Towards an Understanding of the Neurophysiology of Cough in Humans

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy

in the Faculty of Medical and Human Sciences.

2012

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School of Translational Medicine
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ABSTRACT
Towards an Understanding of the Neurophysiology of Cough in Humans
The University of Manchester
Dr Emma C.Y. Hilton
Submitted for the Degree of Doctor of Philosophy
March 2012

Rationale
Chronic cough (cough >8 weeks) is common, leads to an impaired quality of life, and is difficult to treat. Despite intensive investigation, ~40% of patients referred to a specialist cough clinic will remain resistant to treatment targeted at peripheral triggers such as reflux disease, rhino-sinusitis or airways inflammation. An improved understanding of underlying mechanisms in such patients would facilitate drug development.

I propose that there are several important similarities between pain and cough that can be exploited better to understand underlying mechanisms. In chronic pain, a long-lasting up-regulation of afferent pain processing may be generated by changes within the central nervous system, mediated by the NMDA receptor and/or by impaired inhibitory mechanisms. A similar central neuronal up-regulation of cough may also be responsible for the pathogenesis of chronic cough (CC).

Methods
A series of experimental studies were performed to address this hypothesis. Firstly, the anti-tussive and analgesic effect of ketamine, an NMDA receptor antagonist, was investigated in CC patients and healthy controls (HC). Pain thresholds were measured using electrical stimulation in the oesophagus, pharynx and chest wall. Cough sensitivity was measured using standard capsaicin cough challenges. Secondly, I designed and tested novel capsaicin cough challenges in CC patients, asthmatics (A) and HC. ED50 (dose inducing and least 50% maximal cough frequency) and Cmax (maximal cough frequency) was compared by group and gender. Finally, I investigated 2 independent mechanisms of cough inhibition.

Results
(i) CC patients, but not HC, had cough induced by oesophageal electrical stimulation, whilst pain thresholds were similar. Ketamine had a significant analgesic effect but no antitussive effect in CC or HC.

(ii) CC patients had both cough hypersensitivity (lower ED50) and cough hyper-responsiveness (higher Cmax) on full capsaicin dose-response curves.

(iii) Both a painful cold stimulus applied to the hand and conscious cough suppression significantly inhibited capsaicin-induced cough responses in CC and HC.

Conclusions
CC patients exhibited increased oesophageal sensitivity to cough, but not pain, providing evidence for a process of central sensitisation in the brainstem. Higher capsaicin-induced cough frequencies in CC may also be mediated by an increased gain within the CNS, possibly because of failed tonic inhibitory mechanisms. Furthermore, CC patients may have poorer conscious control of coughing. In conclusion, an improved understanding of mechanisms in cough will provide a strong scientific rationale for the development of novel therapeutics.
Declaration

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

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Dedication

To my wonderful husband, Dave.
### Abbreviations

<table>
<thead>
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<tr>
<td>95% CI</td>
<td>95% confidence intervals</td>
</tr>
<tr>
<td>A</td>
<td>Asthmatics</td>
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<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ASI</td>
<td>Anxiety sensitivity index</td>
</tr>
<tr>
<td>ASIC</td>
<td>Acid-sensing ion channel</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine-5'-triphosphate</td>
</tr>
<tr>
<td>B1/2</td>
<td>Bradykinin receptor 1/2</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
</tr>
<tr>
<td>BTS</td>
<td>British Thoracic Society</td>
</tr>
<tr>
<td>BVS</td>
<td>Body vigilance scale</td>
</tr>
<tr>
<td>C1</td>
<td>Dose of tussive agent inducing at least 1 cough</td>
</tr>
<tr>
<td>C2</td>
<td>Dose of tussive agent inducing at least 2 coughs</td>
</tr>
<tr>
<td>C5</td>
<td>Dose of tussive agent inducing at least 5 coughs</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>CC</td>
<td>Chronic cough</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin gene related peptide</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>COX2</td>
<td>Cyclooxygenase 2</td>
</tr>
<tr>
<td>CQLQ</td>
<td>Cough-specific quality of life questionnaire</td>
</tr>
<tr>
<td>CS</td>
<td>Central sensitisation</td>
</tr>
<tr>
<td>Cs/hr</td>
<td>Cough sounds per hour</td>
</tr>
<tr>
<td>Cs/min</td>
<td>Cough sounds per minute</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DNIC</td>
<td>Diffuse noxious inhibitory controls</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>ENT</td>
<td>Ear nose and throat</td>
</tr>
<tr>
<td>EP2</td>
<td>Prostaglandin EP2 receptor</td>
</tr>
<tr>
<td>FEV1</td>
<td>Forced expiratory volume in 1 second</td>
</tr>
<tr>
<td>FGID</td>
<td>Functional gastro-intestinal disorders</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
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<td>GEE</td>
<td>Generalised estimating equations</td>
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<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>GORD</td>
<td>Gastro-oesophageal reflux disease</td>
</tr>
<tr>
<td>NERD</td>
<td>Non-erosive reflux disease</td>
</tr>
<tr>
<td>H+</td>
<td>Proton</td>
</tr>
<tr>
<td>HADS</td>
<td>Hospital anxiety and depression scale</td>
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<tr>
<td>HC</td>
<td>Healthy controls</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>IEM</td>
<td>Ineffective oesophageal motility</td>
</tr>
<tr>
<td>IQR</td>
<td>Inter-quartile range</td>
</tr>
<tr>
<td>LCQ</td>
<td>Leicester cough questionnaire</td>
</tr>
<tr>
<td>LOS</td>
<td>Lower oesophageal sphincter</td>
</tr>
<tr>
<td>LPR</td>
<td>Laryngo-pharyngeal reflux</td>
</tr>
<tr>
<td>mA</td>
<td>Milliamps</td>
</tr>
<tr>
<td>Mg</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>Nav</td>
<td>Voltage-gated sodium channel</td>
</tr>
<tr>
<td>NCCP</td>
<td>Non-cardiac chest pain</td>
</tr>
<tr>
<td>NFR</td>
<td>Nociceptive flexion reflex</td>
</tr>
<tr>
<td>NGF</td>
<td>Nerve growth factor</td>
</tr>
<tr>
<td>NK1</td>
<td>Neurokinin receptor 1</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>nTS</td>
<td>nucleus of the solitary tract</td>
</tr>
<tr>
<td>P</td>
<td>Phosphate</td>
</tr>
<tr>
<td>P2X/Y</td>
<td>Purinoceptor 2</td>
</tr>
<tr>
<td>PAG</td>
<td>Peri-aqueductal grey</td>
</tr>
<tr>
<td>PASAT</td>
<td>Paced auditory serial attention test</td>
</tr>
<tr>
<td>PCS</td>
<td>Pain catastrophising scale</td>
</tr>
<tr>
<td>PD20</td>
<td>Provocative dose causing a 20% decrease in FEV1 from baseline</td>
</tr>
<tr>
<td>PEFR</td>
<td>Peak expiratory flow rate</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A/C</td>
</tr>
<tr>
<td>PND</td>
<td>Post-nasal drip</td>
</tr>
<tr>
<td>PSS</td>
<td>Perceived stress scale</td>
</tr>
<tr>
<td>PTc</td>
<td>Pain thresholds chest wall</td>
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<td>PTo</td>
<td>Pain thresholds oesophagus</td>
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<td>PTp</td>
<td>Pain thresholds pharynx</td>
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<td>QRN</td>
<td>Glutamine-arginine-asparagine</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
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</tr>
<tr>
<td>RAR</td>
<td>Rapidly adapting receptors</td>
</tr>
<tr>
<td>RVM</td>
<td>Rostro-ventral medulla</td>
</tr>
<tr>
<td>SAR</td>
<td>Slowly adapting receptors</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>SNOT</td>
<td>Sino-nasal outcome test</td>
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<td>SP</td>
<td>Substance P</td>
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<td>STAI</td>
<td>State trait anxiety index</td>
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<td>TrkA/B</td>
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<td>TRPA1</td>
<td>Transient receptor potential cation channel member A1</td>
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<td>TRPM8</td>
<td>Transient receptor potential cation channel member M8</td>
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<td>TRPV1</td>
<td>Transient receptor potential cation channel member 1</td>
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<td>TTXr</td>
<td>Tetrodotoxin-resistant sodium channel</td>
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<tr>
<td>UNDW</td>
<td>Ultra-sonically nebulised distilled water</td>
</tr>
<tr>
<td>UOS</td>
<td>Upper oesophageal sphincter</td>
</tr>
<tr>
<td>Utc</td>
<td>Urge-to-cough</td>
</tr>
</tbody>
</table>
Acknowledgements

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The Author

I graduated with an MBChB from the University of Manchester in 2003. After completing 1 year as a Pre-Registration House Officer and a further 2 years as a Senior House Officer in General Medicine, and passing Membership of the Royal College of Physicians (2006), I commenced specialist training as a Respiratory Physician (2007). I was awarded an MRC Clinical Research Training Fellowship to complete the majority of this thesis (2009).
Introduction
1 Background

Cough is the most common presenting symptom at primary care consultation and an estimated 4 billion U.S dollars per year is spent world-wide on cough remedies[1]. Despite this, “over-the-counter” cough medications are no more effective than placebo[2], and there is a lack of effective cough suppressants. Furthermore, coughing can substantially reduce health-related quality of life[3]. For example, patients report urinary incontinence, chest pain, syncope, hoarse voice, musculo-skeletal chest pain, depression, anxiety and social embarrassment (see table 1 and appendix 1.1).

Although cough has been extensively studied in animal models, very little of this knowledge has been translated to humans[4]. This thesis aims to better understand the neurophysiology of cough in humans.

