter systems. Adapted from Benarroch(139).

Current diagnostic classification of IBS is based upon symptoms in the absence of a demonstrable structural or biochemical abnormality (140). This has inevitably created an inherently heterogeneous group of patients. The management of pain in IBS is particularly problematic (141). Coupled with variation in clinical response and a high placebo response has represented significant challenges in the development of efficacious interventions (142). A potential approach is personalised management based on individual features as advocated by the introduction of the multidimensional clinical

profile for the management of functional GI disorders (143). Although recent studies have provided important insights into the pathophysiology of IBS, many of the methods used are often labour intensive, invasive, and expensive. In contrast, performing a conditioned pain modulation paradigms in IBS patients is straightforward, reproducible and inexpensive and requires only a minimal amount of specialist equipment (144). Recent evidence suggests that assessment of conditioned pain modulation may allow for the individualisation of pain treatments in other conditions of chronic pain; for example, Wilder-Smith et al. and Landau et al., showed that baseline conditioned pain modulation might predict postsurgical neuropathic pain (145-149).

S. Sugimine et al. showed in a placebo-controlled study that the effect of pregabalin on conditioned pain modulation was strongly correlated with initial conditioned pain modulation ($r = 0.73$, $p < 0.0001$), the lower the initial conditioned pain modulation was, the more positive effect pregabalin had on conditioned pain modulation, oppositely, participant with initially high conditioned pain modulation may have a reduction in conditioned pain modulation after receiving pregabalin(150). Conditioned pain modulation has also been shown to predict analgesic response to centrally acting medications targeting noradrenergic pathways in diabetic neuropathy(151). Interestingly, Niesters et all showed that Tapantadol (acts via opioid and noradrenergic pathways) could improve CPM in patients with diabetic neuropathy after 4 weeks of treatment(152).

Neuromodulatory analgesic agents, such as tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI), have an established role in the management of IBS however, the number needed to treat remains relatively large (153-156). The exact

mode of action remains unclear but may be due to the interference with specialised brain networks involving emotional and cognitive processing of pain or by engaging descending pathways to alter pain transmission at the level of the dorsal horn of the spinal cord; both mechanisms can, in turn, alter conditioned pain modulation (157). Outcome studies investigating the relationship between conditioned pain modulation in IBS and response to neurotransmitters might offer an objective method to predict the efficacy of those medications and result in a more acceptable number needed to treat.

There is a trend that patients diagnosed with Rome III criteria may have a more diminished conditioned pain modulation that those diagnosed with Rome II. Although the difference was not statistically significant, this may be due to a type 2 error, where only three studies used Rome III while nine studies used Rome II. According to the Rome II criteria, abdominal pain or discomfort must be present for at least 12 weeks in the last 12 months. In contrast, Rome III states that these symptoms must be present in the last three months with the start of symptoms of at least six months before the diagnosis. Notably, studies that have compared these criteria have shown that patients diagnosed using Rome III have significantly more severe abdominal pain and alteration in bowel habit than those diagnosed using Rome II (158).

In contrast to the Rome III criteria, the Rome IV criteria have removed the term "discomfort" from the definition (159). While the prevailing reasons for this change were largely semantic, i.e. several languages do not have a word for discomfort, there are likely large variations in patients' interpretation of this term (160). Coupled with changes in the temporal definition of abdominal pain, rising to weekly in Rome IV from 3 times monthly in Rome III, it is likely that this will lead to a reduction in the population

prevalence of IBS as the new diagnostic criteria will represent a more severe subgroup (161, 162). It is plausible to propose that a more severe IBS phenotype, characterised by greater pain, may have heightened deficiencies in conditioned pain modulation and thus may become a more salient pathophysiological feature of the disorder. This possibility requires confirmation in future studies.

Limitations

This study is not without limitations. Firstly, this meta-analysis was confined to study conditioned pain modulation in IBS and thus extrapolation to other functional gastrointestinal disorders, where visceral pain is a defining feature, is uncertain although similar deficiencies in conditioned pain modulation have been reported in patients with functional dyspepsia and functional abdominal pain (52, 54). Secondly, within the literature to date, there is a female bias in the recruited subjects (88%), and therefore generalizability to male patients is less certain. A previous meta-analysis reported that deficiencies in conditioned pain modulation in males is less than in females and may provide insights into the female preponderance in some types of IBS (163). The neurobiological basis of gender differences both in IBS and conditioned pain modulation is incompletely understood. It has been proposed that differences in pain sensitivity throughout the menstrual cycle may explain some of this variability (114). However, nine out of eleven studies included controlled for the stage of the menstrual cycle, thereby lessening the potential effect on our overall results. Thirdly, the identified studies were undertaken in tertiary care centres, so there is likely some inherent selection bias towards a more severe disease phenotype which potentially limits the external validity.

Fourthly, although, impaired conditioned pain modulation indicates a top-down dysregulation of pain control, it may not be able to differentiate between spinal, bulbar, cortical or emotional drivers of this dysregulation. Finally, we also demonstrated considerable heterogeneity between studies, particularly concerning differences in testing paradigms and outcome measures. However, Lewis *et al.* did not find that difference in the conditioning stimulus type, or test stimulus type significantly affects the study effect size (51). Nevertheless, our results show a clear association between IBS and impaired conditioned pain modulation, although no comment can be made on the direction of causality, which requires future longitudinal study.

Concluding remarks

In conclusion, this meta-analysis supports the hypothesis that conditioned pain modulation is diminished in IBS. This deficit is likely due to a combination of a dysregulated descending pain modulation and central sensitisation. In future, conditioned pain modulation paradigms could be used to improve homogeneity in clinical trials, although the international consensus is needed on the specific parameters of such paradigms such as the use of rectal distention vs somatic pain, the type of conditioning stimulus and controlling for possible confounding factors (164). An interesting potential of this technique may be the personalisation of neuromodulatory therapy in patients with IBS, which may improve outcomes and warrants further studies.

Chapter 4

The Effect of Experimentally Induced Oesophageal Hypersensitivity on Conditioned Pain Modulation

Introduction

Pain is a common experience that drives behaviour and plays an important role in survival. Pain experience may differ from individual to individual; however, depending on the circumstances, such experience may also differ within the same individual. The difference is sometimes quite remarkable; for example, it has been reported that soldiers may report little pain to gun-shot wounds during the battle(38). Such variability in pain experience is possible due to a complex pain control system that intervenes at multiple levels; from pain conduction at the periphery, transmission to the central nervous system, to the processing of such stimuli at cortical levels.

One of the mechanisms of interest in this study is the descending inhibition of pain that may account for some on the variation in pain experience and may be used as a gateway to therapeutic pain regulation in the future. Descending inhibition refers to the ability of central nervous system structures (such as brainstem, limbic system, cortical regions) to inhibit pain at the level of the spinal cord.

One way of assessing descending inhibition is by measuring the Conditioned Pain Modulation (CPM). CPM refers to the endogenous pain inhibition of a specific stimulus when a second pain stimulus is applied simultaneously but in another region of the body. It is evaluated by assessing participant's pain threshold to a specific stimulus (test stimulus), then reassessing it after applying a second painful stimulus, also called conditioning stimulus. Following the principle that ''pain inhibits pain''. CPM was formally known as Diffuse Noxious Inhibitory Control (DNIC) described by Le Bars, Dickenson and colleagues (47). DNIC is a specific term that refers to a brainstem mediated mechanism; thus, Conditioned Pain Modulation was adopted as an alternative term to incorporate the psychophysiological factors important in shaping this type of pain control in humans(48).

In healthy humans, there is a significant increase in pain threshold to the test stimulus after applying a second conditioning stimulus(49). Conditioned pain inhibition is thought to be mediated via Diffuse Noxious Inhibitory Control system. In rats, DNIC is thought to be mediated via neurones in the subnucleus reticularis dorsalis (SRD)(50). However, human studies suggest the involvement of other nuclei such as the Periaqueductal grey and structures that allow for interactions with higher structures such as the autonomic nervous system, emotional centres, past experience and other factors (51, 165, 166).

CPM is reduced (i.e. reduction in pain threshold to the test stimulus after application of the conditioning stimulus is less pronounced) in a variety of chronic pain conditions such as Functional Abdominal Pain, Irritable Bowel Syndrome, Functional Dyspepsia, etc. (52-

54). This reduction in CPM has a large effect size of in various types of painful conditions as suggested by a meta-analysis (51).

Descending pain modulation dysregulation may play a key role in visceral pain hypersensitivity where there is an exaggerated response to a potentially painful stimulus or sometimes without an obvious stimulus. Pain hypersensitivity is thought to take place at one or more levels, such as:

- Peripheral: enhanced transduction of painful signals as seen during local injury or inflammation (peripheral sensitisation)
- Central: at the level of the dorsal horn of the spinal cord where pain signals are integrated and amplified before being projected to higher structures (central sensitisation)
- Cortical level: during the perception (interpretation) phase

Current literature of CPM in visceral pain cannot fully explain the relationship between visceral pain hypersensitivity and CPM for several reasons:

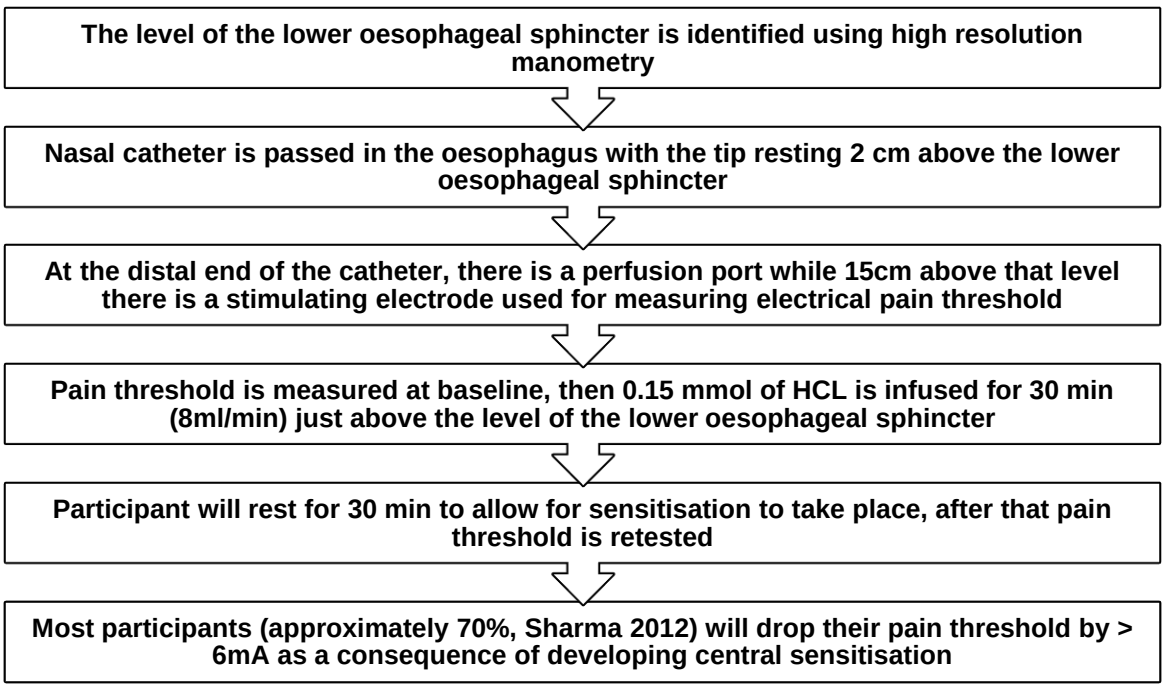
- In chronic visceral pain conditions, is not possible to assess the baseline CPM before the condition started; thus, it is difficult to say whether the reduction in CPM preceded visceral pain or it is a consequence of it
- Unlike acute painful conditions, in chronic visceral pain conditions such as functional gastrointestinal disorders, the painful condition does not completely resolve with time, thus, it is not possible to assess CPM in those patients after the painful condition has resolved.

- Unlike other types of pain, where the insult stimulus is well-known and potentially treatable, the insult stimulus in visceral pain is often vague and non-targetable

For all the above reasons, a better human experimental model is needed to investigate the link between CPM and visceral hypersensitivity.

Our group has pioneered a human model to investigate acid-induced experimental oesophageal hypersensitivity. The model allows the study of central sensitisation in healthy humans. Below is a basic description of the model:

Human Model of acid-induced oesophageal hypersensitivity



This model has been validated against saline infusion and has been repeatedly used and validated by our group and others (68, 167-169). The stimulation electrode is placed 15 cm proximal to the acid infusion port to avoid testing the pain threshold at the inflamed section of the oesophagus, thus testing central sensitisation rather than peripheral sensitisation. Previous studies confirmed the absence of pH changes at the level of stimulation electrode (168). This model also allows for the collection of autonomic variables throughout the experiment using ECG signals. Our group has shown a significant association between the development of visceral hypersensitivity and the reduction in parasympathetic tone (168) such that those who reduce their parasympathetic tone the most during acid infusion also sensitise the most; thus, the proposed study provides an exploratory data regarding a possible association between CPM and parasympathetic tone.

In this model, approximately 70% of participants sensitise (drop their pain threshold by > 6mA) to the established dose and period of acid-infusion (168).

CPM response may differ depending on gender as it is less pronounced in females (51). It has also been shown that CPM depends on the phase of the menstrual cycle (170). We accounted for this by studying females only, in their follicular phase of the menstrual cycle. The choice to study females is also because of the predominance of this gender in functional digestive disorders(171).

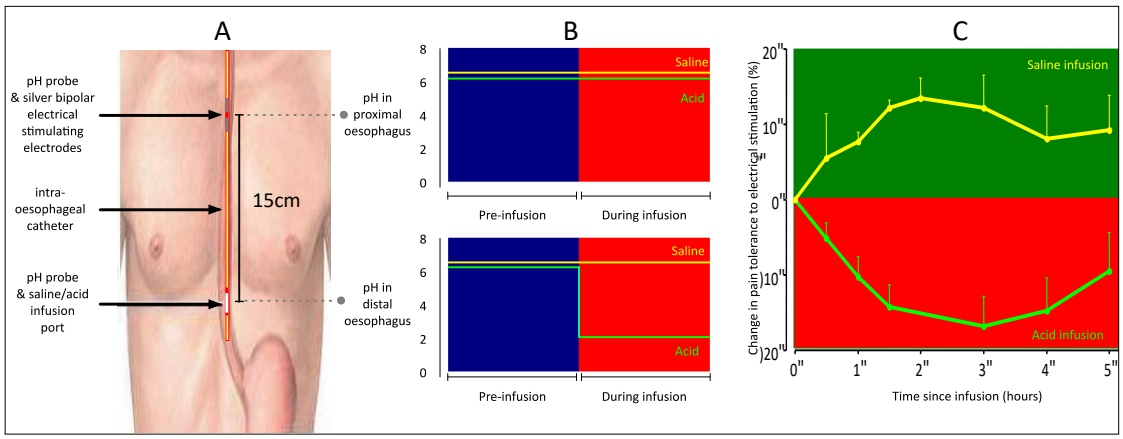


Figure 15: A schematic representation of the oesophageal pain hypersensitivity model. From left to right: -A- a catheter is placed in the oesophagus which has a proximal pH probe and silver bipolar electrical stimulation electrodes to measure oesophageal pain sensitivity and a distal pH probe & infusion port. B – Subjects are randomised to receive either saline or acid infusion. As expected, when saline was infused, pH remained stable in the proximal and distal oesophagus, whereas there is a demonstrable drop in pH in the distal but not the proximal oesophagus during acid. C- Following saline infusion, pain thresholds in the proximal oesophagus -which has not been exposed to acid- show decreased pain sensitivity, i.e. increase in pain thresholds over time (green-shaded area) due to habituation, but following acid infusion, there is increased pain sensitivity, i.e. decrease in pain thresholds over time (red-shaded area) due to central sensitisation. Adapted from Sarker, Aziz et al. Lancet 2000.

Hypothesis

- Baseline CPM can predict the development of experimentally induced oesophageal pain hypersensitivity

Rationale:

If CPM is associated with visceral hypersensitivity, then:

- It can be used as a surrogate maker when evaluating potential treatments for visceral hypersensitivity
- In future studies, therapeutic interventions may target CPM pathways to treat visceral hypersensitivity

Main aims:

- To investigate the relationship between CPM and experimental oesophageal pain hypersensitivity
- To investigate if baseline CPM can predict the development of experimental oesophageal hypersensitivity

Primary outcome measure

CPM before and after acid infusion. CPM is calculated as the percentage change in 'test stimulus' pain threshold (PT) before and during the conditioning stimulus.

Secondary outcome measure

Autonomic measures, including Cardiac Vagal Tone measured for 5 minutes, at baseline and between 25 min-30 min after acid-infusion

Methods

Inclusion criteria

- Women aged 18-65 years
- No chronic pain conditions, including Irritable Bowel Syndrome, fibromyalgia, migraine, and other pain conditions
- Not on any regular medications
- No history of Gastroesophageal Reflux Disease (heartburn less than twice per week)
- No heart conditions such as arrhythmias

Exclusion criteria

- Not meeting all inclusion criteria
- Not able to give informed consent (poor command of English language)
- Pregnancy
- Cold-induced problems such as:
  - Raynauds disease
  - Injuries or skin conditions on the foot
  - Cold-induced skin disorders such as Cold-induced urticaria and similar conditions
  - Known intolerances to cold temperature for other reasons not mentioned here such as arthritis or sickle cell anaemia

We controlled for:

- Gender: we recruited women only
- Menstrual cycle phase: all participants were studied during the follicular phase
- Alcohol consumption, caffeine; restricted 24 hours before the study
- We collected information for co-variate analysis such as age, gender, BMI, other medical conditions, rigorous exercise, sleep deprivation and psychological factors and anxiety using validated questionnaires
- We standardised the investigator-participant interaction by keeping verbal interaction to a minimum after explaining all the steps before commencing the study

Study design

This is a single visit correlational study. Please see below a study flow chart.

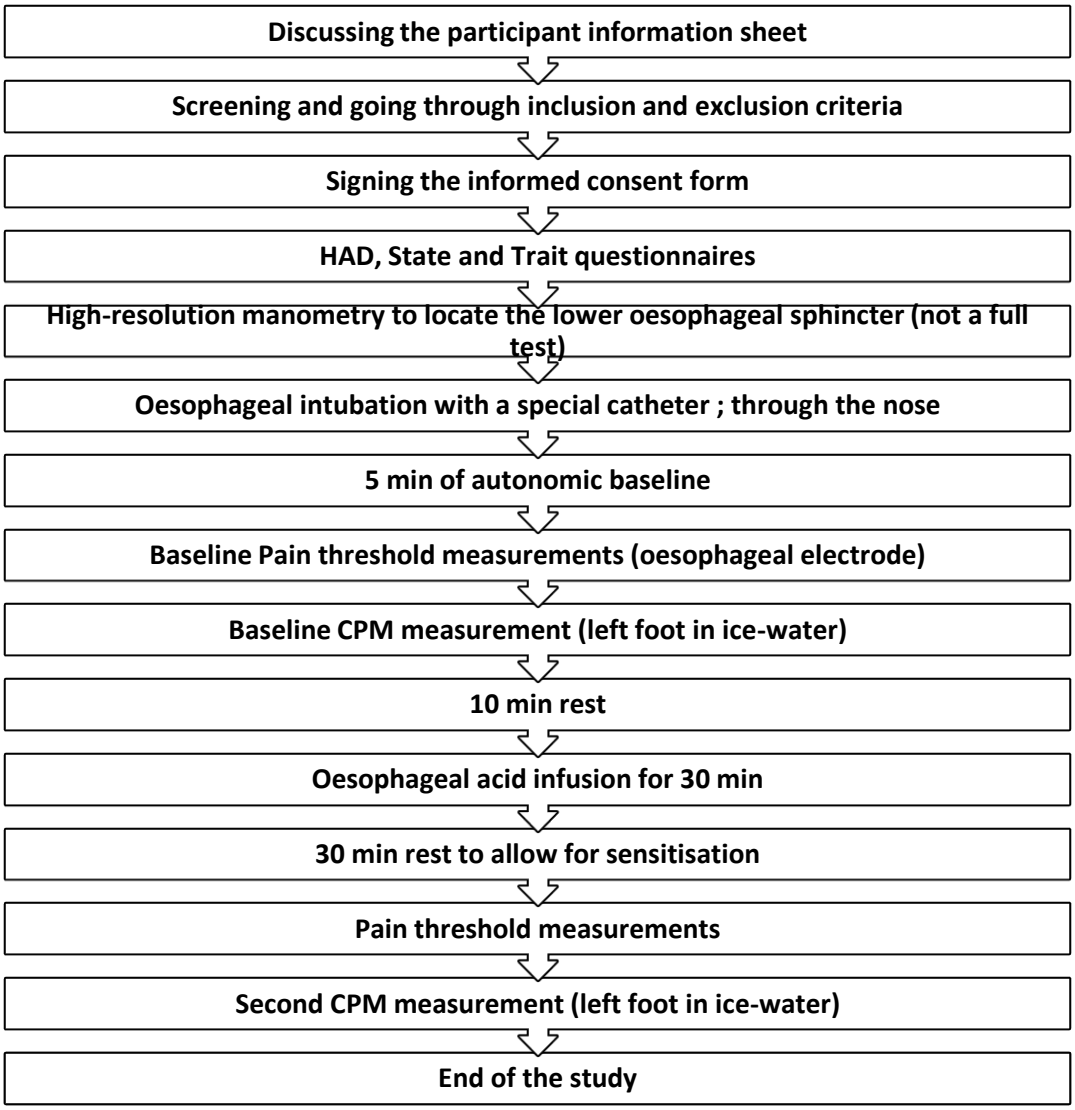


Figure 16: Study flowchart

Interventions

High-resolution oesophageal manometry

To define the anatomical landmarks of the oesophagus (i.e. position of the lower oesophageal sphincter, if the recruited subject has not been studied before using this model), then high-resolution oesophageal manometry (HRM) was performed using

ManoScan 360™ High-Resolution Manometry system from Sierra Scientific . A specialised HRM catheter sheathed in a single-use sleeve was inserted through the nostril into the oesophagus until the distal end of the catheter is resting in the proximal stomach. The catheter is then taped to the nose. Lower oesophageal sphincter (LOS) position, in terms of cm from the nostril, was recorded and used to guide insertion of naso-oesophageal catheters in the next phases of the study. Manometry is a routine diagnostic test.

Autonomic nervous system measurements

At baseline, and continuously after that, measurement of the parasympathetic tone using cardiac vagal tone was made using the non-invasive Neuroscope system (172).

Acid infusion and pain tolerance measurements

Oesophageal intubation

A specialised catheter was inserted through the nose into the oesophagus (3 mm diameter catheter, Unisensor AG, Ch-8544 Attikon, Switzerland). The catheter has a distal infusion port (5cm from the tip) and a proximal stimulation electrode to test pain thresholds (16 cm proximal to the tip). The tip of the catheter will be placed 2 cm above the lower oesophageal sphincter (located by HRM).

Pain tolerance threshold testing

Pain tolerance threshold (PTT) in this protocol is defined as the level of pain when participants report that they cannot tolerate any further increase (Visual analogue scale or VAS of 7 out of 10).

Electrodes were connected to an electrical stimulator and stimuli are delivered at a frequency of 0.5 Hz, using square wave pulses (0.5 s duration), at intensities varying between 0 and 90 mA. The intensity of stimulation was increased incrementally by 2 mA until reaching the pain threshold. The electrical stimulation was immediately stopped when PTT was reached.

Pain tolerance threshold was measured at baseline (T0) and, at 60 minutes (T60) after starting the distal oesophageal acid infusion. The same technique was used to measure pain threshold to assess CPM.

Oesophageal acid infusion

Using the distal port on the oesophageal catheter, we infused 0.15 molar hydrochloric acid (T10-40) (medical grad product, used in practice for IV infusion) using an intravenous pump with a rate of 8 ml per minute for 30 minutes.

CPM testing

CPM was calculated as the percentage change in PTT before and during the conditioning stimulus. Example: If baseline PTT=100mA, PTT during the cold condition stimulus is 120mA, then CPM= +20%.

After obtaining oesophageal electric PTT, we applied conditioning stimulus by placing the left foot in cold water that kept at 2-4 degree Celsius (temperature is kept within the interval by adding ice cubes). Then we tested oesophageal PTT as detailed in the above section while the conditioning stimulus is applied throughout the pain tolerance testing. Cold-stimulus is known to be effective in eliciting a CPM response(51). Traditionally, the conditioning stimulus is applied to a heterotopic (on the other side) place from the test stimulus; thus, the position on the feet was chosen to avoid applying both stimuli to the same spinal afferent's distribution.

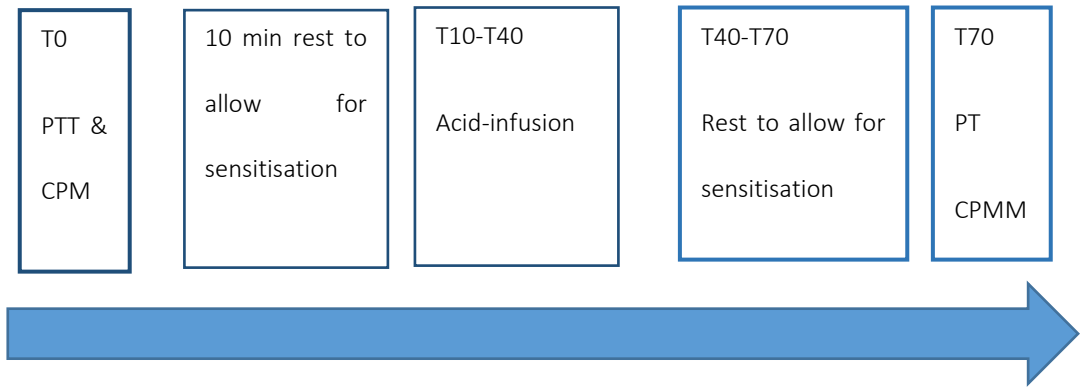


Figure 17: Timing of selected study interventions

Statistical considerations

Statistical analysis

We used partial correlation method using SPSS 25 IBM. We statistically corrected for age, height and weight.

Sample size calculation

Using a regression model with an estimated effect size of CPM in chronic pain conditions to be 0.78 (adopted from a meta-analysis (51), 16 participants must complete the study to have 80% power and 0.05 alfa level.

Ethical approvals

This study was approved by Queen Mary, University of London Ethics committee. Approval reference: QMERC2017/72.

Results

Relationship between baseline CPM and sensitisation

There was a strong and significant correlation between baseline CPM and the degree of sensitisation, r=0.695, *p*=0.008. Delta PTT below refers to the post-acid infusion pain threshold as a percentage from baseline pain threshold. For example, participants who reduce their PTT by 20 percent, will have a Delta PT of 80%, while participants who

reduce their PTT by 10 percent have a Delta PT of 90%. Thus, more sensitisation takes place, the lower Delta-PT will be.

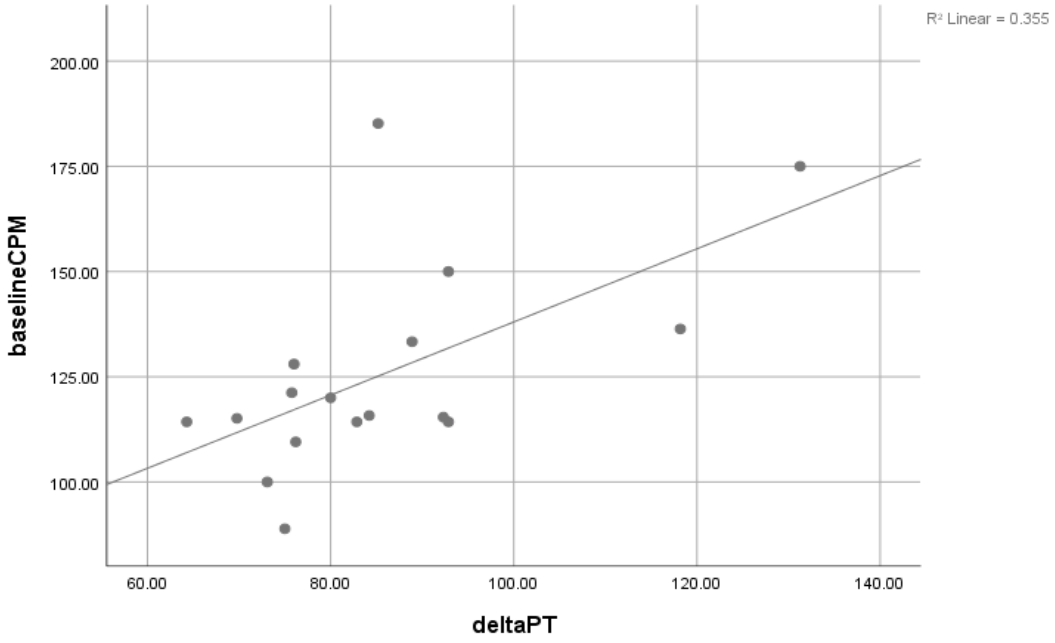


Figure 18: Partial correlation between baselines conditioned pain modulation (CPM) and delta pain threshold.

Table 8 Partial correlation between baselines conditioned pain modulation (CPM) and delta pain threshold adjusted for age, height and weight

Control Variables			Baseline CPM
Age & Height (cm) & Weight (kg)	Delta -PT	Correlation	.695
		Significance (2-tailed)	.008
		df	11

Relationship between change in CPM and sensitisation

There was an inverse correlation between the direction of CPM and sensitisation, r= -0.558, *p*= 0.047. The higher degree of sensitisation correlated with a larger increase in CPM from baseline. This may suggest that participants who sensitised the most attempted to increase their CPM the most. However, these participants had a low CPM at baseline.

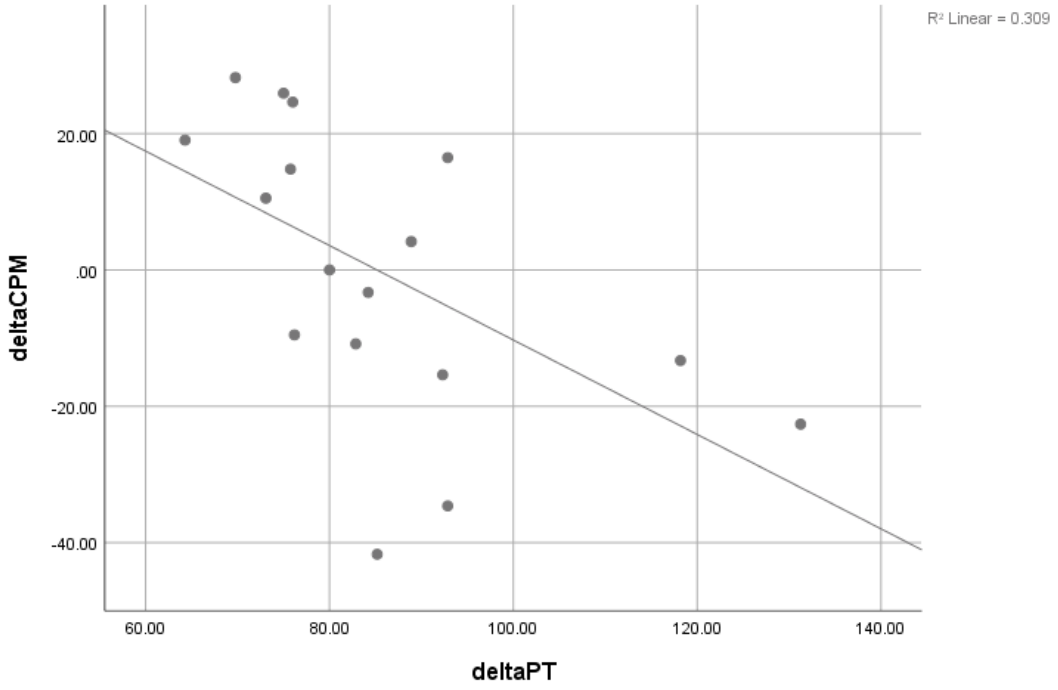


Figure 19: Correlation between change in CPM and change in pain threshold after oesophageal acid infusion.