Table 1- Impact of chronic cough on quality of life

<table>
<thead>
<tr>
<th>Health-related quality of life</th>
<th>Symptoms</th>
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<tbody>
<tr>
<td>Physical</td>
<td>Retching/vomiting</td>
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<tr>
<td></td>
<td>Syncope</td>
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<td></td>
<td>Urinary incontinence</td>
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<td>Chest pains</td>
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<td>Herniae</td>
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<td>Hoarse voice</td>
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<td>Exhaustion</td>
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<td>Headaches</td>
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<td>Psychological</td>
<td>Depression</td>
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<td>Embarrassment</td>
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<tr>
<td>Social</td>
<td>Social withdrawal</td>
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<tr>
<td></td>
<td>Loss of employment</td>
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<tr>
<td></td>
<td>Break-down of relationships</td>
</tr>
<tr>
<td></td>
<td>Isolation</td>
</tr>
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<td></td>
<td>Frequent medical consultations</td>
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</tbody>
</table>

1.1.1 Defining cough

Cough is commonly defined as an explosive sound[5]. This characteristic sound is generated by forced expiration against a closed glottis, and is followed by opening of the glottis to allow high velocity expiration that removes aspirated material or retained secretions from the lungs. Chronic cough is defined by the British Thoracic Society (BTS) as any cough lasting longer than 8 weeks[6].
1.1.2 Epidemiology of cough

In the large European Community Respiratory Health Survey (ECRHS), cough prevalence varied by region[7]. For example, the prevalence of dry cough ranged from 5.5% in Göteborg, Sweden to 16% in Bordeaux, France. The prevalence of productive cough ranged from 3.8% in Cambridge, UK to 27.4% in Dublin, Ireland. In a postal questionnaire survey conducted in Leeds and Bradford (UK), 7% of primary care patients aged 40-49 years reported cough at least weekly or daily in the previous 2 months that was interfering with activities of daily living[8].

Epidemiological studies have additionally highlighted gender differences in cough. The Tucson Children’s Respiratory Study found that although males were more likely to develop cough in childhood, the reverse was true from the age of 16 years onwards[9]. No studies have specifically investigated the role of puberty. In the ECRHS, nocturnal and dry cough related significantly to female gender[7]. The French Epidemiological Study on the Genetics and Environment of Asthma (EGEA) also found that nocturnal cough was more prevalent in females when compared to males[7].

1.1.3 Clinical management of chronic cough

The BTS recommend a systematic approach to identify underlying causes of chronic cough[10]. Providing a chest radiograph and lung function tests are normal, the commonest underlying triggers to be investigated and treated include asthma syndromes (classical asthma, cough-variant asthma or eosinophilic bronchitis), gastro-oesophageal reflux disease and rhinitis[3, 10]. This pathological triad was first described by Irwin in 1981, with a reported 98% of patients responding to targeted treatment of underlying afferent cough triggers[11]. However, high treatment success rates have not been replicated in recent studies. Indeed, up to 40 % of chronic cough patients attending a specialist clinic have no identifiable triggers or else do not respond to treatment[12], and are therefore diagnosed as idiopathic or treatment-resistant chronic cough.

1.1.4 Measuring cough

Cough-related quality of life can be measured using validated questionnaires including the Leicester Cough Questionnaire (LCQ)[13] and the Cough-Specific Quality of Life Questionnaire (CQLQ)[14]. These questionnaires are validated
research tools designed to capture the effects of cough symptoms on the physical,
psychological and social aspects of a patient’s life.

A more objective measure of cough severity is 24 hour cough frequency[5, 15, 16].
An ambulatory cough monitor is worn by the patient for 24 hours in their own home or
work environment. A microphone records all sound which can later be manually
analysed for the number of explosive cough sounds. High cough frequency
correlates moderately with a worse cough-related quality of life. This device does not
measure cough intensity which may be of additional importance to patients.

Cough sensitivity is measured experimentally using inhaled tussive agents e.g.
capsaicin and citric acid. The dose of tussive agent inducing at least 2 or 5 coughs is
known as the C2/C5. These end-points are reproducible to within 2 doubling doses
over the short term (14 days) and long term (6 months), but are not used clinically
because they correlate only moderately with 24 hour cough frequency[15], and do
not clearly differentiate healthy subjects from patients with chronic cough[17].
2 Current understanding of the neurophysiology of cough

Current understanding of the neurophysiology of cough is largely derived from animal studies, with surprisingly limited knowledge in humans. Coughing is elicited by noxious mechanical or chemical stimuli activating pulmonary vagal afferent fibres that terminate in the nucleus tractus solitarius (nTS) in the brainstem[18]. Relay neurones then project to a nearby ‘central cough generator’ where efferent nerves innervating the glottis, diaphragm and inter-costal muscles generate coughing. An additional complexity that cannot be explored in animals is the perceived urge-to-cough; an unpleasant sensation preceding the motor cough response.

Figure 1 – Neurophysiology of cough

Inhaled airway irritants activate vagal afferent fibres, which synapse in the brainstem and project ascending cortical pathways to generate an urge-to-cough sensation and motivate a motor cough response.


2.1 Insights from animal studies

Based on animal studies, conflicting evidence exists for the relative importance of different vagal afferent nerve fibres, made more complex by inter-species differences and the presence or absence of anaesthesia during experiments. Nevertheless, several review articles summarise key characteristics of vagal afferent fibres including rapidly-adapting receptors (RARs), slowly adapting receptors (SARs), c-fibres and putative cough receptors, all of which are likely to mediate cough to some extent[18-20].

2.1.1 Rapidly Adapting Receptors

RAR fibres are intra-pulmonary myelinated fibres arising from nodose ganglia in guinea-pigs. They are activated by mechanical stimuli such as lung collapse, deflation or bronchospasm, but not chemical irritants. However, chemical irritants can activate RAR fibres indirectly by causing broncho-constriction. RAR fibres stimulate parasympathetic output to the airways resulting in reflex bronchospasm and tachyopnea. Whether RAR fibres play a key role in the regulation of cough is now debatable. For example, histamine and methacholine are very robust RAR stimulants, but are only modestly effective at inducing cough. Furthermore, RAR fibres show cyclical activity during respiration, and yet cough is only induced in select circumstances[18].

2.1.2 Slowly Adapting Receptors

SAR fibres are also intra-pulmonary myelinated fibres arising from nodose ganglia in guinea-pigs. They are responsible for the Hering-Breuer Reflex, terminating inspiration and initiating expiration when the lungs are adequately inflated. In contrast to RAR fibres, SAR fibres inhibit parasympathetic output to the airways resulting in reflex bronchodilatation.

Evidence suggesting SAR fibres may play a role in the regulation of cough arises from studies in rabbits showing that inhaled sulphur dioxide, which selectively blocks SAR fibres, reduces the sensitivity and strength of cough evoked by mechanical and chemical stimulation of the tracheo-bronchial or laryngeal mucosa. However, consistent results were not obtained in cats and dogs. Furthermore, activation of
SAR fibres by maintaining continuous positive airway pressure in anaesthetised cats and dogs strengthened the expiration reflex but not cough[21].

2.1.3 Vagal C-fibres

Unmyelinated vagal c-fibres respond to a wide-range of chemical stimuli, but under normal circumstances have a high threshold for activation. They typically express the ligand-gated ion channel Transient Receptor Potential Vanilloid 1 (TRPV1)[22], which is activated by capsaicin (the pungent ingredient in chilli), acid and heat[23]. Inhaled capsaicin reproducibly induces cough in conscious animals and humans[24]. Given that capsaicin is known to be a potent TRPV1 agonist, and that TRPV1 receptors are located on c-fibre terminals, this provides evidence for a role of c-fibres in the regulation of cough.

There are two distinct types of vagal c-fibres innervating the respiratory tract; pulmonary and bronchial c-fibres[25]. In guinea-pigs, pulmonary c-fibres innervate the lungs, receive their main blood supply from the pulmonary circulation and are derived from nodose and jugular ganglia. In contrast, bronchial c-fibres innervate the main conducting airways, receive their main blood supply from the bronchial circulation and are predominantly derived from jugular ganglia. Both types of C-fibres respond directly to capsaicin and bradykinin. However, only the nodose derived pulmonary c-fibres are activated by ATP, adenosine and serotonin. Jugular-derived pulmonary c-fibres are more likely to express neuropeptides[25]. Activation of proximal airway c-fibres may mediate the “urge-to-cough” sensation[26], and sensitise cough. Conversely, activation of intra-pulmonary c-fibres has an acute inhibitory effect on cough in animals[27].

There are some differences in the physiological properties of vagal c-fibres between animals and humans. Guinea pig vagal c-fibres synthesise neuropeptides peripherally, including substance P (SP) and calcitonin-gene related peptide (CGRP), leading to atropine-insensitive bronchospasm, mucus secretion and neurogenic inflammation. This is known as the “axon-reflex”[28]. The axon-reflex appears to be absent in humans, but in all species a centrally mediated reflex parasympathetic activation following c-fibre stimulation results in bronchoconstriction and a period of apnoea[19].
Spinal afferent c-fibres have similar properties to jugular-derived c-fibres, but their role in regulation of coughing is unknown[29]. Spinal c-fibres from the lower airways converge with cardiac spinal afferent nerves on the same second order neurones in the dorsal horn of the thoracic spinal cord. This central convergence of spinal afferents may be responsible for cardiopulmonary reflexes elicited by bronchopulmonary c-fibre stimulation[30].

2.1.4 Cough Receptor

Canning et al[31] observed that citric acid applied directly to the tracheal mucosa of anaesthetised guinea pigs evoked cough even after capsaicin desensitisation or in the presence of topical capsazepine (a TRPV1 receptor antagonist), indicating a mechanism independent of C-fibre stimulation. To reconcile this and the conflicting evidence both for and against a role for RAR and C-fibres in mediating cough, the existence of a putative cough receptor was proposed[32]. Using extra-cellular action potential recordings, isolated nodose-derived extrapulmonary fibres were easily distinguished from RAR and SARs by a slower conduction velocity, lack of response to tracheal distension, stretch or bronchospasm and insensitivity to ATP. In contrast to c-fibres the neurones were highly sensitive to punctuate mechanical stimuli and acid but were not activated directly or indirectly by capsaicin or bradykinin. It is uncertain whether these cough receptors exist in species other than guinea-pigs[20].