Table 9: Correlation between change in CPM and change in pain threshold after oesophageal acid infusion adjusted for age, height and weight. df: degree of freedom.

Control Variables			Delta PT
Age & Height (cm) & Weight (kg)	Delta CPM	Correlation	-.558
		Significance (2-tailed)	.047
		df	11

Relationship between baseline CPM and baseline cardiac vagal tone

We could not detect a significant correlation between baseline cardiac vagal tone and baseline conditioned pain modulation, r= -0.214, *p*=0.483.

Table 10: Partial correlation between baseline cardiac vagal tone and baseline conditioned pain modulation. df: degree of freedom.

Control Variables			Baseline CPM
Age & Height (cm) & Weight (kg)	CVT baseline	Correlation	-.214
		Significance (2-tailed)	.483
		df	11

Other data

For a summary of other collected data, please refer to the tables below. No statistics were performed to avoid multiple testing.

Table 11: Absolute values of autonomic variables during the interventions

		CVT baseline	CVT during acid-infusion	CVT rest post-acid	HR baseline	HR acid infusion	HR rest
Total	Mean	11.1818	12.3465	13.6312	71.818	69.065	67.506
	Std. Deviation	5.52676	4.60099	5.90688	11.8310	8.8992	8.7209
	Skewness	.867	.946	.835	.931	.456	.487

Table 12: Demographic data of participants and CPM

	Age	Height (cm)	Weight (kg)	Baseline CPM
Mean	26.25	167.13	61.288	125.6856
Std. Deviation	6.137	4.897	7.7476	24.71599
Skewness	1.293	-.257	.778	1.256

Psychological data

All participant had a normal HAD score of less than 7.

Discussion

Functional gastrointestinal disorders are grouped and diagnosed according to clinical criteria rather than physiological and pathological criteria. This is clinically useful, mainly for prognostic reasons and to exclude sinister diseases. However, the clinical classification will inevitably create an umbrella term that groups several distinct pathological mechanisms under the same disease heading. This is particularly problematical in research. For example, if a specific intervention is effective against a subtype of a functional disorder with anxiety as the main feature, then it may be less effective in patients with similar symptoms that are driven by enteric nervous system dysfunction. Detecting an intervention with a moderate effect size in heterogeneous groups is challenging and will need a very large number of participants. For this, we are likely to miss reasonably effective intervention when tested based on symptoms only.

One of the theoretical mechanisms in functional gastrointestinal disorders with pain as the main feature is a dysregulated top-down control (see the previous chapter). This study clearly shows that the status of baseline top-down control measured by Conditioned Pain modulation is a strong predictor of pain sensitisation. Those with relatively effective conditioned pain modulation are less likely to sensitise. On the other hand, those who sensitise the most, increase their CPM in percentage terms the most

from baseline. Although the percentage change is more when compared to those with less sensitisation, they have a lower baseline to start with.

The correlation between baseline CPM and sensitisation is strong and significant, suggesting a relevant underlying mechanism. CPM is a surrogate marker of top-down control. However, it cannot pinpoint the level of dysregulation. CPM is influenced by several structures, spinal, bulbar, autonomic and cortical structures. Given the broad range of control, it is very challenging to pinpoint the dysfunctional part of the chain. However, interventions along the chain are likely to be effective, especially at the top level of the chain, such as cortical structures involved in the processing of pain.

CPM may be a rudimentary way of assessing top-down pain control. However, it may predict participant likely to develop pain hypersensitivity when exposed to a sensitising stimulus. Our study clearly shows this in experimental settings. Wilder-Smith et al. and Landau et al. showed that baseline conditioned pain modulation might predict postsurgical neuropathic pain (149, 173). Those conclusions are in line with our findings.

The foreseeable benefits of incorporating CPM in research and perhaps clinical practice can be summarised as below.

First, CPM can be used to monitor treatment progress in patients with chronic painful conditions, especially if a pharmaceutical agent is used that is known to target descending inhibition.

Second, it can also be used in research to reduce heterogeneity and thus to reduce the sample size needed to test an intervention, likely to enhance top-down control of pain.

Limitations

This was a study undertaken in a stringent laboratory setting that is rarely met in the real clinical world. This study indicates that CMP is a reasonable predictor of developing pain hypersensitivity in healthy volunteers; however, this conclusion will need further testing in clinical settings. Another limitation is that we recruited female participants only, thus extrapolation to male populations may require further testing.

In conclusion, conditioned pain modulation at baseline is a strong predictor of developing experimental pain hypersensitivity in healthy volunteers. Conditioned pain modulation may be a useful tool to predict the efficacy of therapeutic interventions targeting descending inhibitory pathways. Conditioned pain modulation may also be a useful tool in clinical trials of analgesic drugs by reducing heterogeneity of study population.

Chapter 5:

The Effect of Transcutaneous Vagal Nerve Stimulation on Pain- Systematic Review and Meta-Analysis

Introduction

We have previously published an extensive review regarding the clinical uses of Vagal nerve stimulation (174). Vagal nerve stimulation has been used in various pain conditions with some degree of success in experimental studies. My main interest is using vagal nerve stimulation for the central modulation of pain, more specifically, oesophageal pain hypersensitivity. However, oesophageal pain hypersensitivity is a novel application with no publications regarding the use of vagal nerve stimulation in this condition. For this reason, we had to widen our literature search to all types of pain. To understand the degree of evidence of this intervention, we conducted a systematic review of the literature and a meta-analysis of the effect of non-invasive vagal nerve stimulation on pain in general.

Methods

Inclusion criteria

- Prospective randomised controlled studies investigation non-invasive Vagus nerve stimulation in humans in pain conditions
- Clearly measured pain outcomes

Search strategy

The study was conducted according to PRISMA guidelines and registered on PROSPERO web site. Two researchers independently searched Pub Med and Web of science. Those two researchers were students who worked under my supervision to conduct this project. They had training in principles of evidence-based medicine, and literature search. Keywords were agreed to be: vagus nerve stimulation, Or Vagus AND pain. The search was restricted to human studies only. We included all articles published prior to 31.04 2018. There was no restriction on language. The abstracts were screened, full papers were screened if met inclusion criteria.

Data extraction

Using a designed Excel Spreadsheet (Excel 2016, Microsoft, Redmond, USA), Data were extracted independently by two investigators. This data included study identifiers, groups and intervention characteristics, primary and secondary outcomes.

Study methodology quality assessment

A checklist was designed to assess the risk of bias in 6 categories. The risk for each category was set as low (0 points), moderate (1 point) or high (2 points). The categories were:1) blinding of assessors (high risk if not blinded or not clearly stated) 2) use of internationally accepted criteria for underlying clinical condition (high risk if not stated, moderated risk if not internationally validated) 3) matching of treatment and control groups concerning age (low risk if <10%, moderate if 10-20% and high >20%) 4) matching of treatment and control group concerning gender (low risk if <10%, moderate if 10-20% and high >20%) 5) the exclusion of depressive disorders as a known confounder to pain (high risk if not mentioned, moderated risk if mentioned and measured and low risk if excluded or adjusted for) 6) control for other known confounders such as menstrual cycle phase, the time of day of assessment, caffeine or alcohol intake, concomitant medication use (pain-killers), stress or anxiety (the high risk was considered in case of control for one or fewer confounders, moderate risk was assumed when at least two confounders were controlled, and the low risk was attributed to control for three or more confounders.

Statistical analysis

Due to the expected heterogeneity between the included studies, we used a random-effects model using Der Simonian-Lard weights. We used the Hedges g with 95%

confidence intervals to measure effect sizes (175, 176). An effect size of 0.2 is considered small, while 0.5 is medium and 0.8 is large. The effect size is a measure used to estimate the difference between the treatment and the control groups regarding the pain-related effect of vagal nerve stimulation

The Higgins I^2 test is used to determine heterogeneity. It ranges between 0% to 100% where 25%, 50%, and 75% indicate low, medium and high statistical heterogeneity respectively (177). Publication bias was assessed by visual analysis of the funnel plot. Comprehensive Meta-analysis, Biostat, New Jersy, USA, Version 2 software was used to perform the meta-analysis and produce the Forest plot.

Results

Search results:

Search results are summarised in Figure 20.

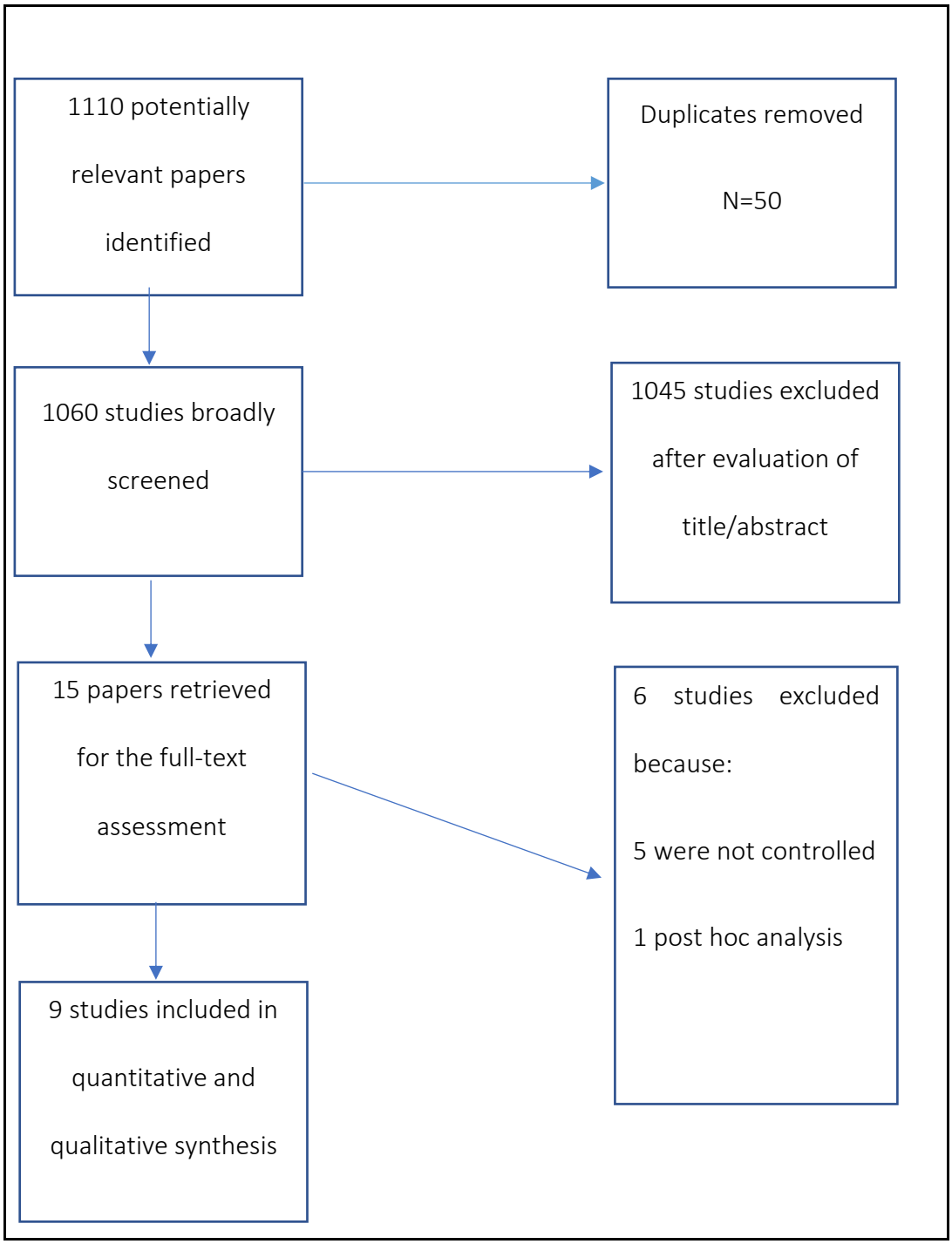


Figure 20: Flowchart of search results.

Characteristics of the included studies

Characteristics of the included studies are summarised in the table below.

Characteristics of the included studies. PT, pain threshold; PI, pain intensity; PFSD, pain-frequency-duration score; Ech, episodic cluster headache; Cch, chronic cluster headache. PENFS: Percutaneous electric nerve field stimulation. PW : pulse width

Study	Kovacik, 2017(8)	**Laqua, 2014(5)**
Study design	Randomized controlled double-blind trial	Randomized controlled crossover
Disease in question	Abdominal pain-related functional GI disorders	Healthy
Stimulation parameters	-PENFS: dorsal and ventral aspects of the ear within 1-1,5mm of vascular branches -3-2 volts with a rectangular pulse wave - frequencies (1 ms pulses of 1 and 10 Hz) every 2 s. -cycles of 2 h on and 2 h off for 120 (5 days). -5 days per week with 2 days off during each of the 4 weeks	- auricular concha bilaterally -for 30 min - 2 Hz/100 Hz bursts - 0,2MS PW
Painful stimulus used	No	microprocessor-controlled device Neurometer
Main outcomes	-abdominal pain score -worst pain ratings -reduction in worst pain of 30% and more from baseline to 3 week of treatment(n)	Electrical pain thresholds
Main results	active>sham	Active=placebo

Study	Busch, 2013(7)	Frokjaer ,2016(3)
Study design	Randomized controlled double-blind crossover trial	Randomized controlled single-blind crossover trial
Disease in question	healthy	healthy
Stimulation parameters	-25Hz -0.25ms pw, -1.6 mA +/-1.5 mA - left concha at the inner side of the tragus -during the period of one hour	- 30 Hz - 250 ms pw - 1,46mA+/-0,73mA (final) - During the period of 1 hour - left concha
Painful stimulus used	- a series of contact heat pulses -pinprick stimulators, -pressure gauge device, -TSA 2001-II thermal sensory testing device	handheld electronic pressure algometer
Main outcomes	1) tonic heat pain intensity 2)mechanical pain thresholds 3) pressure pain thresholds 4) mechanical pain intensity 5) thermal pain thresholds 6)WUR	1) Bone pain threshold 2) Muscle pain threshold
Main results	Active>sham Active>sham Active>sham Active>sham Active=sham Active=sham	Active>sham Active=sham

Study	Silberstein,2016 ACT1(4)	Silberstein, 2016 EVENT(4)
Study design	Randomised controlled crossover	Randomised controlled double-blind
Disease in question	Attacks of cluster headache: chronic and episodic	Chronic migraine headache
Stimulation parameters	-Up to 1 month, ussig GammaCore device -to the right side of the neck - only one attack could be treated in 12 hours, maximum of 5 attcks	GammaCore device -2 minutes of self-administered stimulation delivered 5-10 minutes apart at three prespecified times every day
Painful stimulus used	No	No
Main outcomes	1)- response rate at 15 minutes -responder for more than 50% of treated attacks -pain-free for more than 50% of treated attacks 2) -sustained response rate at 15-60 minutes -change in attack duration 3) -Pain intensity -rescue medication use in the first hour after the first attack -duration of the first attack	Number of headache attacks per 28 days
Main results	1)Active>sham in a cohort of episodic CH 2)Active>sham in all subjects and cohort of episodic CH 3) Active=sham	Active=sham

Study	**Goadsby, 2018(2)**
Study design	Randomized controlled double-blind trial
Disease in question	Episodic and chronic cluster headache,
Stimulation parameters	2 weeks duration with a min of 6 hours between treatment sessions , using GammaCore device (neck-cervical vagus)at the time of attack onset
Painful stimulus used	No
Main outcomes	1- the proportion of all treated attacks that achieved pain-free status within 15 minutes 2- mean proportion of treated attacks per subject that achieved responder status within 30 minutes 3-mean proportion of treated attacks per subject that achieved pain-free status within 30 minutes 4- mean change in pain intensity from attack onset to 15 and 30 minutes
Main results	1- active>sham in eCH group;active=sham in total and in cCH group 2- active>sham in total cohort; active=sham in eCH and cCH groups 3- active=sham 4- active>sham in eCH group; active=sham in total and in cCH group

Bias assessment

Methodological quality assessment is summarised in table 13. Visual analysis of the funnel plot did not suggest publication bias, see figure 21.