The central termination site of cough receptors has recently been characterised using novel micro-injection techniques and retrograde labelling in anaesthetised guinea-pigs[33]. Micro-injection of glutamate antagonists (nonNMDA and NMDA) into a discrete area of the nTS (0.8mm lateral and ~0.8mm rostral to the obex), selectively abolished cough evoked from the larynx/trachea without affecting basal respiratory rate. Using retrograde tracers (DiI and fast blue), this nTS area was confirmed as the likely primary relay site for cough receptors. Injection of DiI into the discrete location of the nTS regulating cough, and simultaneous injection of fast blue into the tracheal mucosa, co-labelled a sub-set of nodose ganglia-derived neurons.

2.1.5 Good cough and bad cough?

It has been proposed that there are 2 distinct vagal afferent pathways mediating cough[34]. Nodose-derived extra-pulmonary Aδ-fibres, or cough receptors, are likely to act as the first line of airway defence (good cough) because, regardless of the
conscious state of the animal, they are robustly activated by acid/punctuate stimuli. In contrast, C-fibres are recruited mainly under conditions of airways inflammation to enhance cough sensitivity, and therefore may be responsible for excessive coughing associated with respiratory disease (bad cough). Selective pharmacological targeting of c-fibre mediated cough e.g. via TRPV1 receptor antagonism would therefore be predicted to abolish the abnormal c-fibre mediated cough whilst preserving protective Aδ-mediated cough.

This attractive viewpoint may be somewhat over-simplified, and in reality a complex interaction between vagal afferent fibres is likely to occur. For example, just like Aδ-fibres, c-fibres are sensitive to acid and may therefore play an additional protective role against aspiration[35]. Furthermore, after exposure to neurotrophic factors released during allergic or viral induced inflammation, Aδ-fibres undergo a phenotypic switch leading to novel expression of TRPV1[36]. Although the functional significance of these phenotypic changes is unknown, it is possible that in these circumstances Aδ-fibres become up-regulated by inflammation in a manner similar to c-fibres. Neural plasticity of vagal afferents in airway disease is discussed further in section 3.3.2.

2.2 Insights from human studies

Regardless of the debate surrounding afferent cough pathways in animals, clinically there appear to be 2 types of cough. First is a violent cough response to noxious airway stimulation e.g. aspiration, which is immediate and impossible to control, best described as a “reflex” cough. Second is a “sensory-driven” cough whereby inhaled irritants generate a graded urge-to-cough sensation which motivates cough, but can be consciously controlled[37]. Indeed, two thirds of chronic cough patients describe a sensation of an irritation/tickle in the throat prior to coughing[38]. In a recent review article, it was suggested that reflex cough and sensory-driven cough may form a continuous spectrum of coughing, ranging from mild sensations under complete voluntary control to intense sensations that are impossible to control, see appendix 1.2[37].

2.2.1 Measurements of cough sensitivity

Measures of cough sensitivity have proved to be useful research tools. The dose of inhaled capsaicin or citric acid inducing at least 2/5 coughs within 15 seconds of inhalation (C2/C5) is a reproducible end-point in health and disease[24], and shows
moderate correlation with cough frequency and quality of life questionnaires in chronic cough patients[15, 16]. Patients with cough-variant asthma, gastro-oesophageal reflux disease and idiopathic chronic cough have heightened cough sensitivity to inhaled capsaicin compared to healthy controls[17], which is reported to decrease after successful treatment of the cough[39]. However, there is considerable overlap of C2/C5 in healthy subjects and patients with respiratory disease. This could hint at the importance of other undiscovered patho-physiological mechanisms.

Ultrasonically-nebulised distilled water (UNDW or fog) can also be used to evoke cough, either as a single dose or as gradually increasing outputs. Using this method 15–20% of subjects may not cough even at maximal attainable fog output, although capsaicin and citric acid will induce cough in the same subjects[24].

Several factors have been reported to influence cough sensitivity. For example, cough sensitivity is increased in females compared to males[40], and is temporarily sensitised during viral upper respiratory tract infection[41]. In contrast, cough sensitivity is down-regulated by regular tobacco smoking[24]. The molecular mechanisms responsible for the latter observation are unknown. Ethnicity has no significant effect on cough sensitivity[24].

2.2.2 Mechanistic insights from measurement of cough sensitivity

Higenbottam et. al. demonstrated a lack of cough response during inhalation of UNDW in heart-lung transplant patients. Such patients are completely deinnervated below the level of the tracheal anastamosis. Therefore, this indicates that cough in humans might be dependent on airway (not laryngeal) afferent nerves[42]. Furthermore, cough appears to be mediated by vagal rather than spinal afferent nerves. Compared to healthy controls, patients with low cervical spine injury have preserved capsaicin cough thresholds[43].

Afferent fibres activated by UNDW that are responsible for evoking cough are restricted to large airways[44]. In contrast, capsaicin evokes cough when nebulised into the large or small airways[44]. This provides clues that distinct afferents are activated by different types of stimuli. Based on animal studies it is likely that capsaicin, a selective TRPV1 agonist, stimulates c-fibres[45]. Given that bradykinin may sensitise TRPV1 receptors[23] and accumulates in the presence of increased
angiotensin-converting enzyme (ACE)[46], it is interesting that patients taking ACE inhibitors have enhanced cough sensitivity to capsaicin, but not to UNDW[44].

A study by Lavorini et al.[47] sought to clarify the precise vagal afferent nerve mediating experimentally evoked coughing in humans. Given that reflex respiratory responses elicited by RAR and c-fibre stimulation include hyperpnoea and rapid shallow breathing respectively, the authors proposed that monitoring breathing pattern following capsaicin or fog inhalation should provide insights into the afferent fibre stimulated. At threshold doses, both capsaicin and fog increased inspiratory minute ventilation and inspiratory drive in keeping with direct or indirect RAR activation.

2.2.3 Bronchial tone and cough

Cough is a common symptom in patients with obstructive lung disease and so it may be hypothesised that bronchial constriction would increase cough sensitivity, particularly since RARs are normally activated in these circumstances. In fact, airway responsiveness and cough sensitivity are likely to operate by entirely different neural pathways.

Doherty et al. showed that chronic asthmatics and COPD patients not necessarily complaining of cough had lower capsaicin cough thresholds than healthy controls[48]. However, in asthmatics, there was no significant correlation between baseline percent predicted FEV1, baseline PD20 to histamine or PEFR variability and capsaicin cough response. Furthermore, median C5 was unchanged after histamine challenge despite a mean reduction in FEV1 of 0.6L[48]. Similarly, Fujimura et al showed no significant correlation between PD20 and cough response to inhaled capsaicin in asthmatics and patients with sino-bronchial syndrome[49]. In mild-moderate asthmatics, objective 24 hour spontaneous cough frequency bears no significant correlation with FEV1[50]. In healthy volunteers, bronchial dilatation does not affect measured C5 to capsaicin[51], but does reduce cough frequency to UNDW[52].

2.2.4 Cortical control of cough

A major limitation of animal experiments is that the cortical control of coughing cannot be studied. Consequently little is known about important cortical areas
influencing cough. In humans, conscious suppression of cough is possible unless the urge-to-cough becomes irresistible. Conversely a cough can be psychogenic, for example a “habit cough”, which is most commonly diagnosed in children[53]. It is therefore highly likely that cough can be modified by descending cortical pathways[54]. In cerebral disease, these descending cortical pathways can be disturbed. For example, right handed patients with left sided middle cerebral artery stroke demonstrate weak or absent voluntary cough despite an intact afferent cough response to nebulised tartaric acid[55].

Cough can be attenuated during anaesthesia and sleep. Cough frequency is consistently reduced during the night compared to the daytime[56] and cough responses of enflurane-anaesthetised subjects to tracheal irritation are suppressed[57]. The effect of sleep or anaesthesia on cough could be caused by reduced conscious perception of the urge-to-cough or by activation of endogenous inhibitory mechanisms. In rats, endogenous inhibitory mechanisms are more active during sleep[58].

2.3 The Urge-to-cough

The urge-to-cough is an unpleasant sensation of airway irritation perceived prior to coughing that can be rated on a modified Borg scale following inhalation of tussive agent. The magnitude of urge-to-cough sensation increases dose-dependently during capsaicin challenge, always precedes cough and shows linear correlation with cough intensity measured by cough frequency, airflow pattern and expiratory muscle EMG activity[59].

Functional Magnetic Resonance Imaging (fMRI) during inhalation of sub-threshold doses of capsaicin provided insight into which areas of the cortex are activated during urge-to-cough sensations. Mazzone et.al.[60] found a significant correlation between magnitude of signal change and magnitude of urge-to-cough in the right primary somato-sensory cortex, corresponding to an area receiving sensory information from the throat. A significant correlation was also observed in the anterior mid-cingulate cortex and supplementary motor area, both of which may be associated with cough inhibition. However, capsaicin has a large number of other side-effects including oral burning and irritation of the eyes which could explain the wide-spread cortical activation observed. A more carefully controlled functional MRI study of low-intensity urge-to-cough evoked by citric acid inhalation performed by
Smith et al. demonstrated bilateral activation in a discrete area of the somatosensory cortex (unpublished).

Compared to males, females demonstrate a steeper log-log relationship between citric acid dose and reported urge-to-cough magnitude, indicating a sensitisation of afferent pathways[61]. This could explain the predominance of females with chronic cough, but the mechanisms responsible are unknown.

The urge-to-cough may also be up-regulated during viral upper respiratory tract infection (URT). Dicpinigaitis et al. performed serial capsaicin cough challenges on healthy subjects during and after a viral URTI[62]. During viral infection, both urge-to-cough threshold and cough threshold were significantly reduced compared to 4-8 weeks after recovery. The difference between capsaicin cough threshold (dose inducing at least 1 cough, C1) and urge-to-cough threshold was also calculated (CΔ), and was found to be similar during and after viral infection. Taken together, these findings suggest that up-regulation of cough occurs via afferent pathways, and that an increase in urge-to-cough sensation drives a proportional increase in motor cough response. The relationship between the urge-to-cough and motor cough response has not been studied in chronic cough patients.