Table 13: Bias assessment scores.

Study	Blinding of outcomes assessors	Patients selection	Patients/controls matching for age	Treatment/control matching gender	Exclusion of depressive disorders	Control for confounders
Busch,2013	0	0	0	0	0	1
Frokjaer,2016	0	0	0	0	2	0
Goadsby,2018	0	0	0	0	2	2
Kovacik,2017	0	0	0	0	2	1
Laqua,2014	2	0	0	0	2	2
Napadaw,2012	2	0	0	0	2	1
Silberstein,2016 ACT1	0	0	0	0	2	2
Silberstein,2016 EVENT	0	0	0	0	2	2
Usichenko,2017	2	0	0	0	2	2

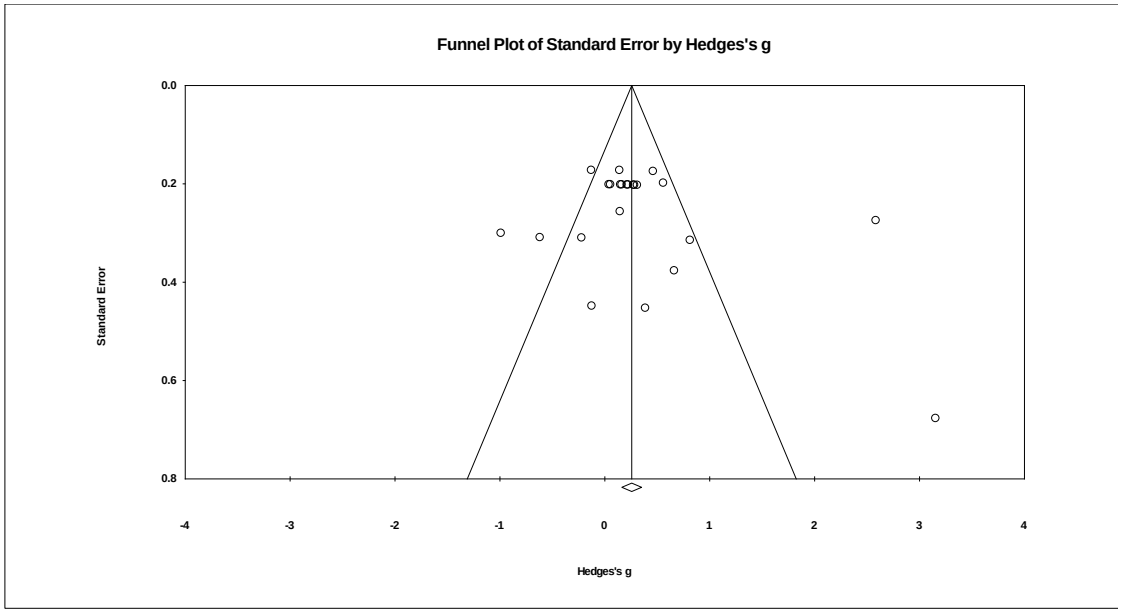


Figure 21: Funnel plot of the included studies, the visual inspection does not indicate a significant publication bias

Heterogeneity of included studies

There was a large heterogeneity in the included studies; Q-value 134.08, I squared=83.59, *p*-value < 0,001

The overall effect of vagal nerve stimulation on pain

Non- invasive vagal nerve stimulation had a small but significant effect on pain; Hedges g = 0.217, *p*=0.005 see figure 22.

Meta Analysis

Study name	Subgroup within study	Time point	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Busch V et al, 2013	Combined	Single time point	0.331	0.066	0.004	0.202	0.460	5.034	0.000
Frokjaer et al, 2016	Combined	Single time point	0.135	0.319	0.102	-0.491	0.761	0.422	0.673
Goadsby et al, 2018	Combined	Combined	-0.279	0.273	0.074	-0.813	0.256	-1.022	0.307
Kovacic K et al, 2017	PFSD worse pain	Combined	0.656	0.201	0.040	0.262	1.050	3.265	0.001
Laqua R et al, 2014	Combined	Combined	0.091	0.221	0.049	-0.343	0.524	0.409	0.682
Napadow V et al, 2012	Tonic deep-tissue mechanical PI	Combined	0.677	0.378	0.143	-0.064	1.418	1.791	0.073
Silberstein et al; ,2016 ACT one	Mean PI at 15 min Ech+Cch	Single time point	0.146	0.173	0.030	-0.194	0.486	0.843	0.399
Silberstein et al; ,2016 ACT1	Combined	Single time point	0.168	0.123	0.015	-0.074	0.410	1.364	0.173
Silberstein et al; ,2016 EVENT	Mean change in the number of headache days/ week	Single time point	0.151	0.257	0.066	-0.354	0.655	0.585	0.558
Usichenko T et al, 2017	Heat pain thresholds	Single time point	-0.215	0.311	0.097	-0.824	0.395	-0.691	0.490
			0.217	0.076	0.006	0.067	0.367	2.836	0.005

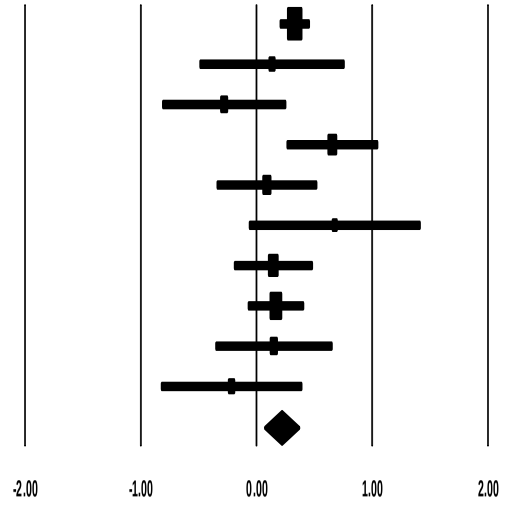
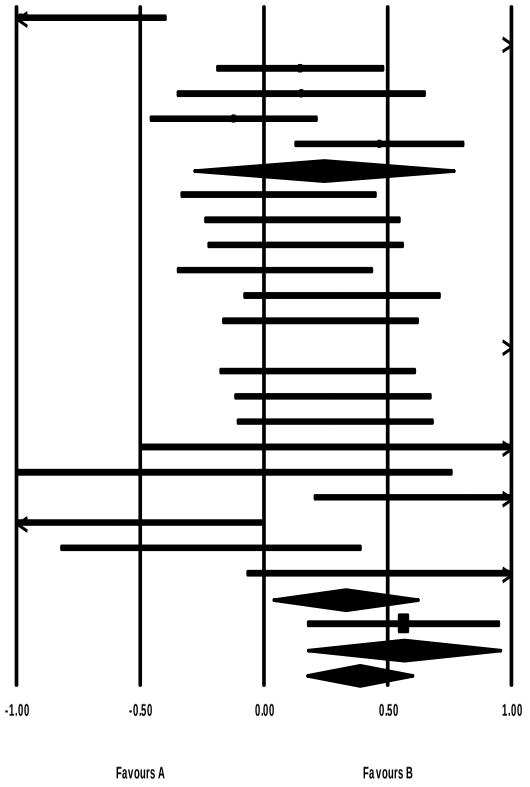


Figure 22: Forest plot of the effect size of transcutaneous vagal nerve stimulation on pain using a random effect model. The overall effect size was 0.217 (95% CI 0.067-0.367, p=0.005). Favour A indicate sham, Favour B indicates vagal nerve stimulation.

Effect modification by type of pain

Vagal nerve stimulation appeared to have a significant effect on somatic and visceral pain but not on headache. The overall Hedges' g of 0,564 (0,173-0,955; *p*= 0,005) was observed in visceral pain whereas the effect size of somatic pain was 0,328 (0,033-0,623; p value=0,029), see the figure below.

Group by Type of pain	Study name	Subgroup within study	Time point	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Headache	Goadsby et al, 2018	Mean change in PI in Cch	15 min	-0.984	0.301	0.091	-1.574	-0.393	-3.263	0.001
Headache	Goadsby et al, 2018	Mean change in PI in Ech	15 min	3.160	0.678	0.460	1.831	4.489	4.660	0.000
Headache	Silberstein et al; 2016 ACT one	Mean PI at 15 min Ech+Cch	Blank	0.146	0.173	0.030	-0.194	0.486	0.843	0.399
Headache	Silberstein et al; 2016 EVENT	Mean change in the number of headache days/week	Blank	0.151	0.257	0.066	-0.354	0.655	0.585	0.558
Headache	Silberstein et al; 2016 ACT1	Cch response rate at 15 minutes	Blank	-0.123	0.173	0.030	-0.463	0.217	-0.708	0.479
Headache	Silberstein et al; 2016 ACT1	Ech response rate at 15 minutes	Blank	0.467	0.176	0.031	0.122	0.811	2.657	0.008
Headache				0.240	0.269	0.072	-0.286	0.767	0.894	0.371
Somatic	Busch V et al, 2013	Cold PT contralateral	Blank	0.059	0.203	0.041	-0.338	0.456	0.292	0.770
Somatic	Busch V et al, 2013	Cold PT ipsilateral	Blank	0.155	0.203	0.041	-0.242	0.553	0.765	0.444
Somatic	Busch V et al, 2013	Heat PT contralateral	Blank	0.168	0.203	0.041	-0.229	0.566	0.830	0.407
Somatic	Busch V et al, 2013	Heat PT ipsilateral	Blank	0.044	0.203	0.041	-0.353	0.441	0.219	0.827
Somatic	Busch V et al, 2013	Mechanical PT contralateral side	Blank	0.315	0.204	0.042	-0.084	0.714	1.546	0.122
Somatic	Busch V et al, 2013	Mechanical PT ipsilateral side	Blank	0.229	0.203	0.041	-0.169	0.627	1.126	0.260
Somatic	Busch V et al, 2013	Pressure PT contralateral	Blank	2.590	0.276	0.076	2.050	3.130	9.399	0.000
Somatic	Busch V et al, 2013	Pressure PT ipsilateral side	Blank	0.217	0.203	0.041	-0.181	0.615	1.069	0.285
Somatic	Busch V et al, 2013	Tonic heat PT contralateral side	Blank	0.278	0.203	0.041	-0.120	0.677	1.369	0.171
Somatic	Busch V et al, 2013	Tonic heat PT ipsilateral side	Blank	0.288	0.204	0.041	-0.111	0.687	1.416	0.157
Somatic	Frokjaer et al, 2016	Bone PT	Blank	0.393	0.454	0.206	-0.496	1.282	0.866	0.387
Somatic	Frokjaer et al, 2016	Muscle PT	Blank	-0.118	0.449	0.202	-0.999	0.763	-0.263	0.793
Somatic	Laqua R et al, 2014	Electric PT antinociceptive response	Blank	0.819	0.316	0.100	0.200	1.438	2.594	0.009
Somatic	Laqua R et al, 2014	Electric PT nociceptive response	Blank	-0.612	0.310	0.096	-1.220	-0.004	-1.974	0.048
Somatic	Usichenko T et al, 2017	Heat pain thresholds	Blank	-0.215	0.311	0.097	-0.824	0.395	-0.691	0.490
Somatic	Napadow V et al, 2012	Tonic deep-tissue mechanical PI	15-post stimulation	0.669	0.378	0.143	-0.072	1.409	1.770	0.077
Somatic				0.328	0.150	0.023	0.033	0.623	2.182	0.029
Visceral	Kovacic K et al, 2017	PFSD worse pain	1 week	0.564	0.199	0.040	0.173	0.955	2.827	0.005
Visceral				0.564	0.199	0.040	0.173	0.955	2.827	0.005
Overall				0.385	0.110	0.012	0.170	0.599	3.509	0.000



Forest plot showing Hedges'g effect size and 95%CI of effect of VNS on pain according to different types of pain. PT, pain threshold; PI, pain intensity; PFSD, pain-frequency-duration score; Ech, episodic cluster headache; Cch, chronic cluster headache; Favours A, sham control; Favours B, active stimulation; CI, confidence interval

Discussion

Our study shows that there is a small but statically significant effect of non-invasive vagal nerve stimulation on pain. It is no surprise that the included studies had significant heterogeneity. Studies differed in conditions being treated, location of vagal nerve stimulation, stimulation parameters, and primary endpoints. Despite that, there is a modest but significant effect size when compared to sham.

Subgroup analysis showed that vagal nerve stimulation reduces visceral pain and somatic pain but not headache. These data are based on a few studies with marked heterogeneity. For example, the data for visceral pain is based on one study only(178). In contrast, there is marked heterogeneity in studies addressing pain in chronic headache. This review included one study addressing chronic migraine and two other studies on cluster headache. Cluster headache can be further subdivided to chronic cluster and episodic cluster headache.

With regards to somatic pain, the overall effect size was statistically significant in favour of vagal nerve stimulation, but Hedges' g of individual studies varied depending on pain modalities. The small number of studies did not allow for further subgroup analysis of mechanical and thermal modalities separately; instead, we could only extrapolate on the overall effect size on somatic pain. Although the "mechanical" pain modalities were found to be more significantly reduced by vagal nerve stimulation, tonic pain models showed promising results with regards to both mechanical and heat paradigms. These findings suggest a role of a central mechanism of action of vagal nerve stimulation as the

tonic pain paradigms test temporal summation, which is central nervous system-specific (179).

The included studies used various device locations. Frokjaer et al., applied the stimulator exclusively on the cymba conchae, whereas in other studies the device was placed on the cavity conchae, tragus or dorsal and ventral aspects of the ear. These areas could be additionally supplied with the great auricular and trigeminal nerves that may reinforce the vagal nerve stimulation via connections to the spinal trigeminal nucleus (3, 180).

Stimulation of the tragus, dorsal and ventral aspects of the ear tended to show positive results, while studies that used cervical stimulation tended to be negative. These observations must be accepted with caution because the effect size may reflect several factors such as different disease populations, duration of stimulation, parameters of stimulation and many other factors.

Laqua et al. and Usichenko et al. studies did not show an overall difference between active and sham intervention with regards to experimentally induced electrical and heat pain, respectively (5, 6). However, the detailed subgroup analysis of participants with vagal nerve stimulation suggested a decreased pain threshold indicating a pro-nociceptive effect. Those results could be potentially explained either a type one error or a paradoxical effect in susceptible individuals.

The exact mechanics are unknown. Kovacic et al., (8), demonstrated a hypoalgesic effect of percutaneous electric nerve field stimulation on patients with functional abdominal pain. The authors postulate that this effect might be driven via stimulation of brainstem nuclei involved in pain pathways, such as the Nucleus of the Solitary Tract (NTS). This stimulation is likely to be anatomically medicated via vagal nerve stimulation (VNS). The other studies also showed a similar antinociceptive effect of VNS on various types of pain. The effect is seemingly independent of the stimulation parameters. To our knowledge, no human study has investigated precise parameters to elicit a specific response but rather used known parameters to avoid habituation and remain safe.