In contrast, the urge-to-cough is down-regulated in elderly patients with cerebral vascular disease and recent history of aspiration pneumonia[63]. In 8 patients with previous aspiration pneumonia, urge-to-cough intensity rated at 2 doubling doses below citric acid induced C2 and C5 was significantly lower compared to age-matched healthy controls. Smokers also demonstrate lower citric acid induced C2/C5 and a less steep log-log relationship between citric acid dose and urge-to-cough intensity compared to non-smokers, indicating a desensitisation of afferent cough pathways[64].
3 Sensitisation of cough and pain: similar underlying mechanisms?

The neural mechanisms responsible for sensitisation of cough in patients with chronic cough are poorly understood, but similarities to the mechanisms underlying the development of chronic pain are proposed.

3.1 Sensitisation of pain

Under normal conditions, a transient noxious stimulus, such as a pin-prick, activates peripheral nociceptors (c-fibres and Aδ-fibres), which synapse in the dorsal horn of the spinal cord and evoke immediate reflex withdrawal[65]. In addition, dorsal horn neurons decussate and project ascending cortical pathways, to generate an unpleasant “pain” sensation to warn the individual of impending harm[66].

Sensitisation of pain can occur after prolonged or intense noxious stimuli e.g. a burn injury to the hand, and is characterized by lowered pain thresholds and increased magnitude of pain[66]. This sensitisation occurs both at and surrounding the injury site, and serves to prevent further harm, to allow time for healing. Importantly, sensitisation will normally be reversible, but if it persists, even after any tissue injury has resolved, on-going pain hypersensitivity can become pathological.

Although pain hypersensitivity is partly generated by lowered thresholds for activation in peripheral nerves, known as peripheral sensitisation[65], it is predominantly caused by changes that manifest within the central nervous system (CNS), known as central sensitisation (CS)[67]. Indeed, changes within the CNS are fundamental to the development of pain hypersensitivity. Once CS has been established, the normal relationship between stimulus and pain is disturbed, and pain is reported out of proportion to tissue injury. Therefore, although pain may appear to arise from the periphery, it is actually generated by an increased gain within the CNS.

3.2 Sensitisation of cough

Just like pain, cough can also become sensitised e.g. following a viral infection, and is characterized by lowered cough thresholds[17, 41, 62] and increased magnitude of cough frequency[68]. A temporary sensitisation of cough should be reversible once the viral infection has resolved. On the contrary, in chronic cough patients, cough sensitisation appears to persist and therefore becomes pathological. In some
chronic cough patients, excessive cough appears to arise spontaneously without any history of a sensitising event.

There is evidence that cough hypersensitivity could be generated by lowered thresholds for activation in vagal nerves (peripheral sensitisation)[69]. However, a growing body of evidence now also favours a role for central sensitisation (CS), discussed below. As with pain, once CS is established, the normal relationship between stimulus and cough is disturbed, and cough is disproportionate to the degree of airways disease. Therefore, although the cough may appear to arise from the airways in chronic cough patients, it is actually generated by an increased gain within the CNS.

The underlying mechanisms proposed for peripheral and central sensitisation of pain are discussed, and the evidence supporting a role for these mechanisms in relation to cough is highlighted.

3.3 Peripheral sensitisation

3.3.1 Peripheral sensitisation in pain

Transducer receptor/ion channel complexes located at peripheral nociceptor terminals convert noxious thermal, mechanical or chemical stimuli into an action potential signal[70]. Inflammatory mediators (prostaglandin E2, serotonin, bradykinin, epinephrine, adenosine) and neurotrophic factors released during tissue damage or by inflammatory cells activate various intra-cellular transduction pathways, ultimately leading to phosphorylation of peripheral transducer receptors/ion channels. This increases the excitability of the nociceptor terminal membrane reducing the amount of depolarisation required to initiate an action potential, and is partly responsible for hyperalgesia at the injury site (primary hyperalgesia), known as peripheral sensitisation[70], see Figure 2.

3.3.2 Peripheral sensitisation in cough

Analogous to the phenomenon of peripheral sensitisation in somatic tissue, airway inflammation leads to increased excitability of vagal nerve terminals such that previously sub-threshold stimuli become threshold for action potential generation[71].
Exposure of vagal afferent nerves to sensitising inflammatory agents is able to lower the mechanical threshold for inducing cough. Ricco et al. isolated trachea preparations from immunised guinea-pigs to quantify neural activity before and after antigen challenge. With electrophysiological recording techniques, they demonstrated no evidence of direct vagal nerve activation after antigen exposure, but a 4 fold decrease in the mechanical threshold for action potential generation, thus indicating sensitisation of an unidentified airway mechanical transducer[72]. Another in vivo experiment, using an isolated innervated tracheal preparation from a guinea pig, demonstrated a lowered mechanical stimulation threshold of airway jugular-derived Aδ-fibres following exposure to prostaglandin E2[69].

Cough may also be sensitised by the TRPV1 receptor present on vagal c-fibres. TRPV1 is a ligand-gated, heat sensitive, cation channel directly activated by capsaicin. The TRPV1 pore is open at temperatures >42°C under normal conditions. This threshold is reduced to 30°C when tissue pH is low, as would occur in airways inflammation. Furthermore, endogenous inflammatory mediators such as bradykinin and prostaglandin E2 activate G-protein-coupled receptors and subsequently directly or indirectly sensitise TRPV1[23].

Autacoids such as prostaglandin E2, bradykinin and histamine may additionally modulate neuronal excitability by altering current flow through ion channels. One example of this is the tetrodotoxin-resistant (TTXr) sodium channel. Studies on guinea-pigs, using local anaesthetic to block TTXr selectively, suggest these currents contribute to overall excitability of jugular-derived vagal afferent neurones[73].
Figure 2 – Transduction and peripheral sensitisation

A

Transducer channels

Voltage-gated channels

Nav1.7, 1.8 & 1.9

Nav1.6, 1.7 & 1.8

TRPV1-4

TRPM8

TRPA1

ASICs

P2X3

Transducer

Potential

Generator

Potential

Action potential

TRANSUCTION

B

Sensitisers

Receptors

Signal transduction

Bradykinin

PGE2

NGF

H+

ATP

B1/B2

EP

TrkA

ASIC

PKC

PKA

PI3K

ERK

pTRPV1

TRPV1

Reduced

threshold

Increased

Excitability

PERIPHERAL SENSITISATION

Modified from Woolf et al. 2007[65].

A. Transduction. Noxious stimuli activate various transducers located on the peripheral nerve terminal (TRPV1-4, TRPM8, TRPA1, ASICs, P2X3), which depolarize the cell. Nearby voltage-gated sodium channels (Nav1.7, 1.8 & 1.9) are subsequently activated, and may generate an action potential. B. Peripheral sensitization. Inflammatory mediators (bradykinin, PGE2, NGF, H+, ATP) activate their corresponding receptors on the nerve terminal (B1/B2, EP, TrkA, ASIC, P2Y) thereby activating intra-cellular pathways leading to phosphorylation and trafficking of TRPV1 receptors, and phosphorylation of voltage-gated sodium channels, increasing the sensitivity of the nerve terminal.
3.4 Central sensitisation

3.4.1 Central sensitisation (CS) of pain

The hallmark features of CS to pain were first described in rats[74]. Mustard oil was applied to the rat’s hind paw to activate c-fibres, and intracellular recordings of dorsal horn neurons were recorded at baseline and in response to noxious/innocuous stimulation of the hind limb, both within and outside of the normal receptive fields. Several changes in the dorsal horn neurons were noted after mustard oil application including spontaneous action potential discharge, increased action potential firing frequency in response to noxious stimuli, lowered thresholds for action potential initiation and a widening of receptive fields. These neurophysiological observations corresponded remarkably well with the clinical features of patients with chronic/neuropathic pain i.e. spontaneous pain, increased pain in response to painful stimuli (hyperalgesia), pain in response to innocuous mechanical stimuli (allodynia) and dispersed pain at sites distant from the original injury. Therefore it was proposed that these findings could be directly relevant to the development of chronic pain in humans[75].

The underlying molecular mechanisms responsible for central neuronal hyperexcitability of pain pathways have now been extensively studied. In particular, the induction and maintenance of CS is dependent on the N-Methyl-D-Aspartate (NMDA) receptor [66].

3.4.2 The role of the NMDA receptor in central sensitisation of pain

The NMDA receptor is a calcium permeable ligand-gated ion channel, activated by glutamate and located throughout the CNS[76]. It is a tetramer containing two obligatory NR1 subunits, and two modulatory NR2/3 subunits (NR2A, NR2B, NR2C, NR2D, NR3A or NR3B). NMDA receptors have a large extracellular N-terminal region, 3-transmembrane domains (M1, 3 and 4) and a pore-forming domain (M2) containing the QRN site, which is critical for calcium permeability. M3 and M4 share a large extracellular loop which, together with the N-terminal, forms the agonist binding domain[76].

In the dorsal horn, the most common NMDA complex is NR1-NR2A/B, located on the post-synaptic cell[66]. At resting membrane potential, the NMDA receptor holds a voltage gated magnesium block preventing channel opening. Transient pain e.g. pin-
prick briefly activates c-fibres, thereby signalling the central release of the excitatory neurotransmitter glutamate, which only activates ligand-gated AMPA and kainite receptors, see Figure 3.

Repetitive, low frequency, noxious c-fibre stimulation leads to the central release of glutamate and neuropeptides e.g. substance P (SP). SP binds to post-synaptic neurokinin receptors (NK1) to produce a cumulative depolarisation which is sufficient to remove the voltage-gated magnesium block on the NDMA receptor, allowing the channel to open[67]. This causes a gradual “wind-up” of action potential discharge in the central neurones with each successive stimulus, see Figure 4. The clinical correlate of wind-up is temporal summation characterised by an increase in reported pain intensity to each successive stimulation, and is also mediated by the NMDA receptor[77]. This is a form of activity-dependent post-synaptic hyper-excitability lasting several tens of seconds and which is fully reversible. Wind-up is believed to be a pre-cursor to the development of CS.

Intense or prolonged noxious stimulation e.g. intra-dermal capsaicin injection or burn injury evokes a barrage of c-fibre activation. This can lead to a longer-lasting central neuronal hyper-excitability, known as central sensitisation (CS), see Figure 5. As for wind-up, sustained post-synaptic depolarisation removes the voltage-gated magnesium block on the NMDA receptor, allowing the cell to become permeable to calcium. Raised intra-cellular calcium is a key trigger for various intra-cellular pathways, ultimately leading to phosphorylation of the NMDA receptor and AMPA receptor, as well as increased trafficking of AMPA receptors to the cell surface[66]. NR1 phosphorylation by protein kinase A (PKA) or protein kinase C (PKC) increases the response of NMDA receptors to glutamate. NR2 phosphorylation increases channel opening and prevents endocytosis. PKC reduces the magnesium block on NMDA receptors, and increases the probability of channel opening. This all has the net effect of increasing synaptic strength[66].

An increase in the excitability of one synapse may spread to adjacent synapses. Afferent nerves, including c-fibres and Aβ-fibres, convey sensory information from the injury site and surrounding area and converge on the same centrally sensitised neurone[78]. This leads to hyperalgesia and allodynia surrounding the injury site (secondary hyperalgesia/allodynia), see Figure 5.
Although CS can persist for several hours, it is normally fully reversible. Persistent tissue inflammation or nerve injury may lead to the development of a more permanent state of central up-regulation, mediated by transcriptional changes in the dorsal horn[79]. The expression of some proteins is increased e.g. c-Fos, NK1, TrkB and COX2[66]. In other cases, novel genes are expressed. For example, Aβ-fibres (myelinated somatic mechanoreceptors) undergo a phenotypic switch and begin to express substance P and BDNF, therefore becoming capable of eliciting pain or even generating central sensitisation[67].
Synapse in the dorsal horn of the spinal cord. Brief noxious stimulation e.g. pin-prick activates c-fibres leading to the central release of excitatory neurotransmitter, glutamate (blue circles). Glutamate binds to post-synaptic AMPA and NMDA receptors. However, only AMPA receptors are activated. At resting membrane potential, NMDA receptors hold a voltage-gated magnesium (Mg) block preventing ion channel opening.
Figure 4  Wind-up

Repetitive c-fibre activation leads to the central release of glutamate (blue circles) and neuropeptides e.g. substance P (yellow circles). Substance P binds to post-synaptic NK1 receptors, to produce cumulative membrane depolarisation. The magnesium block on the NDMA receptor is released, and the channel opens. This activity-dependent increase in post-synaptic excitability is short-lasting (tens of seconds) and fully reversible, known as wind-up or temporal summation.
Figure 5 – Central sensitisation

Intense and sustained noxious stimuli e.g. intra-dermal capsaicin injection, evokes a barrage of c-fibre activation. This leads to a longer-lasting central neuronal hyperexcitability, known as central sensitisation. One mechanism inducing central sensitisation involves the NMDA receptor. Sustained post-synaptic depolarisation relieves the magnesium block (as for wind-up) allowing an influx of calcium into the post-synaptic cell. Calcium triggers several key intra-cellular pathways which lead to (i) the phosphorylation of NMDA receptors and AMPA receptors (P), increasing receptor activity and (ii) trafficking of AMPA receptors to the cell surface and finally (iii) transcriptional changes. This contributes to the longer-lasting maintenance of central sensitisation. Spread of hyper-excitability in the post-synaptic cell to adjacent synapses is responsible for the development of hyperalgesia/allodynia surrounding the injury site, known as secondary hyperalgesia/allodynia.
3.4.3  **NMDA-receptor mediated CS in healthy volunteer experimental pain models**

Healthy volunteer models of CS facilitate the testing of anti-hyperalgesic drugs. These models were developed based on the evidence that secondary hyperalgesia (increased pain surrounding the injury site) is a centrally mediated phenomenon. For example, intra-dermal capsaicin produces a large area of secondary hyperalgesia around the injection site [80, 81]. Other methods for inducing secondary hyperalgesia include topical capsaicin [82], burn injury [83], trans-cutaneous electrical stimulation [84] and acid infusion into the oesophagus [85]. In all these models, the NMDA receptor antagonist, ketamine, reduces the area of secondary hyperalgesia, confirming that CS of somatic and visceral pain is NMDA receptor mediated in humans.

3.4.4  **Evidence that NMDA receptors mediate cough centrally**

Several animal studies provide evidence that NMDA receptors in the nTS mediate the cough reflex. Injection of NMDA receptor antagonist into a discrete location in the nTS of anaesthetised spontaneously breathing rabbits abolished cough evoked by mechanical stimulation of the main carina [86]. In anaesthetised guinea-pigs, micro-injection of NMDA receptor antagonist into a discrete region of the nTS where cough receptors terminate abolished cough evoked from the trachea [87]. Memantine, a use-dependent low-affinity NMDA receptor antagonist, almost completely abolished cough evoked by citric acid in a conscious guinea-pig model, see appendix 1.3.

3.4.5  **Evidence for central sensitisation of cough**

Mazzone and Canning proposed that central sensitisation of the cough reflex could be operating in the brainstem [88]. They investigated the synergistic interaction of vagal afferents in the regulation of the cough in anaesthetised guinea pigs [89]. Direct administration of capsaicin or bradykinin to the trachea of anaesthetised guinea-pigs did not directly evoke cough, but reduced the threshold for electrical stimulation of cough from the trachea. This was replicated by micro-injection of capsaicin or substance P into the nTS. This suggests that direct activation of airway c-fibres is insufficient to initiate cough in anaesthetised animals, but sensitises cough in the nTS in the brainstem. Subsequent experiments have shown that intra-oesophageal administration of capsaicin also sensitises coughing subsequently
evoked by electrical stimulation of the trachea, providing further evidence for central convergence of oesophageal and vagal afferent fibres, and a possible role for CS.

CS in the brainstem leading to a widening of receptive fields for evoking cough could also explain some of the clinical features of chronic cough. Extra-pulmonary disease, such as oesophageal reflux, is commonly implicated as an underlying cause or trigger of cough. However, in the most recent and rigorous studies, objective quantification of acid and non-acid reflux in chronic cough patients has shown that on average, the number of reflux events over 24 hours is within the normal range[90]. Careful analysis of these data has demonstrated that a proportion of patients (48%) have a statistically significant positive temporal association between distal reflux followed by cough. This suggests that physiological amounts of distal reflux may be directly triggering cough, and strengthens the evidence for a role of CS, with probable convergence of oesophageal and pulmonary vagal afferent fibres on centrally sensitised brainstem neurons [37, 91]. This will be further discussed in section 4.

3.4.6 Phenotypic switch of vagal afferent fibres

There is evidence that vagal fibres may, in select circumstances, undergo a phenotypic switch and express novel genes. Thus, previously silent afferent fibres may be additionally recruited to elicit cough. For example, under normal circumstances, substance P (SP) is almost exclusively produced by nociceptive non-myelinated vagal c-fibres. However, in a guinea-pig model of allergic inflammation, the proportion of myelinated nerve fibres expressing SP was greatly increased compared to control [92]. Neurotrophic factors (e.g. NGF) are capable of regulating mRNA expression encoding neurokinins, and could be responsible for these transcriptional changes if expression were up-regulated[93]. More recent work shows that nodose-derived Aδ-fibres develop a novel expression of TRPV1 following direct tracheal application of brain-derived neurotrophic factor (BDNF)[36].
4 Oesophageal hypersensitivity

As discussed above, in the presence of a sensitised cough reflex, distal oesophageal reflux may directly evoke cough[90]. The mechanisms responsible for oesophageal hypersensitivity to cough are unknown, but central convergence of pulmonary and oesophageal vagal afferent fibres on sensitised brainstem neurones is thought to be the most likely explanation[91, 94]. Limited evidence suggests that chronic cough patients do not also demonstrate oesophageal hypersensitivity to pain, because they do not tend to complain of typical reflux symptoms such as heartburn[91]. However, this has not been confirmed. Mechanisms responsible for oesophageal hypersensitivity to pain and cough are now discussed.

4.1 Oesophageal hypersensitivity to pain: evidence for central mechanisms

4.1.1 Neural afferent pathways

Oesophageal pain is predominantly mediated by spinal afferent fibres, but vagal afferent fibres may also contribute[95]. Spinal afferents are thought to encode the location and intensity of noxious stimuli in the oesophagus, but synapse centrally over large numbers of segments in the thoracic spinal cord[96, 97]. This extensive central convergence of visceral and somatic afferent fibres explains why oesophageal discomfort is poorly localised, and in many cases difficult to distinguish clinically from cardiac chest pain. Vagal afferent fibres most densely innervate the upper oesophagus[91, 98] and contribute to the autonomic sensations associated with visceral pain including nausea, fullness or bloating[96]. Noxious chemical stimuli, such as acid, may preferentially activate vagal rather than spinal afferents[98].

4.1.2 Oesophageal hypersensitivity to pain

The majority of patients with troublesome heartburn and/or regurgitation i.e. symptomatic gastro-oesophageal reflux disease (GORD) have no macroscopic evidence of oesophagitis[99]. This is known as non-erosive reflux disease or NERD. Approximately 20% of NERD patients have normal amounts of acid reflux on 24 hour pH testing, but demonstrate a positive temporal association between acid reflux episodes and symptoms[99], suggesting that in a proportion of NERD patients neuronal hypersensitivity of the oesophagus is a key pathogenic mechanism.
Several experimental studies confirm that NERD may have a specific patient subgroup with evidence of oesophageal hypersensitivity. NERD patients with normal 24 hour pH monitoring showed significantly lower pain thresholds to electrical stimulation of the distal oesophagus compared to NERD patients with abnormal 24 hour pH monitoring and healthy controls[100]. Furthermore, in some studies, NERD patients with normal 24 hour pH testing demonstrate mechanical hypersensitivity in the oesophagus[101, 102], although this was not confirmed by others[103].

Non-cardiac chest pain (NCCP) is diagnosed in patients with symptoms of chest pain in the absence of cardiac disease. Around 50% of such patients demonstrate lowered oesophageal pain thresholds compared to controls[104].