Despite considerable progress in our understanding of the neurobiology of vagal afferents, a mechanistic appreciation of how VNS exerts a seemingly diverse beneficial effect remains lacking. However, we would propose two factors that may explain this. Firstly, the possibility of publication bias. This is less likely for one reason; there are a plethora of possible combinations of stimulation parameters such as frequency, wavelength, wave morphology, current intensity, shape of the electrodes, size of the electrodes, individual differences in skin resistance, proximity of nerve afferents to the electrodes, duration of stimulation, time of the day of stimulation and finally anatomical variations in skin innervation. To find a specific stimulus, the proportion of negative studies to positive studies must be large. Thus, even with large publication bias, negative studies should considerably outnumber positive ones, which is not what we see. Secondly, it is plausible to suggest that the effect of VNS is non-specific. The hypoalgesic effect may be driven via sending nonspecific signals at the level of the brainstem. These

signals will have a ''competing for effect'' with incoming pain stimuli or perhaps triggering a nonspecific reflex that activates descending pain inhibition or even results in a nonspecific release of inhibitory neurotransmitters. The effect is therefore independent of the stimulation parameters or perhaps type of the stimulation as long as such stimuli reach specific brainstem nuclei involved in pain pathways. If this hypothesis is sound, the hypoalgesic effect could be induced by the stimulation of any of the cranial nerves. For example, Kovacic et al used an enlarged auricular field that encompasses areas supplied the great auricular and trigeminal nerves that may, in turn, stimulate the vagal nerve via communications with the spinal trigeminal nucleus(8). For anatomical considerations, the vagus nerve is perhaps one of the best candidates due to its extensive network. If this is the case, then we can argue that the shape of the electrode, exact location or parameters, etc. are less relevant if they avoid habituation and remain safe.

The exact neurobiology of how a competing stimulus prevents incoming pain signals at the level of the brainstem remains to be elicited. In Melzack and Wall's ''gate-control theory of pain'', competing (non-noxious) stimulus can inhibit noxious stimulus at the level of the spinal cord (41). Our hypothesis shares many aspects of this theory, with the evident difference that the level of inhibition is at the level of the brainstem rather than the spinal cord. It is evident that this conceptual hypothesis needs significant tuning and extensive testing.

In summary, this literature review and meta-analysis suggests that vagal nerve stimulation has an anti-nociceptive property. While the effect size was modest but

remained statistically significant. There is important heterogeneity in those included studies which may limit subgroup analysis at this stage.

In conclusion, there is enough literature data in favour of the anti-nociceptive effect of noninvasive vagal nerve stimulation that warrant further investigations and clinical trial in various pain conditions.

Chapter 6

Effect of Transcutaneous Vagal Nerve Stimulation on Reversing Acid-Induced Oesophageal Pain Hypersensitivity

Introduction

Oesophageal pain is a major global cause of disability, healthcare-seeking and reduction in quality of life (181). Chronic oesophageal pain is a symptomatic feature of disorders such as erosive oesophagitis, non-erosive reflux disease and non-cardiac chest pain. The latter has been estimated to account for approximately 700,000 consultations in the accident, and emergency departments with care costs to the National Health Service estimated to be in the order of £83 million *per annum*. Patients often display heightened sensitivity to intra-oesophageal stimuli, which is referred to as oesophageal pain hypersensitivity (182, 183). However, the experience of oesophageal pain is highly individual with a multitude of factors including physiological and psychological factors proposed to account for this variability (172).

Amongst the physiological factors, autonomic nervous system (ANS) plays an important role. The ANS is a bidirectional, hierarchically controlled brain-body nexus that integrates the external environment with the internal milieu. The ANS has been postulated to play a pivotal role in the modulation of pain through its multiple interactions that occur at the

level of the periphery, spinal cord, brainstem, and forebrain (184). The ANS has two broadly antithetic branches, the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). The primary neural substrate of the PNS is the vagus nerve.

Previously, we have sought to determine the role of ANS in modulating oesophageal pain hypersensitivity using a safe, well-validated clinical model of oesophageal pain (40), where participants were randomised to receive saline or acid infusion in the lower (distal) oesophagus. Following acid infusion into the distal oesophagus, pain thresholds to electrical stimulation are reduced in both the exposed (distal) and the (upper (proximal)) non-acid exposed oesophagus, the latter most likely as sequelae of central sensitization.

Work from our group has demonstrated that during distal oesophageal acidification, there is a rise in SNS tone and a fall in PNS tone (167). Interestingly, we have also demonstrated that participants who decreased their PNS tone the most developed a heightened degree of oesophageal pain hypersensitivity (168). Despite progress in identifying the mechanisms that account for development and maintenance of the oesophageal pain hypersensitivity, translation into efficacious drug treatments has remained limited, notwithstanding concerns regarding safety and side effects (185). Therefore, it is not surprising that several psychological and alternative/complementary interventions, such as cognitive behavioural therapy and yoga, have been employed in the management of oesophageal pain syndromes (186).

A common feature of these interventions is the conscious control of breathing frequency and depth. Deep breathing has been proffered as a method of inducing analgesia, possibly through increasing PNS tone (187). These data and ours provided a rationale for the suggestion that the PNS may have analgesic properties in the oesophagus. We have recently examined this possibility using the oesophageal pain hypersensitivity model where participants undertook either, deep breathing to physiologically increase PNS tone or normal (sham) breathing during oesophageal acidification. Deep breathing significantly increased cardiac vagal tone *(figure 24 panel A)* and prevented the development of oesophageal pain sensitivity (188), (*figure 24 panel B).* It has been proposed by our group that other methods of increasing PNS tone, for instance electrically, have a similar effect (submitted, *QMERC2014/5*).

Electrical vagal nerve stimulation (VNS) was first used in humans in 1988 and is an efficacious treatment for drug-resistant epilepsy (189). Traditional VNS is undertaken in a procedure where a bipolar helical electrode is placed around the cervical vagal nerve, which is connected to a pulse generator placed in a subcutaneous pocket in the chest, not dissimilar to a cardiac pacemaker, *(figure 25 panel A)*. However, this method of VNS necessitates surgical implantation with its attendant risks and complications (190). Recently, an external transcutaneous VNS (t-VNS) system, consisting of an earplug-like electrode to interface with the concha of the outer ear and a handheld battery-powered electrical stimulator, has become commercially available (NEMOS system(CE marked)), *(figure 25 panel B)*. The auricular branch of the vagus nerve innervates the concha of the ear and is located directly under the skin, making it a suitable target for transcutaneous stimulation. t-VNS has been demonstrated to be safe, well-tolerated and has a high degree of user-friendliness. A preliminary study has reported that t-VNS reduces

sensitivity to heat pain in healthy volunteers (7). Furthermore, recent studies have demonstrated that t-VNS patterns of brain activation, as determined by functional magnetic resonance imaging, were similar to those evoked by traditional VNS (191). Thus, VNS *per se* represents an attractive proposition for investigating the role of the PNS in oesophageal pain and t-VNS specifically, a viable, safe and acceptable technology for achieving this.

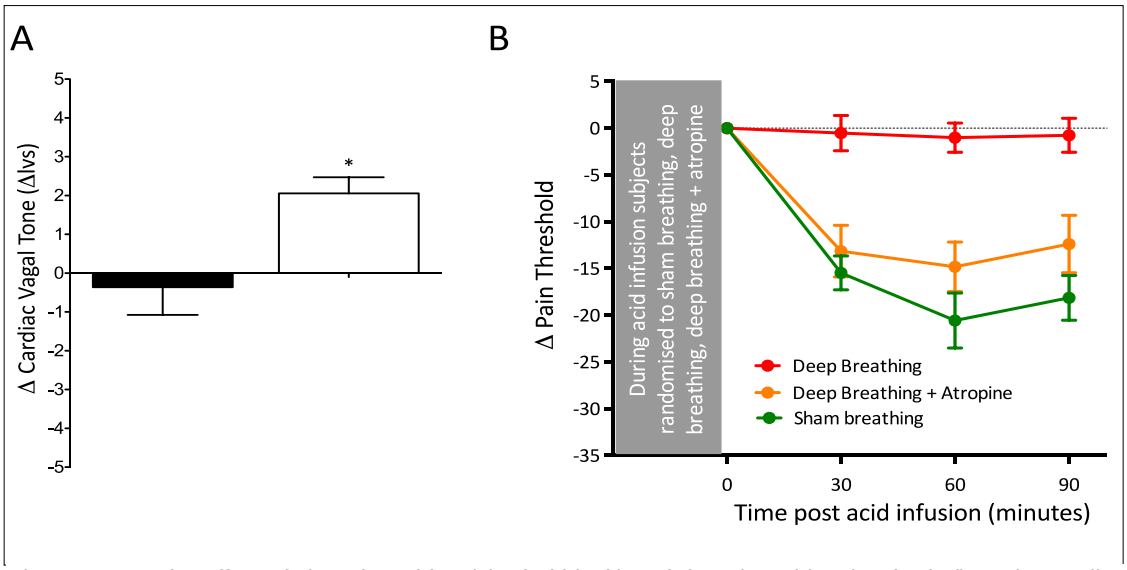


Figure 24: A- The effect of sham breathing (shaded black) and deep breathing (unshaded) on the cardiac vagal tone (PNS tone) indicating that deep breathing increases vagal tone (p<0.0001).

B- The effect of sham breathing, deep breathing and deep breathing + atropine on change in pain thresholds. Mixed effects regression modelling showed a coefficient of effect for deep breathing of 9.94 (95% CI 8.3-11.6, p= 0.0001). Atropine blocked the analgesic effect of deep breathing. Adapted from Botha et all, Gut 2014.

We have recently examined the possibility of using the proposed tVNS device to prevent the development of acid-induced oesophageal hypersensitivity in our human model described above, where participants undertook either active tVNS, to increase PNS tone or sham tVNS during oesophageal acidification. tVNS significantly increased cardiac vagal tone and prevented the development of oesophageal pain sensitivity (manuscript

submitted, QMUL Ethics approval number QMERC2014/5) (figure 26). Although the latter study shows that tVNS prevents the development of oesophageal hypersensitivity when used simultaneously with acid infusion, in many patients, acid-induced oesophageal hypersensitivity is due to Gastro-oesophageal reflux disease, where sensitisation is already established and, therefore, it is important to determine whether the tVNS can reverse sensitisation that has been established. If proven, the ability to reverse oesophageal pain sensitivity gives tVNS a dual mechanism of action (i.e. prevention and reversal of oesophageal hypersensitivity), thus strengthening the rationale for translation into clinical practice. The focus on enhancing the reversal of oesophageal pain sensitivity is especially important when taking into consideration the larger therapeutic window to use this intervention when compared with prophylactic stimulation during reflux episodes only. The pivotal experiments evaluating the role of VNS in reversing acid-induced oesophageal pain hypersensitivity have not been conducted. Our aim is, therefore, to use the model above of acid-induced oesophageal pain hypersensitivity, to determine the effect of t-VNS on the reversal of pain hypersensitivity after it has already been established following acid infusion.

Hypothesis

We, therefore, hypothesised that t-VNS reverses the development of acid-induced oesophageal pain hypersensitivity by increasing PNS tone.

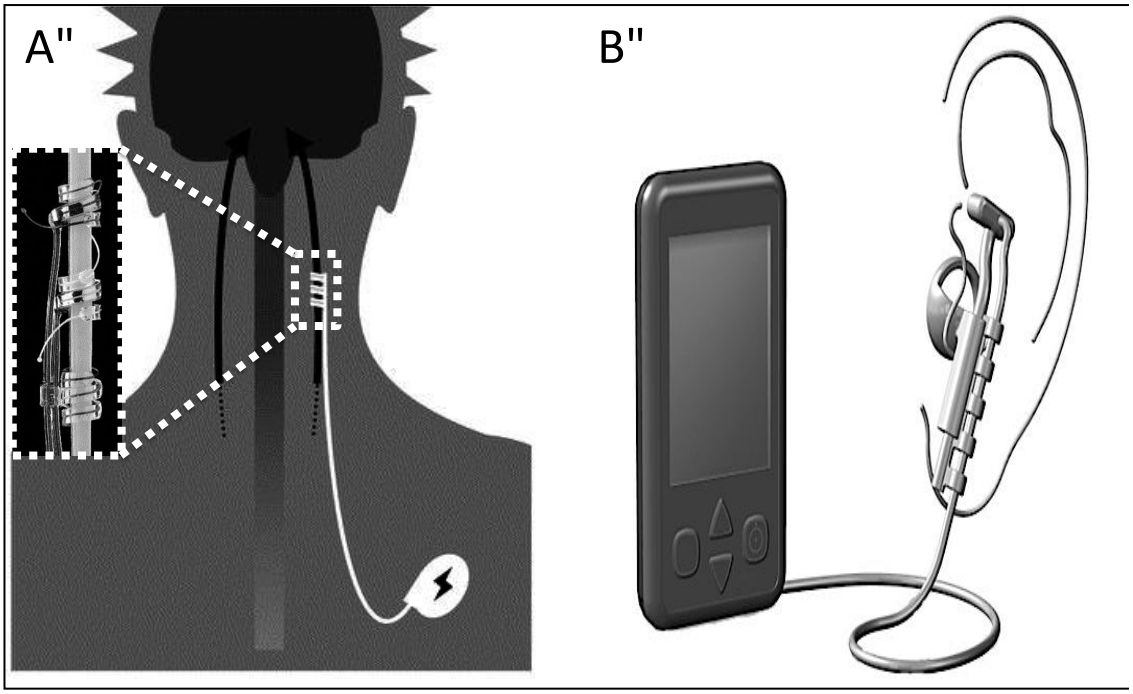


Figure 25: Electrical vagal nerve stimulation. Panel A (left) depicts a traditional invasive vagal nerve stimulation whereas panel B demonstrates external non-invasive transcutaneous vagal *nerve stimulation (NEMOS system).*

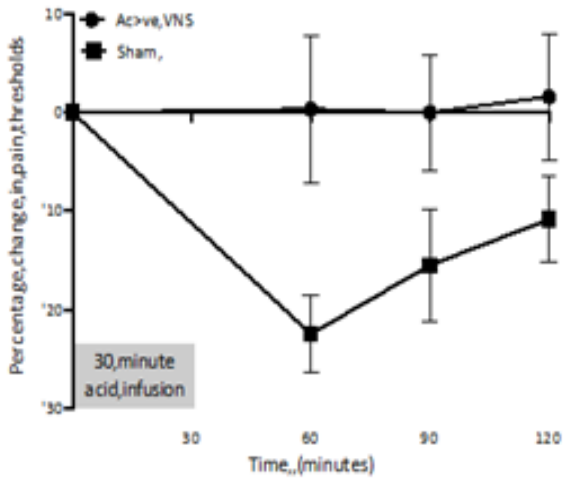


Figure 26– The effect of active and sham tVNS on the development of oesophageal pain hypersensitivity, derived from change in pain thresholds. Mixed effects linear regression, controlling for age and gender, demonstrated that VNS prevented the development of acid-*induced esophageal hypersensitivity in comparison to sham stimulation (coefficient 15.4mA /unit time (95% confidence interval 8.8 to 22.2), p=0.001)*

Methods

We proposed to undertake a study evaluating the use of t-VNS in our validated model of oesophageal pain hypersensitivity. The study flowchart is summarised in *figure 28*.

Sample size

Based on our extensive experience with this model of acid-induced oesophageal hypersensitivity, we have produced summary data demonstrating that subjects have a mean reduction in pain thresholds of -14.3% +/- standard deviation of 16.4% at T120 relative to baseline. Seventeen subjects would provide 90% power at a two-sided significance level of 0.05 to detect a difference of 14.3%, i.e. to prevent sensitisation in an AB/BA crossover design.

Study design

A flowchart presentation of the study design is presented in figure 27.

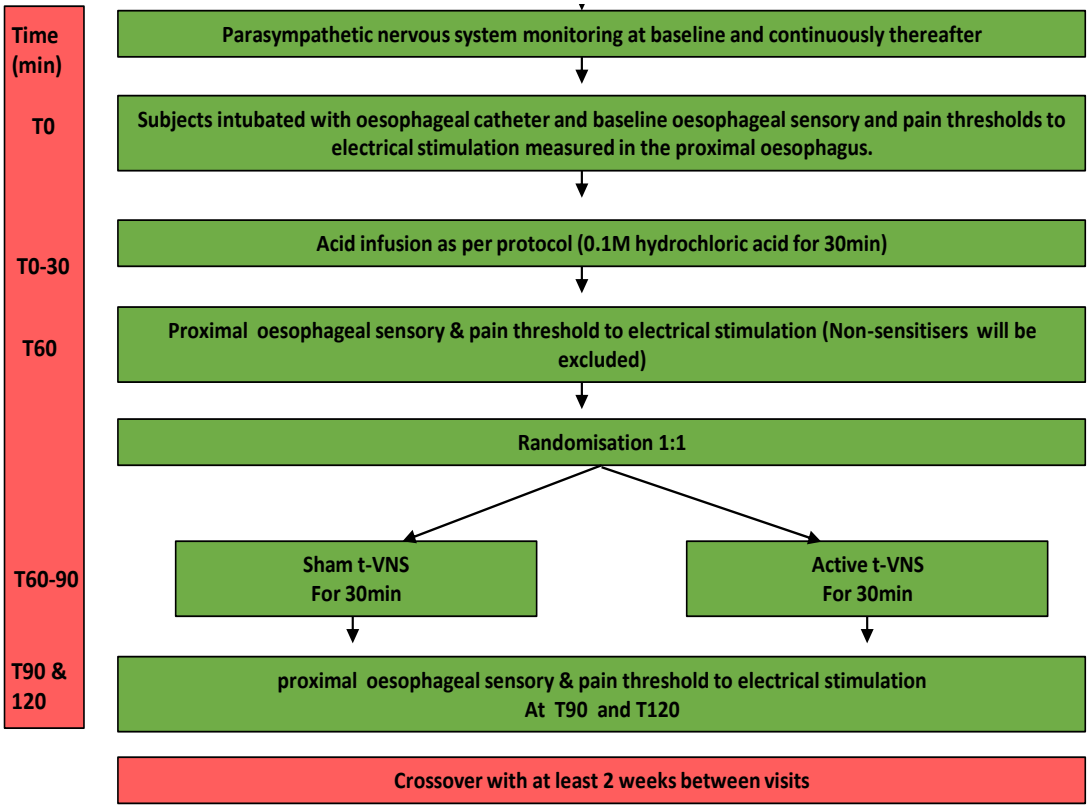


Figure 23: Study flowchart

Visit 1

Psychological profiling and autonomic nervous system measurements

Validated questionnaires assessing anxiety (Hospital Anxiety and Depression Scale and Spielberger State/Trait anxiety) were completed. To define the anatomical landmarks of the oesophagus (i.e. position of the lower oesophageal sphincter, if the recruited subject has not been studied before using this model), then high-resolution oesophageal manometry was undertaken first to define this. At baseline, and continuously after that,

measurement of PNS tone were made using the non-invasive Neuroscope system, a technologically advanced biosignals acquisition system that allows for validated measurement of efferent (cardiac vagal tone) of the PNS (172). This monitoring also provides an additional safety aspect to the study.

Randomisation Procedures

The randomisation of subjects to either active or sham intervention was performed using approved statistical software (www.randomization.com). Subjects were not told which intervention (active/sham) they have been randomised to.

High-resolution Manometry

The ManoScan 360™ High-Resolution Manometry system from Sierra Scientific was used. A specialised HRM catheter sheathed in a single-use sleeve was inserted through the nostril into the oesophagus until the distal end of the catheter is resting in the proximal stomach. The catheter is then taped to the nose. Lower oesophageal sphincter (LOS) position, in terms of cm from the nostril, were recorded and used to guide insertion of further catheters in the next steps of the study.

Acid infusion and pain tolerance measurements

Intra-oesophageal intubation of participants was undertaken using a specialised catheter, containing a distal infusion port and a pair of silver-silver chloride bipolar ring electrodes (3 mm diameter catheter (Unisensor AG, Ch-8544 Attikon, Switzerland). Oesophageal sensory testing was performed via a pair of silver-silver chloride bipolar ring electrodes (inter-electrode distance 1 cm), situated 16 cm proximal to the tip of a 3 mm

diameter catheter. Following identification of the lower oesophageal sphincter, using high-resolution manometry, electrical stimulation was performed 17 cm proximal to the lower oesophageal sphincter. Electrodes were connected to an electrical stimulator, and stimuli are delivered at a frequency of 0.5 Hz, using square wave pulses (0.5 s duration), at intensities varying between 0 and 90 mA. The intensity of stimulation was increased incrementally by two mA, and each subject were asked to report both, the sensory threshold (visual analogue scale (VAS) of 1 out of 10), and when they cannot tolerate any further increase (VAS of 7 out of 10), that is defined as pain tolerance threshold (PTT). The electrical stimulation was immediately stopped when PTT is reached.

Electrical sensory and pain tolerance testing were undertaken in the proximal oesophagus at baseline (T0) and, at 60 minutes (T60), 90 minutes (T90) and 120 minutes (T120) after starting distal oesophageal acid infusion of 0.15M hydrochloric acid (T0-30) (Stepping Hill Hospital, Stockport) using a syringe pump (KDS Scientific 100, Linton Instrumentation, Palgrave, UK). At T60, non-sensitisers were excluded. Non-sensitisers are defined as having a post-acid infusion reduction in upper oesophageal PTT of ≤ 6 mA at T60, as previously defined by Sharma et al. (169).

Active and sham vagal stimulation

To evaluate the effect of t-VNS on enhancing the recovery of OPH, after acid infusion participants were randomised, in a single-blinded manner (participants were blinded to the position of the active intervention in the study), to receive either active t-VNS (placed on the region of the outer ear supplied by the auricular branch of the vagus) or sham t-VNS (t-VNS module placed on the area located below the tragus, supplied by the Great Auricular nerve) (Figure 28). The device delivers rectangular pulses (250 μS, 25 Hz) (7)

(NEMOS, Cerbomed GmbH, Erlangen, Germany (CE marked). Both intervention (active/sham) lasted for 30 minutes (T60-T90). We then repeated pain tolerance measurements at T60, T90 and T120 (Figure 29). The sham intervention was adopted after it was recently validated in a published clinical trial using tVNS and paced deep breathing to modulate gastroduodenal motility, only active stimulation and not sham significantly increased vagal tone (192).

Blinding

Participants were blinded to the position of the active intervention in the study and were told that we are measuring the effect of stimulation on two distinct nerves. Analysis of results was performed by an investigator blinded to the type of intervention. Participants were told that we are studying the effect of electrical stimulation on 2 distinct nerves.

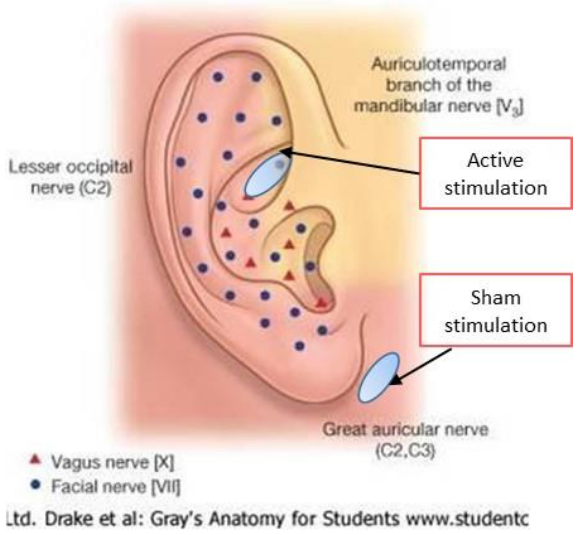


Figure 28: Nerve supply of the outer ear. The ellipse markings show the proposed positioning of the electrodes for active and sham stimulation.

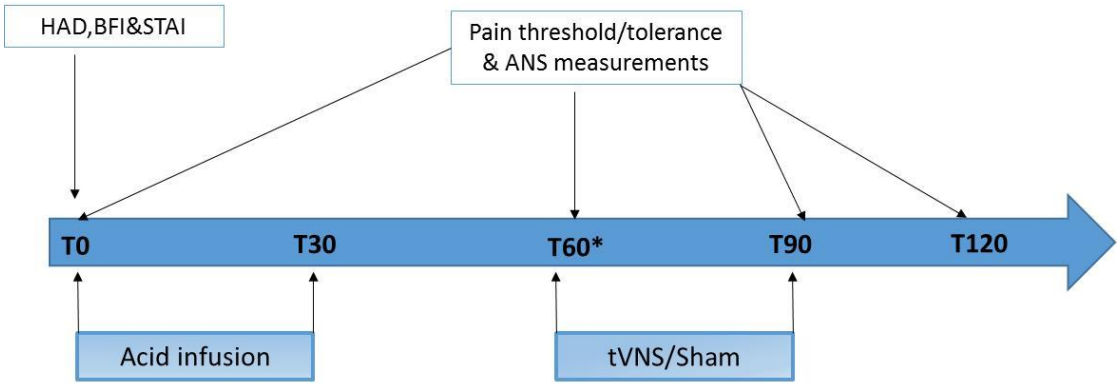


Figure 29: Timeline representation of the proposed interventions in each visit

Following a period of no less than two weeks, to reduce any potential carryover effect, participants were crossed over and restudied to receive the intervention they did not receive in visit 1.

Schedule of assessment:

Assessment	Visit 1	Visit 2
Medical and social history taking	x	
Questionnaires	x	x
Inclusion and exclusion criteria	x	x
High-resolution Manometry	x	
ANS monitoring	x	x
PTT measurements	x	x
Oesophageal acid-infusion	x	x
Exclusion on non-sensitizers	x	
Randomisation	x	
Active/sham tVNS	x	x

Primary outcome measure

- Pain tolerance to electrical stimulation at T120

Secondary outcome measures

- Pain tolerance thresholds at T60, T90

- Effect of t-VNS on ANS variables

Data Analysis

Changes in pain tolerance thresholds were analysed using linear mixed-effects regression model with a maximum restricted likelihood (fixed effects: time, interventions, i.e. active t-VNS vs sham t-VNS; random effect = subject) with T0 thresholds accounted for in the model as zero to yield a regression coefficient for intervention effect. All analyses were two-sided, and a statistical criterion of $\alpha<0.05$ were adopted. Analyses were conducted using the propriety software Stata /SE version 10.1 for Windows (College Station, TX, USA) and SPSS 20, (SPSS Inc., Chicago, IL).

Participants

Healthy subjects, aged 18-41, were recruited from the staff and local population around Queen Mary University of London by advertisement and poster only or from our existing databases of healthy volunteers who have previously agreed to have their contacted details included in the database and expressed interest in being informed of other studies in the institute. Vulnerable groups were not be approached. We recruited participants to compensate for dropouts until the sample size was met.

Inclusion criteria:

1. Healthy volunteers, aged 18-60, from the staff of Queen Mary, University of London and the local population.

2. Inclusion was determined based on availability, with no prior selection bias included. They should be able to attend the Wingate Institute for at least 2 x 2.5-3 hours sessions.

Exclusion criteria:

1. Participants are unable to provide informed consent (e.g. not English speaking)

2. Participants with any systemic disease or medications that may influence the autonomic nervous system (e.g. beta-agonists or Parkinson's disease)

3. Participants with a history of cardiovascular conduction problems

4. Participants who are pregnant

5. Participants who have tinnitus

6. Participants with cochlear implants

7. Those with reflux disease

8. Those on medication, whether prescribed or over the counter, including acid reduction medication

Results

Demographics

Twenty-five participants (12 male, mean age 26.4 years, range 19-41) were recruited. In total, 7 participants (4 male, mean age 27.7 years, range 19-36) were excluded with five classified as non-sensitizers, and a further 2 participants did not tolerate naso-oesophageal intubation, *see flowchart below*. This was an expected rate of non-sensitization based on our previous work, thus leaving, 18 participants (8 male, mean age 26 years, range 19-41).

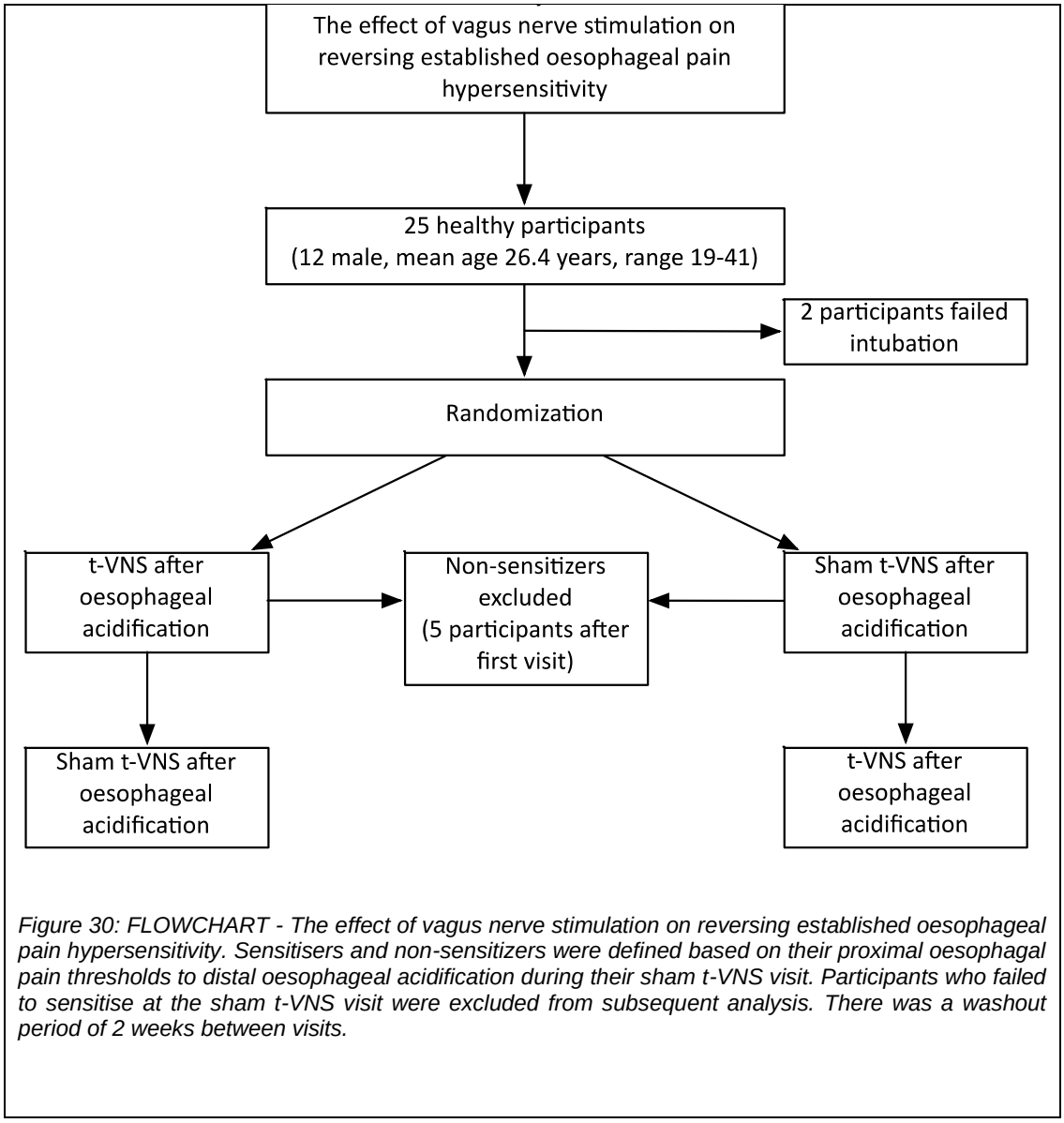


Figure 30: FLOWCHART - The effect of vagus nerve stimulation on reversing established oesophageal pain hypersensitivity. Sensitisers and non-sensitizers were defined based on their proximal oesophagal pain thresholds to distal oesophageal acidification during their sham t-VNS visit. Participants who failed to sensitise at the sham t-VNS visit were excluded from subsequent analysis. There was a washout period of 2 weeks between visits.