4.1.3 The role of central sensitisation in oesophageal pain hypersensitivity

To investigate whether central sensitisation (CS) is an underlying mechanism in the development of oesophageal hypersensitivity, a human model of CS of the oesophagus has been established. Secondary hyperalgesia, a centrally mediated clinical manifestation of CS, was induced in healthy volunteers by infusing high concentration acid into the distal oesophagus,[105] to reduce pain thresholds to electrical stimulation of the non-acid exposed proximal oesophagus for up to 5 hours, see Figure 6. The NMDA receptor antagonist, ketamine, intravenously administered before and after acid infusion attenuated and reversed the development of acid-induced secondary hyperalgesia in the proximal oesophagus compared to placebo[85]. Therefore, the NMDA receptor appears to be crucial in the induction and maintenance of CS of the oesophagus in this model.

In NCCP patients with no evidence of gastro-oesophageal reflux disease (GORD)[105], the effect of distal oesophageal acid on proximal oesophageal pain thresholds was even more exaggerated. In contrast, NCCP patients with confirmed GORD (by 24 hour pH studies) did not show a significant reduction in proximal oesophageal pain thresholds following distal acid infusion in this model, although baseline pain thresholds were lower than controls indicating resting oesophageal pain hypersensitivity[106]. Secondary allodynia was demonstrated after treatment for 6 weeks with 20 mg bd omeprazole in a limited number of the same patients. The hypothesis was that a “ceiling” level of central sensitisation is established in these patients, and that acid-suppressing medication partially reverses this.
Another study of healthy subjects investigated the effect of distal oesophageal acid infusion on distal oesophageal pain thresholds to thermal, mechanical and electrical stimulation. Acid infusion did not induce pain, but subsequently reduced pain thresholds to all types of stimuli, indicating that a process of sensitisation (peripheral or central) had occurred. Importantly, the oesophageal acid infusion potentiated the Nociceptive Flexion Reflex (NFR), a polysynaptic spinal cord-mediated pain reflex (see section 5.1.1), providing further evidence for the convergence of somatic and oesophageal visceral afferent nerves and indicating that the acid had initiated CS.

**Figure 6 - Healthy volunteer model of central sensitisation of the oesophagus**

A naso-oesophageal catheter is inserted, and positioned so that an infusion port is 3cm above the lower oesophageal sphincter. Acid is then infused directly into the lower oesophagus. Immediately following the acid infusion, pain thresholds to electrical stimulation are determined in the non-acid exposed upper oesophagus. In healthy volunteers, the acid infusion reduces pain thresholds in the upper oesophagus. Oesophageal hypersensitivity at a site distant from the original injury is a demonstration of secondary hyperalgesia, known to be mediated by central sensitisation.
4.2 Oesophageal hypersensitivity in chronic cough

There are several lines of evidence that activation of oesophageal vagal afferents may sensitise and/or directly evoke cough in humans, suggesting that central mechanisms may be responsible for the association between reflux and cough.

4.2.1 Oesophageal acid infusion increases cough reflex sensitivity

Oesophageal acid infusion rapidly reduced capsaicin cough thresholds in a group of 9 patients (7 female, 2 male) with abnormal gastro-oesophageal reflux and chronic cough. This effect was not observed in healthy controls (n=18) or patients with gastro-oesophageal reflux, but no cough (n=16)[107].

Wu et al.[108] randomised 7 mild asthmatics with normal gastroscopy and no cough to receive acid or saline infusion into the lower oesophagus 1 week apart. The dose of inhaled capsaicin inducing 3 coughs was significantly reduced during acid infusion compared to saline. No proximal acid reflux was detected during acid infusion, reducing the likelihood of aspiration.

4.2.2 Oesophageal acid infusion may directly induce cough

Ing et. al[109] investigated 22 patients with cough and abnormal GOR demonstrated by 24 hour pH monitoring. Patients received acid or saline infusion into the distal oesophagus for 15 minutes in random order and were compared to 12 matched controls. In the cough patients, cough frequency increased from a median of 8.3 during saline infusion to 36.5 during acid infusion. There was no significant change in cough frequency during acid infusion in healthy controls. Other investigators have not shown an increase in cough frequency during oesophageal acid infusion in chronic cough patients with abnormal gastro-oesophageal reflux[110].

4.2.3 Temporal association of reflux and cough

In chronic cough patients, the temporal association between reflux and cough was carefully investigated using objective 24 hour cough monitoring with synchronised objective 24 hour impedance/pH monitoring, the gold-standard for detection of acid and non-acid reflux episodes[90]. Although the mean number of reflux episodes was within the normal range (67.7 episodes over 24 hours), there was a positive temporal association between reflux and cough in the majority of patients (71.8%). In 47.9%,
reflux tended to precede cough more often than would be expected by chance alone, indicating that reflux may be directly triggering cough. Reflux-cough association positive patients had a significantly more sensitive cough reflex than reflux-cough association negative patients. However, they had an overall similar number of reflux episodes, and no evidence of an increased prevalence of macroscopic oesophagitis at gastroscopy. Therefore, the association between reflux and cough appeared to be independent of the amount of reflux, providing further support for a process of central rather than peripheral sensitisation.

4.2.4 Evidence against a role for micro-aspiration

Micro-aspiration could theoretically directly trigger cough from the airways or else cause a degree of chronic airways inflammation which sensitises cough. However, pepsin levels in broncho-alveolar lavage fluid as a marker of gastric micro-aspiration was not elevated in chronic cough patients with and without gastro-oesophageal reflux disease compared to healthy controls[111]. Furthermore, chronic cough patients have no more laryngeal/pharyngeal reflux than healthy controls[112], making micro-aspiration an unlikely pathogenetic mechanism in chronic cough.
5 Inhibitory mechanisms

In addition to sensitisation of afferent pathways, a failure of descending inhibitory mechanisms may contribute to the pathogenesis of chronic pain, and by analogy chronic cough.

5.1 Inhibition of pain

The brain modulates spinal cord excitability via descending inhibitory and facilitatory pathways. The limbic system and hypothalamus project to the midbrain periaqueductal grey (PAG) with final common output through the rostral ventral medulla (RVM). Reciprocal firing of RVM “on” or “off” cells projecting to the dorsal horn promote or inhibit nociceptive transmission respectively[113].

5.1.1 Measuring endogenous inhibition

One mechanism that is thought to reflect endogenous pain inhibition is known as Diffuse Noxious Inhibitory controls or DNIC. This is a c-fibre mediated lower brainstem inhibitory mechanism that is activated by applying a painful stimulus to one part of the body (conditioning stimulus), to inhibit pain perceived elsewhere (test stimulus)[114]. Lack of endogenous inhibitory mechanisms, such as DNIC, are thought to partly explain why some individuals are more susceptible to the development of chronic pain conditions[115]. Indeed, several studies have identified deficient DNIC mechanisms in patients with fibromyalgia[116], chronic headaches[117] and irritable bowel syndrome[118] compared to healthy controls. DNIC mechanisms also tend to decline from middle age onwards[119] and are less effective in women[116, 120], consistent with the observation that chronic pain syndromes are more common in middle age women. The development of chronic post-thoracotomy pain can be independently predicted by magnitude of DNIC response pre-operatively[121], raising the possibility that a DNIC protocol could be a useful clinical test.

In human DNIC studies, pain is measured using subjective pain intensity scales, functional Magnetic Resonance Imaging, somato-sensory evoked potentials[115] or the Nociceptive Flexion Reflex (NFR)[114]. The NFR was pioneered by Jean-Claude Willer in the 1980’s as an objective measure of pain. It is a spinal reflex whereby the electromyographic response of the biceps femoris muscle is measured following
painful electrical stimulation of the sural nerve. Conditioning stimuli including immersion of a limb in painful hot/cold water, ischaemic limb pain and noxious pinch to the nasal septum were all successful at attenuating the NFR and raising pain thresholds. It has subsequently been found that experimentally induced breathlessness can act as a noxious conditioning stimulus to inhibit the NFR[122].

Distraction is not thought to be responsible for the inhibitory effect of a conditioning stimulus in a DNIC paradigm[123]. However, manipulation of expectations can alter the magnitude of DNIC effect. In an experiment by Goffaux et. al. healthy subjects were instructed that applying the conditioning stimulus would either lead to enhanced pain (hyperalgesia) or reduced pain (analgesia)[124]. Expectations of hyperalgesia completely blocked DNIC, suggesting that DNIC can be modulated by cortical pathways.

5.2 Inhibition of cough

One well described inhibitory cough mechanism is voluntary suppression. Indeed, healthy subjects are able to suppress capsaicin and citric acid induced cough[125-127]. In contrast, chronic cough patients demonstrate poorer voluntary control over experimentally induced coughing[127], see appendix 1.4.

Endogenous mechanisms of cough inhibition are less well investigated. It has been suggested that the endogenous release of endorphins in the nTS in the brainstem may inhibit afferent transmission to reduce cough[126], but this has not been confirmed. In this respect it is interesting that μ-opioid receptor agonists, such as morphine, are known to activate endogenous inhibitory pain pathways[115], and could therefore plausibly also activate endogenous inhibitory cough pathways. In fact, morphine is probably the most effective cough suppressant currently available. In a randomised controlled trial in patients with treatment-resistant chronic cough, morphine reduced subjective cough scores and cough-specific quality of life[128]. In healthy volunteers, systemically administered morphine inhibited capsaicin induced cough[129].
6 General Hypothesis and Aims

I hypothesise that there are 3 overlapping neural mechanisms that contribute to the pathogenesis of chronic cough. These include:

(i) Central sensitisation of afferent cough pathways
(ii) Failure of endogenous inhibition of cough
(iii) Lack of conscious control over the urge-to-cough

Two or more of these mechanisms may be operating interactively. For example, failed endogenous inhibition of cough would pre-dispose to the development of central sensitisation, and impaired conscious control would worsen cough severity. A clinical test to identify the predominant mechanism(s) for each individual would assist in determining the most appropriate treatment.