The most common symptom reported with acid infusion was nausea (4/18, 22.2%). Absolute threshold data at (T0) and after acid infusion (T60, T90, T120) are shown in *Table 14*. There were no differences in absolute values of PTT at T0 or T60 in participants receiving t-VNS or sham VNS (T0 mean (SD) t-VNS 38.7mA (12.6) vs sham t-VNS 37.3mA (15.7), *p*=0.69, T60 t-VNS 28.7mA (11)) vs sham t-VNS 27.2mA (11.2), *p*=0.55). Relative to the T60 time-point, there was an increase in PTT with t-VNS at T90 of 3mA (95% CI 1 - 5.1) in comparison to sham t-VNS of 0.7 mA (95% CI -1 – 2.3). Similarly, at T120, there was an increase in PTT with t-VNS of 3.8mA (95% CI 1.5 - 6.1) in comparison to sham t-VNS, 1.3mA (95% CI -0.4 – 3). Mixed-effects regression showed a significant effect for t-VNS (coefficient 17.3mA /unit time (95% CI 9.8 - 24.7), *p*=0.0001), *see Figure 31*.

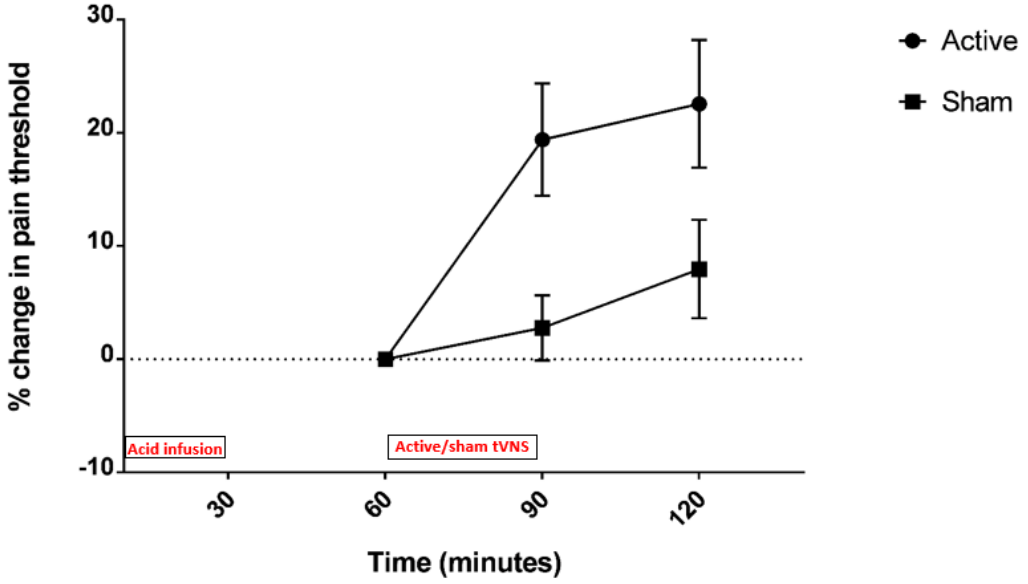


Figure 3124: The effect of t-VNS and sham t-VNS on the reversing established oesophageal pain hypersensitivity, derived from the paired change in pain thresholds (mean ± standard error of the mean), in the proximal oesophagus at T60, T90 and T120, with mixed-effects regression showing a coefficient of 17.3mA /unit time (95% CI 9.8 - 24.7), p=0.0001.

Table 14: Absolute values for proximal oesophageal PTT before (T0) and after (T60, T90 and T120) acid infusion with (a) t-VNS and (b) sham t-VNS delivered after acid infusion.

a. Pain tolerance thresholds – t-VNS after oesophageal acidification				
	T0	T60	T90	T120
Pain thresholds: mean (SD) mA	38.7 (12.6)	28.7 (11.0)	32.1 (16.5)	34.5 (20.7)
b. Pain tolerance thresholds– sham t-VNS after oesophageal acidification				
Pain thresholds: mean (SD) mA	37.3 (15.7)	27.2 (11.2)	30.3 (12.1)	32.9 (13.9)

Effect of tVNS on cardiac vagal tone

Using a Repeated Measures general linear model, I could not find a statistically significant effect of vagal nerve stimulation on cardiac vagal tone, see table 15, figure 32 and table 16.

Table 15: Absolute values of cardiac vagal tone during the different epochs of the experiment

	Active	Sham
Baseline	9.61	11.30
Acid infusion	9.8	9.42
Sensitization	12.17	11.30
Stimulation/sham	10.58	12.55
Post Stimulation/sham	11.16	13.78

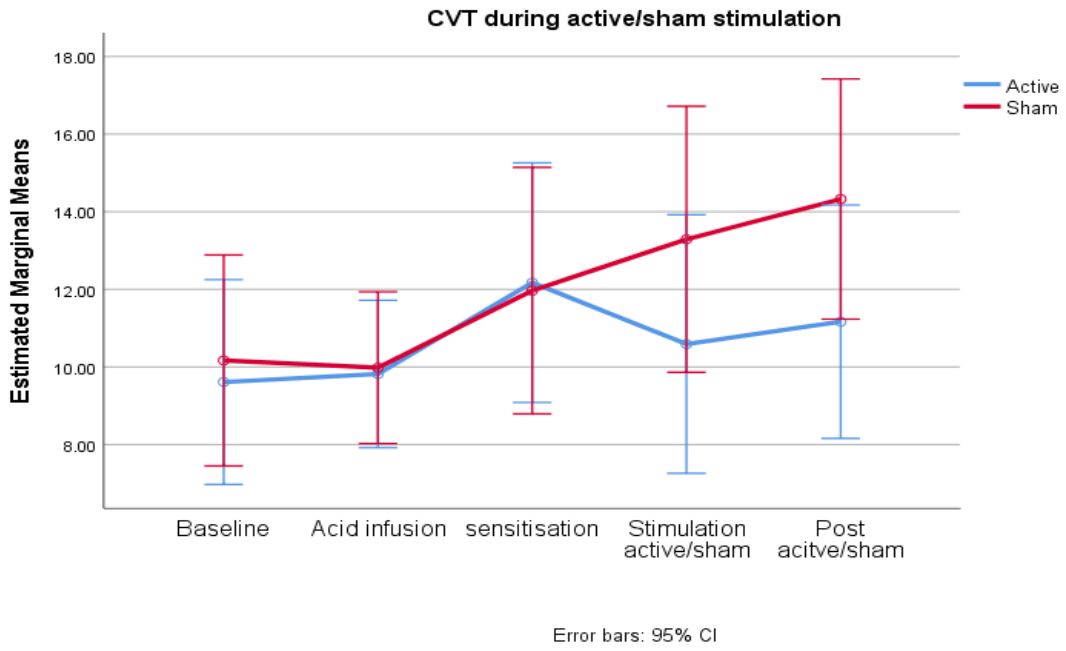


Figure 32: Effect of active/sham transcutaneous vagal nerve stimulation on cardiac vagal tone.

Table 16: Repeated Measures general linear model failed to detect a statistically significant effect of vagal nerve stimulation on cardiac vagal tone. df: degree of freedom, tVNS: transcutaneous vagal nerve stimulation.

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Intercept	22362.624	1	22362.624	197.503	.000
tVNS	71.080	1	71.080	.628	.434
Error	3736.475	33	113.227		

Relationship between cardiac vagal tone and sensitisation

There was no significant correlation between change in cardiac vagal tone and the degree of sensitisation, see figure 33.

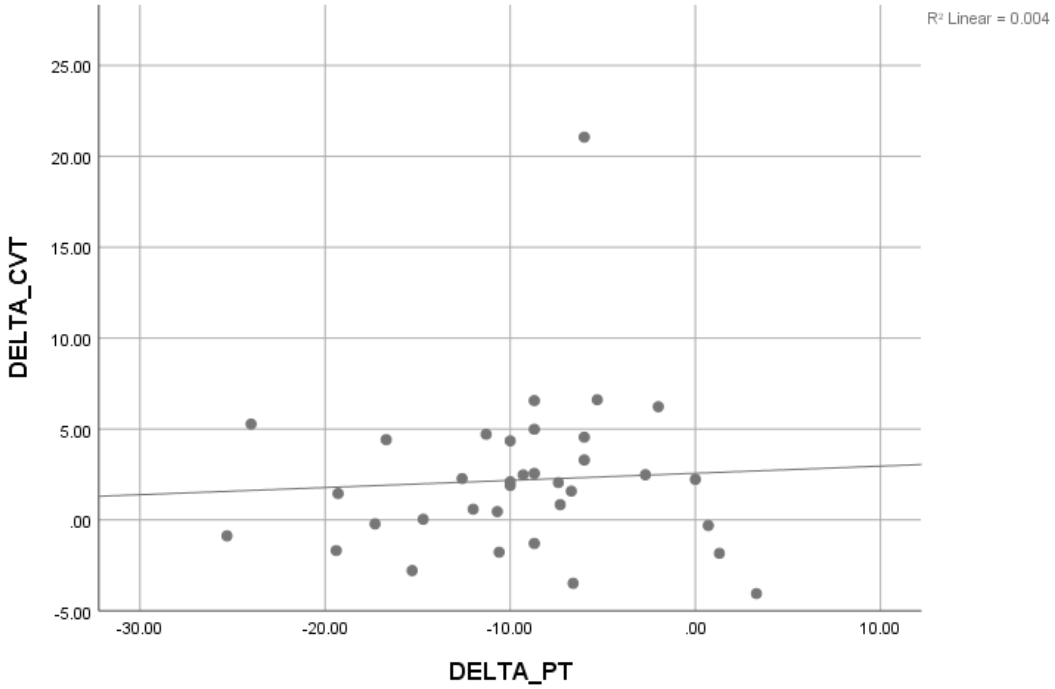


Figure 25: Correlation between the change in cardiac vagal tone (DELTA_CVT) and change in pain threshold (DELTA_PT). Pearson's correlation was 0.06, p=0.731.

DISCUSSION

This study suggests that t-VNS reverses established acid-induced oesophageal pain hypersensitivity. This effect is likely mediated by vagal modulation of the central nociceptive network. It is also possible, although less likely that the effect could also be mediated in part via an anti-inflammatory pathway(193, 194). During systemic inflammation, the CNS is activated by vagal afferents. Following input integration, the coeliac ganglion activates the vagal efferent, which acts to modulate the immune response in the spleen, leading to a triggering of splenic adrenergic neurons which in turn cause a release of noradrenaline and subsequently acetylcholine (ACh(194)). ACh binds to the alfa-7nACh receptor localised to macrophages, which in turn decreases the release of inflammatory cytokines, including tumour necrosis factor. Thus, it is thought that the vagal nerve is a key modulator of inflammation. Furthermore, during GI inflammation or pain, the vagal afferent fires to the CNS, which in turn leads to the activation of the vagal efferent which targets myenteric neurons of the intestinal wall. This leads to the subsequent release of ACh from enteric neurons, serving a similar immunomodulatory pathway as to when systemic inflammation occurs(194).

The articular branch of the vagal nerve stimulated in this study, is a pure afferent nerve (195); thus, most likely, the anti-nociceptive effect seen in this study is centrally mediated. The anti-inflammatory effect of vagal activation require efferent modulation which is not seen in this study.

Functional neuroimaging studies suggest that t-VNS modulates areas of the brain associated with central pain neuromatrix such as the thalamus, orbitofrontal cortex, cerebellum, hypothalamus, medulla and the limbic system (196, 197). For instance,

implanted VNS has been shown to result in the insula and cortical activation. Those areas have been observed to be important in mediating acid-induced oesophageal pain in healthy participants and in patients with gastro-oesophageal reflux disease (198-200). Moreover, t-VNS is associated with an increase in insula activity and a reduction in the amygdala and hippocampal activity (201). It has been recently illustrated how higher resting parasympathetic CVT conveys greater network connectivity in several subcortical regions implicated in descending analgesia, including the anterior insula, amygdala and hypothalamus, suggesting a prospective neural mechanism for t-VNS induced anti-nociception (202).

In previous studies, our group has demonstrated that the oesophageal hyperalgesia that develops in the non-acid exposed proximal oesophagus most likely occurs due to central sensitisation at the dorsal horn of spinal cord (203). Central sensitisation reflects enhanced nociception through three broad mechanisms, namely, temporal summation, increased activation of nociceptive facilitatory pathways or impairment of descending pain inhibitory pathways. Dysfunction within the descending pathways may particularly promote and maintain central sensitisation (204). Within the brainstem, primary afferent vagal fibres terminate in the nucleus tractus solitarius, which also contributes to descending inhibitory pathways which form a spinal-bulbo-spinal anti-nociceptive circuit (205). The central analgesic effect of VNS has been proposed to increase such descending pain modulatory pathways (206). However, other studies have shown that the vagus nerve modulates nociceptive processing in both the spinal cord and the brain.

For instance, nociceptive transmission at the spinal dorsal horn can be inhibited by the electrical stimulation of abdominal vagal afferents (207, 208).

The antinociceptive effect could also be in part mediated via a local anti-inflammatory effect. The vagus nerve effect on inflammation is mediated by acetylcholine or noradrenaline, also known as the cholinergic anti-inflammatory pathway (209-211). In the context of t-VNS, several studies have shown short term stimulation exerts an anti-inflammatory effect (212, 213). Besides, the vagus nerve interacts with the hypothalamic-pituitary-adrenal axis, which results in the release of cortisol inhibiting the proliferation of pro-inflammatory cells (194). Following acid-induced oesophageal cell injury, there is an influx of inflammatory mediators whose function is to repair squamous epithelium.

Our findings have several therapeutic implications. Heartburn and chest pain are common symptoms in functional oesophageal disorders which are mediated, in part, by oesophageal hypersensitivity (214, 215). Although proton pump inhibitors (PPIs) are the gold standard for the treatment of gastro-oesophageal reflux disease, a substantial proportion of such patients fail to respond (216). Non-pharmacological interventions are increasingly being sought to treat chronic pain disorders. Coupled with the data from our study, and an established favourable safety profile, t-VNS could represent an attractive non-invasive neuromodulatory intervention that warrants further study in this group.

This is particularly important given that we demonstrated that t-VNS could reverse established oesophageal hypersensitivity.

Our study is subject to several limitations. Within any cross-over design, there is potential for a carryover effect, although we attempted to ameliorate this by using at least two weeks between study visits (217). We used electrical stimulation to investigate visceral oesophageal pain which may be considered non-physiological. The main aim of this study is to investigate pain hypersensitivity caused by central sensitisation. An electric stimulus can bypass the several types of nociceptive receptors to initiate an afferent signal transmitted to the dorsal horn of the spinal cord, where central sensitisation is thought to take place which makes it a convenient stimulus modality to use and the model used in our study has been well validated to induce secondary hyperalgesia most likely due central sensitisation. Finally, this is a study in young, healthy volunteers. Further studies are needed to see if this applies to patients with evidence of oesophageal pain hypersensitivity.