In a series of clinical experiments, I aim to investigate neural mechanisms that contribute to the pathogenesis of chronic cough. I will seek evidence supporting a role for central sensitisation and failed endogenous inhibition or conscious control of cough.
General Methodology
7 General Methodology

7.1 Subjects

7.1.1 Healthy controls

Healthy subjects were recruited by poster advertisements displayed around the hospital and from volunteer databases. They had normal lung function and no current or past history of respiratory disease, chronic pain, irritable bowel syndrome, chronic headaches, reflux disease, post-nasal drip or psychiatric illness.

7.1.2 Chronic cough patients

Chronic cough (CC) was defined as a cough lasting longer than 8 weeks. Chronic cough patients were recruited from a tertiary referral specialist cough clinic (University Hospitals South Manchester, UK) where they had undergone several investigations (lung function, bronchial challenge testing, nasendoscopy, CT scan, bronchoscopy, 24 hour impedance/pH monitoring), and had received 8 week treatment trials for gastro-oesophageal reflux disease (GORD), asthma, eosinophilic bronchitis and/or nasal disease as indicated. None of the patients had demonstrated a good response to standard treatment.

7.1.3 Asthmatics

Asthmatics were physician-diagnosed, with documented clinical evidence of bronchial hyper-reactivity (positive methacholine challenge and/or reversibility in FEV1 >12%). Asthma patients had well-controlled mild/moderate asthma (FEV1 >75% predicted, British Thoracic Society treatment step 1-3, no courses of oral steroids within the previous 4 weeks) and were able to omit long-acting bronchodilators prior to participation.

7.1.4 Exclusion criteria

Exclusion criteria were recent upper respiratory tract infection (<4 weeks), pregnancy or breast-feeding, current tobacco smokers or ex-smokers with >10 pack year smoking history, diabetes mellitus, use of opiates or ACE inhibitors, use of any centrally acting medication which could alter the sensitivity of the cough reflex, significant on-going medical or psychiatric illness, a history of drug or alcohol abuse and for chapter 1, allergy to ketamine.
7.1.5 Ethics approval and clinical trial registration

All studies were approved by a UK Research Ethics Committee and registered as a clinical trial on a freely accessible online database. All participants gave written, informed consent.

7.2 Cough measures

7.2.1 Objective cough frequency

Twenty-four hour objectively recorded cough frequency was measured in all study participants in chapter 1 and chapter 3.

A piezo-electric sensor was applied to a shaved chest wall just below the sternal notch and an air microphone was attached to the patient’s lapel. These were connected to the custom built VitaloJAK Cough Monitor® (Vitalograph Ltd, UK). Twenty-four hour digital sound recordings were collected (8 KHz, 16 bit wav format), stored on a 4GB data card and transferred to a personal computer where they were archived on compact disc. The recordings were analysed using an audio editing package (Cool Edit 2000, Syntrillium). Periods of silences were first removed by a custom written programme. Explosive cough sounds, including those induced during cough challenges, were then manually counted by a trained observer, and cough rates expressed as cough sounds/hour (cs/hr). This is a validated, repeatable method for measuring objective cough frequency [5, 15].

7.2.2 Cough challenges

Capsaicin was used to induce cough in all experiments conducted in this thesis. An advantage of capsaicin is that it is extremely safe for use in humans[130]. Furthermore, it evokes cough by a single clearly defined mechanism, by activating TRPV1 receptors present on vagal c-fibres. In contrast, citric acid may evoke cough by more than one mechanism of action (vagal c-fibres and Aδ-fibres)[130]. Another tussive agent is ultrasonic distilled water (fog). However, in a reported 15-20% of subjects fog inhalation fails to evoke coughing, and in some individuals causes significant broncho-constriction[130].

In chapter 1, the capsaicin cough challenge was performed using the single-breath, doubling dose method, delivered by a dosimeter (Koko dosimeter, Ferraris Ltd) with
inspiratory flow rate limitation as recommended in European Respiratory Society guidelines [131]. Serial doubling concentrations of capsaicin ranging from 0.48μM to 1000μM were administered 1 minute apart with 3 interspersed placebo inhalations (normal saline) to which both researcher and subject were blinded. The number of coughs, defined as explosive sounds, which occurred in the 15 seconds after each inhalation was recorded and the test stopped when the subject coughed at least 5 times. The lowest concentration of inhaled capsaicin inducing 2 and 5 coughs was recorded, known as C2 and C5 respectively. To ensure consistency the same nebuliser pot was used throughout the experiment for each individual, and re-calibrated at regular intervals. Spirometry was performed before and after the challenge.

In chapter 2 and 3, the capsaicin cough challenge design was modified to construct full dose-response curves, and is fully described in chapter 2, section 2.2.

7.2.3 Urge-to-cough

In chapter 2 and 3, urge-to-cough intensity after each dose of inhaled capsaicin was rated on a modified Borg Scale, ranging from 0-10 (no urge to cough to maximum urge-to-cough)[59].

7.3 Psychological Questionnaires

Psychological symptoms can profoundly affect the processing of pain[132], but is it unknown how psychological factors may interact with the urge-to-cough and/or cough frequency evoked by inhaled capsaicin. One study found that a higher level of obsessional symptoms were associated with increased difficulty in suppressing virally induced cough[133]. Therefore, given that CC patients have previously been shown to have higher levels of anxiety and depression when compared to HC[134, 135], any identified group differences in urge-to-cough could plausibly be explained by psychological symptoms. Therefore, it was believed to be important to measure and control for these variables where possible.

In chapter 2 and 3, the validated State-Trait Anxiety Index (STAI, see appendix 2.6) and Hospital Anxiety and Depression Scale (HADS, see appendix 2.1) were completed by all participants under guided supervision.
7.4 Disease specific Questionnaires

7.4.1 ROME III questionnaire

This self-report questionnaire was used to screen all participants in chapter 1 and 3 for Functional Gastro-Intestinal Disorders (FGID) using the ROME III criteria, see appendix 2.7.

7.4.2 Sino-nasal Outcome Test and Reflux Symptom Score

All subjects in chapter 2 and 3 completed a Sino-nasal Outcome Test (appendix 2.2) and Reflux Symptom questionnaire (appendix 2.10) to determine whether they were symptomatic of reflux disease or post-nasal drip syndrome.

7.4.3 Cough specific Quality of Life Questionnaire

CC patients in chapter 3 completed a Cough-Specific Quality of Life questionnaire (appendix 2.5) to indicate the degree to which the cough was impacting on quality of life, as a marker of cough severity.

7.5 Statistical methods: Generalised Estimating Equations

Each experiment conducted in this thesis contains repeated measures data. That is, where measurements are made on groups of subjects under different experimental conditions and/or over time. In this situation, there is random variation within subjects (between experimental conditions or time points) and random variation between subjects (each subject differs from one another). It is ordinarily the case that within-subject measurements are far less variable than between-subject measurements. Indeed, repeated measures within the same subject tend to be correlated with each other. For example, a subject with low cough thresholds at baseline is more likely to have low cough thresholds at other time-points or under other experimental conditions. This dependency of repeated measures should not be ignored or else standard errors are over-estimated and the power of the study to detect statistically significant changes over time or under different experimental conditions is reduced[136].

Generalised Estimating Equations (GEE) is a widely used statistical technique first introduced by Liang and Zeger in 1986[137] that is used to fit mathematical random effects models to repeated measures data. GEE has already been applied in
respiratory research. For example, a European Respiratory Society/American Thoracic Society workshop on longitudinal analysis (1995) discussed the use of GEE models to compare the rate of decline of FEV1 in current smokers to that of non-smokers[138].

Given that repeated measures within the same subject under different experimental conditions or separate time points are correlated, the GEE model accommodates a correlation structure. GEE are termed a marginal model because it uses the mean values per subject as the outcome and adjusts the standard error appropriately using a robust sandwich estimator to allow for within-subject correlations. The correlation structure needs to be pre-specified. For data that are correlated within-subjects over time, an "autoregressive" correlation structure is preferred, whereas within-subject observations that are equally correlated are “exchangeable”[139]. A data structure that conforms precisely to a normal distribution is not required for GEE models. Furthermore, count data, such as cough frequency, can be reliably modelled using a poisson distribution.

In comparison to GEE, the use of repeated measures ANOVA is limited because it requires complete data sets and normally distributed outcome variables, and only has a crude adjustment for the correlated data over time. However, the main limitation of GEE models are that it treats the correlation structure as a nuisance. Therefore, where the between and within individual variability is of particular interest, multi-level modelling is preferred.

All GEE models performed in this thesis were conducted by myself under the supervision and guidance of Julie Morris, Honorary Lecturer in Medical Statistics.
CHAPTER 1

The Effect of Ketamine, an NMDA-Receptor Antagonist, on Cough and Pain Sensitivity in Chronic Cough Patients and Healthy Controls
8 Introduction

8.1 Rationale

Despite minimal lung pathology, chronic cough (CC) patients demonstrate lowered cough thresholds\[17\], and report excessive cough in response to normally innocuous stimuli e.g. talking on the telephone\[140\]. Airway neuronal hypersensitivity may be responsible for these observations. Whilst both peripheral and central mechanisms of sensitisation would reduce thresholds for activation of cough, central neuronal hyper-excitability would additionally amplify cough response to a broad range of stimuli, such that coughing is disproportionate to the degree of airway injury. If CNS mechanisms were responsible for CC, this would explain why treatment for peripheral triggers such as reflux, nasal disease or asthma is often unsuccessful. By analogy, central sensitisation is a key mechanism responsible for several chronic pain disorders in which there is limited evidence of peripheral tissue injury e.g. irritable bowel syndrome\[141\] and fibromyalgia\[142\].

One of the hallmark clinical features of central sensitisation in chronic pain is a widening of the usual receptive fields\[74\]. This is due to central convergence of afferent fibres arising from the injury site and surrounding areas onto sensitised neurones. Circumstantial evidence for a similar widening of receptive fields in CC arises from the well documented observation that cough can be triggered from extrapulmonary sites e.g. oesophageal reflux. Indeed, in \(~50\%\) of CC patients distal oesophageal reflux precedes cough more often than would be expected by chance alone, suggesting that reflux is directly triggering cough\[90\]. The direct activation of cough by oesophageal stimuli is likely to arise via central convergence of vagal pathways\[91\], but this has not been confirmed. If hypersensitivity of afferent pathways in CC is specific to cough, but not pain, this would provide further evidence for a central rather than peripheral mechanism of cough sensitisation.