In conclusion, non-invasive vagal nerve stimulation could reverse an experimentally induced oesophageal hypersensitivity in healthy volunteers, under strict laboratory settings. Further studies are needed to see if the effect is significant in patients with clinical manifestations of oesophageal pain hypersensitivity, such as those with hypersensitive oesophagus as defined by the Rome IV classification (214).

Chapter 7:

Summary of findings and future directions

Summary of findings

Pain hypersensitivity is a common finding in functional gastrointestinal disorders. It represents a challenge for several reasons. Firstly, it is a common finding that reduces the quality of life, drives anxiety and causes health care seeking visits (9, 218). One example is oesophageal pain in patients who are adequately treated with acid-reducing medications that may persist in 10- 40 % depending on the study (9). When taking into consideration the prevalence of gastroesophageal reflux disease, ten per cent can add an important burden to the healthcare system and can cause an important reduction in the quality of life.

Functional gastrointestinal disorders are benign in nature; thus, a treatment designed to treat such disorders should have a good safety profile to be justified. Given the chronicity of such conditions and the large prevalence, a proposed treatment should also be reasonably cheap and preferably self-applied.

Another challenge is that functional gastrointestinal disorders are grouped based on clinical symptoms and not by well explained physiopathological mechanisms. Such classification will inevitably result in a large heterogeneity within this group.

This thesis aims to contribute to a better understanding of visceral pain hypersensitivity and to propose feasible interventions to be developed further.

In the 1st chapter, I tried to present a relevant overview of the biology of pain. I introduced concepts such as the pain network, definitions of peripheral and central sensitisation. There are notable observations that are likely to facilitate understanding of pain hypersensitivity. The important role of descending pathways in regulating pain is also discussed. Descending pathways are complex and influenced by several factors ranging from local spinal mechanisms to complex interactions of multiple inputs from emotional, autonomic, hormonal and cortical regions. We also observe an interconnected pain and autonomic network that shares many of its key structures.

Such a complex pain network likely means that several pathways that can override each other depending on the context. This will inevitably mean that there is a heterogeneous group of patients with a similar final complaint e.g. pain.

There are peripheral and central causes of pain hypersensitivity. However, the role of peripheral causes is less evident in functional gastrointestinal disorders. For example, Guy Boeckxstaens and colleagues studied the effect of the mast cell stabiliser / H1 receptor antagonist ketotifen on rectal pain hypersensitivity in IBS. The medication was superior to placebo in abdominal pain reduction in a controlled study; however, there was no difference in the spontaneous histamine and tryptase release measured in the supernatant of rectal biopsies before and after treatment(219). One interpretation is that the effect of ketotifen on pain hypersensitivity was centrally mediated via H1 receptor antagonism.

Descending pain inhibition can be very pronounced, for example, the within-group difference in pain threshold in gunshot wounds in soldiers. This observation highlights the potential of descending pathways in controlling pain that we could potentially target as a therapeutic pathway.

The overall pain experience is complex. It is a result of complex and intricate interaction between the primary stimulus (if there is one), the central pain network, autonomic network, emotional and cognitive centres.

Emotions have an important influence on descending regulation of pain (220). Several emotions express clear autonomic responses. James and Lange suggested that emotions are the by-products of biological feedback from the periphery (63, 64). They argue that for example, an increased heart rate, sweaty hands and shallow breathing will produce emotions consistent with this physical status, such as fear. Regardless of the directionality of the cause and effect between emotion and autonomic response, the association is clearly there. One could suggest that a conditioned reflex is formed between specific emotional responses and physiological status. If this line of thinking is sound, then inducing a physiological state that is consistent with relaxation, will likely induce relaxation. For example, slow deep breathing causes reliable parasympathetic nervous system activation with slowing down of breathing and heart rate. This, in turn, can activate descending pathways to increase the pain threshold. This mechanism of action will also contribute to other non-pharmacological analgesic interventions such as massage therapy. Muscle tone feedback likely contributes to the emotional status; one example on that is benzodiazepines that are both anxiolytics and muscle relaxants.

In chapter 2, I investigate the autonomic effect of two interventions used successfully previously by our group to reduce experimental oesophageal pain. Those are attention or distraction (30)and slow deep breathing(188). In this study, I demonstrated that there is a distinct autonomic pattern between the two interventions.

Slow deep breathing increased the parasympathetic tone; however, this effect was short-lived and ceased immediately after returning to normal breathing. Several plausible mechanisms could mediate the effect of slow deep breathing on pain. First, the explanation proposed by Botha and colleagues suggested that the effect is mediated by an efferent vagal mechanism. This conclusion was supported by diminishing the anti-nociceptive effect of slow deep breathing with concomitant use of atropine. Atropine is known to inhibit the action of acetylcholine that is the main neurotransmitter on the efferent vagal. Although this explanation has its merits, there is one limitation. Atropine is also known to increase heart rate and possibility respiratory rate by an antimuscarinic action. This antimuscarinic action could have increased sensitisation in the atropine group making them more sensitised than the slow deep breathing group. There was no control group to see if the use of atropine alone could increase sensitisation.

The other possible mechanism is by distraction. Slow deep breathing could have a distractive effect on participants. The control group were asked to count their breathing frequency, which could act as a control for distraction. It is not known if the two interventions had the same distractive effect.

Another possible mechanism is an afferent one. Slow deep breathing has well described effects on autonomic reflexes such as the cardiopulmonary coupling which is mediated via the vagal nerve. It is plausible that parasympathetic activation induced by slow deep

breathing may trigger autonomic influence on descending pathway acting on pain receptive filed in the spinal cord, this action will be therefore independent of the efferent vagus.

The other intervention studied in chapter two is attention. This has previously shown to reduce oesophageal pain. The mechanism is likely cognitive and related to diverting attention away from the pain stimulus (30). Some studies suggest that cognitive tasks, such as placebo analgesia could trigger descending pain inhibition(221). However, it is not clear if attention directly triggers descending inhibitory pathways.

Although it appears that slow deep breathing and attention produce distinct autonomic signatures, it is still possible that both can trigger descending inhibition of pain. For that reason, it plausible to suggest a synergistic effect when combining the two interventions. Such a combined action is likely achieved by meditation exercises that combine both breathing techniques and distraction.

In chapter 3, I studied the top-down control of pain. Admittedly, there is no precise way of pinpointing the exact central structure that contributes the most of descending pain modulation. As previously mentioned, the control over descending pathways is intricate. It involves multiple structures that can tap into the system to inhibit or facilitate pain. As a general rule, higher structures can alter the overall balance depending on the situation. For example, chronic anxiety and physically intense sports share similar autonomic

profile, which is a sympathetic nervous system activation. However, the pain threshold set by descending modulation is likely to be different in those two situations (220).

I hypothesised that in functional gastrointestinal disorder, the descending inhibition of pain is impaired. Conditioned pain modulation is a way of measuring this pathway(222). We showed that there is a statistically significant and clinically important difference in descending inhibition between patients with irritable bowel syndrome and healthy controls. Patients were five times more likely to have an impaired conditioned pain modulation than healthy control. This level of significant separation between IBS patients and healthy controls is rarely seen in clinical studies. This odd's ratio is very similar to that of post-infectious IBS, which is now accepted as a distinct entity in IBS (223). These findings have several implications. First, they emphasise the descending pain pathways role in functional pain. Secondly, conditioned pain modulation can help to classify patients by physio pathological factors. Such classification aims to reduce heterogeneity in clinical trials and mechanistic studies. Third, those findings suggest that descending pain modulatory pathways are potential targets in functional visceral pain; this may be achieved by using centrally acting medications such as antidepressants active via the noradrenergic pathways (tapentadol, duloxetine, reboxetine and nortriptyline in low doses) . Finally, conditioned pain modulation can also act as an objective endpoint in clinical trials. Based on the highly significant difference between patients and healthy control, further studies are warranted to better understand the relationship between conditioned pain modulation and pain hypersensitivity.

In chapter 4, I elaborated on the relationship between descending pain modulation and experimental oesophageal hypersensitivity. The main finding was that baseline conditioned pain modulation was a strong predictor of sensitisation to experimental acid infusion. Those findings are in line with the observations of Wider-Smith and colleagues who demonstrated that baseline conditioned pain modulation can predict postsurgical pain (148). Another observation in this study was that participants who sensitised the most also increased conditioned pain modulation the most in percentage terms. However, they had a low baseline; thus, the overall magnitude of descending pain inhibition was low in absolute terms. This suggests a ceiling effect of conditioned pain modulation in those participants.

In chapter 5, I performed a systematic review, to gather information regarding the use of vagal nerve stimulation in pain. There was a small but significant overall effect. This small effect size was predicted because of the important heterogeneity of the studies. Noticeably, there was an important variation in stimulation periods and parameters. There was a positive effect despite the variability of stimulation parameters, site of stimulation, conditioned studied, this suggest a non-specific mechanism. The effect is most likely afferent because of the anatomy of the stimulated nerves. The auricular branch of the vagal is a pure afferent nerve(195). Reassuringly, there was no important side effect reported with the use of this intervention.

In chapter 6, I studied the effect of non-invasive vagal nerve stimulation in a validated human model of oesophageal pain hypersensitivity. I used a device designed to stimulate the auricular branch of the vagal nerve. My results suggest that vagal nerve stimulation

could reverse temporarily acid-induced oesophageal pain hypersensitivity faster than sham stimulation. This effect was independent of the efferent vagal effect.

I suggest that the effect of vagal nerve stimulation is mediated at the level of the central nervous system. Despite a careful review of the published literature on the anti-nociceptive effect of vagal nerve stimulation, the exact mechanism remains elusive. From published literature, we notice a positive effect of vagal nerve stimulation on nociception despite stark variation in stimulation parameters. In my opinion, one possible mechanism of how non-invasive vagal nerve stimulation could exert an anti-nociceptive effect would be through competition of afferent stimulation with ascending pain signals. This conceptual theory would resemble, to some extent, what has been suggested by Melzack and Wall(41). Melzack and Wall suggested a gate-like effect at the level of the spinal cord where non-painful stimuli compete with painful ones resulting in reduced pain transmissions. Several interconnecting steps explain this phenomenon, such as interneurons at the level of the dorsal horn. It is plausible that a similar mechanism may exist at the level of the brainstem where stimulation of the auricular branch of the vagal nerve is transmitted. Non-painful stimuli generated by vagal nerve stimulation may compete with the painful stimuli resulting in reduced transmission. Such a mechanism will explain the non-specific nature of this intervention. Another possibility is that the non-specific nature of vagal nerve stimulation at the level of the brain stem triggers descending pain inhibition, narrowing the receptive field at the level of the spinal cord.

Future directions

Based on the results of my program of the research described in this thesis, there are several studies warranted.

Based on the study in chapter one, we suggest a clinical trial to investigate a combined intervention with distraction and slow deep breathing in patients with evidence of oesophageal reflux hypersensitivity. If effective, this is a safe and low-cost intervention that is likely to improve the quality of life of those patients and reduce costs for healthcare providers. This will be a 6-weeks randomised study. The active group will be assigned to once-daily protein pump inhibitor and specially designed mindfulness exercise with slow deep breathing paradigm using a telephone application for 30 minutes twice daily. Focusing on the present or focusing of breathing can act as a form of distraction. The other group will be assigned to double dose of protein pump inhibitors for six weeks (common clinical practice). The outcome measure will be specific gastroesophageal reflux disease validated questioners and quality of life questioners.

Based on the studies in the third and fourth chapters, there is a need to investigate conditioning pain modulation in patients with visceral pain hypersensitivity to see if simple baseline conditioned pain modulation test can predict patients with oesophageal pain hypersensitivity proved by reflux monitoring (214). This will have a prognostic value in those patients when choosing the appropriate treatment.

It is feasible to investigate the effect of centrally acting medications such as several classes of antidepressants, anxiolytic and muscle relaxants on conditioned pain modulation in patients with oesophageal pain hypersensitivity. Condition pain modulation can be used to predict the success of therapy of medications with a potential

effect of descending pain inhibition. For example, in diabetic neuropathy, conditioned pain modulation could predict response to the serotonin and norepinephrine reuptake inhibitor duloxetine and the mu-opioid receptor agonist and noradrenaline reuptake inhibitor tapentadol(151, 152).

Another future study will aim to investigate the effect of vagal nerve stimulation on conditioned pain modulation in patients with oesophageal reflux hypersensitivity in a sham-controlled trial. We will measure conditioned pain modulation at baseline, then randomise patients to either active transcutaneous vagal nerve stimulation or sham stimulation for 30 min twice per day for six weeks. The main outcome measure will be conditioned pain modulation after active or sham treatment.

Limitations

Applicability to the clinical population

All experimental studies were performed on healthy volunteers that may limit applicability to patients. The reason for choosing healthy volunteers was to avoid the multiple confounders usually found in patients with visceral hypersensitivity. Those patients usually display several concomitant functional pain syndromes which makes it very challenging to interpret data. Controlling confounders in disease population such as the intensity of symptoms, psychological and social factors, medications and other medical conditions would not have been feasible within the timeline of this PhD thesis.

To understand the temporal relationship between conditioned pain modulation at baseline and pain hypersensitivity, it is necessary to measure conditioned pain modulation before and after the disease. In clinical settings, pain hypersensitivity is a

chronic condition; thus, it is not possible to measure conditioned pain modulation before the onset of the disease. Studying conditioned pain modulation in healthy volunteers before and after experimentally induced pain hypersensitivity may help to establish a temporal relationship.

Limitations of selected methods

The acid-induced oesophageal pain hypersensitivity model

This model uses a defined noxious stimulus to induce oesophageal pain hypersensitivity. However, the module is relatively invasive and creates unpleasantness and anxiety. Anxiety and unpleasantness could contribute to sensitisation or hypervigilance to the painful stimulus. A locally defined noxious stimulus may contribute to pain in many diseases such as non-erosive reflux disease, where there is an increased acid exposure in the lower oesophagus. However, most functional gastrointestinal disorders lack a defined sensitising stimulus. This may also affect the applicability of this model to all functional pain conditions.

Electric oesophageal pain as a testing stimulus in Conditioned Pain Modulation paradigm

The use of electric oesophageal pain stimulus has not been previously used in a conditioned pain modulation paradigm. Electrical pain stimulus is separately validated in both the acid-induced oesophageal model and in Conditioned pain modulation paradigm (55, 224). The choice of oesophageal location of the stimulus was chosen for several reasons: 1) Participants had an inserted catheter for acid infusion, which also contain a stimulation electrode. 2) The location is relevant to our clinical question that is

concerned with visceral pain. 3) Choosing another stimulus would have prolonged the study and added another unpleasant experience that is not necessary.

Using transcutaneous vagal nerve stimulation

Then devise used in chapter 6 is the NEMOS device. This device has an electrode that is attached to the concha of the ear. As per chapter 5, there are no robust data to favour specific stimulation parameter. The stimulation was set at the lowest tactile sensation felt by participants. This meant different intensities for different participants. There is marked variability in the subjective experience of the stimulus in between individuals, a tactile sensation for some can be painful for others. We set the intensity at the sensory threshold to avoid an unpleasant sensation. A painful sensation caused by stimulation would have confounded the degree of sensitisation in between individuals.

Conclusions

Studies in this thesis explored the relationship between the central modulation of pain and visceral pain hypersensitivity. I have presented evidence that central control of pain is inhibited in conditions with visceral pain hypersensitivity such as irritable bowel syndrome. I have also demonstrated that baseline top-down control of pain could predict the degree of developing pain hypersensitivity in healthy individuals. I then demonstrated that non-invasive vagal nerve stimulation could reverse experimental pain hypersensitivity, likely by a central mechanism. These studies may be helpful in planning future studies in patients with visceral pain hypersensitivity which may be helpful in understanding underlying pathophysiology and may also have therapeutic implications.

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