Furthermore, an improved understanding of the mechanisms by which cough can become centrally sensitised would be valuable. For example, one key mechanism that mediates central sensitisation of pain is an up-regulation of the NMDA receptor\[143\] in the dorsal horn of the spinal cord. Although cough is mediated by the NMDA receptor centrally in the nTS in the brainstem in animals\[86, 87\], it is unknown whether an up-regulation of the NMDA receptor additionally contributes to central sensitisation.
8.2 Hypothesis

Central sensitisation of cough is an important patho-physiological mechanism in chronic cough patients, and is mediated by the NMDA receptor.

8.3 Aims

(i) To investigate the effect of ketamine, an NMDA receptor antagonist, on capsaicin cough thresholds in chronic cough patients and healthy controls
(ii) To compare visceral (pharyngeal/oesophageal) and somatic (chest wall) pain sensitivity in chronic cough patients and healthy controls
(iii) To investigate the effect of ketamine on visceral and somatic pain sensitivity in chronic cough patients and healthy controls
(iv) To investigate whether pharyngeal/oesophageal stimulation is able to directly evoke cough.
9 Methodology

9.1 Subjects

Twelve healthy controls and 12 chronic cough patients were enrolled. The eligibility criteria are described in section 7.1. The study was approved by a UK Research Ethics Committee (REC:07/H1004/142) and registered at www.clinicaltrials.gov (NCT00858624).

9.2 Study Design

9.2.1 Screening

At screening, subjects underwent the following:

(i) Capsaicin cough challenge (see section 7.2.2) : to ensure that all subjects had a measurable C2/C5
(ii) Completion of ROME III questionnaire (see section 7.4.1): to screen for functional gastro-intestinal (GI) disorders
(iii) Manometry (see section 9.3.1): to measure oesophageal motility and locate the upper and lower oesophageal sphincters
(iv) 24 hour impedance/pH test (CC only, see section 9.3.2): to quantify acid/non-acid reflux.

9.2.2 Randomised visits

Subjects were then entered into a randomised, double-blind, 2-way cross-over study with 2 visits least a week apart, see Figure 7.

At each of the randomised visits, a series of baseline measurements were recorded in the following order:

(i) PASAT (see section 9.3.3): auditory test of attention
(ii) Pain thresholds to electrical stimulation of upper oesophagus, pharynx and anterior chest wall (see section 9.3.4 and 9.3.5)
(iii) Capsaicin cough challenge (see section 7.2.2).

When baseline measurements were completed, a double-blind infusion of ketamine or placebo (see section 9.3.6) was administered over 30 minutes. The PASAT and pain threshold measurements were repeated immediately after the infusion had finished and every 1 hour and 30 minutes thereafter. The cough challenge was
repeated 45 minutes after the infusion had finished and every 1 hour 30 minutes thereafter.

Spontaneous cough frequency was recorded from the beginning of each randomised visit over a 24 hour period (see section 9.3.7).

Finally, 24 hours after the start of the drug infusion, subjects had the following repeat tests:

(i) Capsaicin cough challenge
(ii) Chest wall pain thresholds
Figure 7 - Study Design

PASAT = Paced auditory serial attention test. PT indicates pain thresholds. C2/C5 indicates capsaicin cough challenge. Time is shown in minutes.
9.3 Detailed Description of Procedures

9.3.1 Manometry

Manometry was performed using a water perfused system (Dentsleeve, Oakfield Instruments Ltd, Oxon, UK) to locate the lower and upper oesophageal sphincters and to assess oesophageal motility. All such procedures were performed and interpreted either by a trained oesophageal technician (Miss Helen Jones), or myself. Clinical reports were checked and verified by Professor Lesley Houghton.

The patient fasted for 6 hours prior to the test. After calibration of the equipment, the patient was positioned sitting upright. Local anaesthetic spray was administered nasally and to the throat. Using lubricating jelly, the catheter was inserted nasally and advanced into the oesophagus with the assistance of the patient who was instructed to take regular sips of water. A manometric trace was observed in real time using a computer monitor. The distal sleeve of the catheter was positioned within the high pressure area of the lower oesophageal sphincter (LOS), and the catheter taped to the nose. The patient was then instructed to lie supine. At least 20 water boluses (5ml each) were given to the patient to obtain information about peristaltic activity throughout the oesophagus. The basal pressure within the LOS was calculated, and relaxation of the LOS prior to the arrival of each water swallow observed. On completion of the 20 water swallows, the patient was instructed to sit upright. The catheter was gradually withdrawn to locate the high pressure zone of the upper oesophageal sphincter (UOS). The distance from the nose to the LOS and UOS was documented. Each manometric trace was interpreted by at least 2 oesophageal technicians.

9.3.2 Impedance/pH Monitoring

All chronic cough patients completed 24 hour impedance/pH monitoring to objectively measure reflux disease using the Sleuth® multi-channel intra-luminal impedance ambulatory system (Sandhill Scientific Inc; Highland Ranch, CO, USA). All impedance/pH tests were performed and interpreted either by a trained oesophageal technician (Miss Helen Jones), or myself. Clinical reports were checked and verified by Professor Lesley Houghton.
With the patient sitting upright, the impedance/pH catheter was inserted nasally into the oesophagus as already described. The catheter was positioned so that the distal and proximal pH sensors were 5 cm and 22 cm above the LOS respectively. The catheter was attached to a digital data logger which was carried in a shoulder bag. The patient recorded meal/drink times, periods of recumbency and symptoms on the data logger, and noted additional information on a diary card. Patients were requested not to drink carbonated or acidic drinks e.g. fruit juice throughout the study period (24 hours).

The catheter was removed after 24 hours. The oesophageal data were downloaded onto a password protected personal computer and opened for editing using Bioview Analysis software® (Sandhill Scientific Inc). Patient diary cards were reviewed and meal times and recorded drink periods were marked and excluded from the analysis.

The data were independently manually analysed using Bioview Analysis® (Sandhill Scientific Inc) by two trained oesophageal technicians. A reflux episode was defined as a retrograde drop in impedance from baseline to a 50% threshold, noted in at least two consecutive channels (of the distal four) with the most distal channel remaining below the 50% mark for at least two seconds. A total number of reflux episodes of ≤ 73 is considered normal [147, 148]. Reflux episodes were defined as full column if there was propagation to the most proximal channel without interference of a swallow.

9.3.3 Paced Auditory Serial Attention Test (PASAT)

Ketamine is known to reduce attention which could confound pain threshold measurements. The Paced Auditory Serial Attention Test (PASAT) is a commonly used measure of vigilance [85, 149], requiring subjects to add consecutive numbers presented verbally and to respond orally with the correct value while focusing on the next number in the series. Participants were presented with 60 numbers with 3 seconds between each number. The total score represented the number of correct responses out of 60.

9.3.4 Visceral Pain Thresholds

An oesophageal stimulation catheter (Gaeltec, Dunvegan, Isle of Skye) was positioned without local anaesthetics using the manometric measurements obtained at screening.
The catheter was designed to incorporate two pairs of silver-silver chloride bipolar ring electrodes (inter-electrode distance 1 cm) with each pair of electrodes separated by 7 cm. The distal electrode pair was sited 3 cm below the upper oesophageal sphincter (UOS) in the upper oesophagus, and the proximal electrode pair was sited in the hypopharynx. The positioning of the catheter ensured that the distal pair of electrodes in the upper oesophagus did not stimulate the more sensitive oesophageal transition zone [150]. The catheter was connected to an electrical stimulator (Model DS7; Digitimer Ltd) controlled using a trigger generator (Model DG2, Digitimer Ltd) and electrical stimuli were delivered at a frequency of 0.2 Hz using square wave pulses (500 μs duration). The stimulus intensity was increased by 2 milliampere (mA) to a maximum of 99 mA, and subjects indicated the intensity of the sensation on a verbal descriptor scale (slight sensation, definite sensation, slight discomfort, definite discomfort and pain). This scale has been shown to correlate with the amplitude and latency of oesophageal cortical evoked potentials during electrical stimulation of the oesophagus [151]. All subjects practiced rating pain thresholds in the oesophagus and pharynx prior to the start of the experiment. The lowest stimulus intensity (mA) at which the subject reported pain was recorded, and the mean of 3 readings at baseline and the mean of 2 readings at every other time-point were calculated. Pain thresholds were taken as the lowest stimulus intensity at which subjects reported pain. Participants indicated the perceived location of the painful sensations in the pharynx and oesophagus.

9.3.5 Chest Wall Pain Thresholds

A pair of disposable surface silver-silver electrodes were placed 1 cm apart on the anterior chest wall. The electrodes were connected to an electrical stimulator as for visceral pain thresholds. The intensity of the stimulus delivered was increased by 2 milliamperes (mA) to a maximum of 99 mA, and subjects indicated the intensity of the sensation on a verbal descriptor scale (weak, mild, moderate, strong and intense). The lowest stimulus intensity (mA) at which the subject reported an intense sensation was recorded, and the mean of 3 readings at baseline and the mean of 2 readings at every other time-point was calculated. An "intense" sensation was taken as pain threshold.
9.3.6 Ketamine/Placebo Infusion

A pre-filled syringe was provided by hospital pharmacy containing ketamine diluted to 1mg/ml in normal saline or placebo (normal saline alone). The syringes were coded and identical in appearance to maintain blinding. The order of delivery was randomised by pharmacy. A cannula was inserted into the left ante-cubital fossa and a loading dose of 0.075mg/kg was administered intravenously over 10 minutes followed by an infusion of 0.005mg/kg over 20 minutes via an infusion pump (3100, Smiths Medical). The chosen dose of ketamine was based on published pharmacokinetic data[152] to achieve a stable plasma target concentration of ~100 ng/mL. Previous studies have reported an effect of ketamine on pain hypersensitivity at plasma concentrations of 100-200ng/mL[153, 154]. Ketamine administered at this dose has been shown to reverse acid-induced pain hypersensitivity of the upper oesophagus in healthy volunteers[85]. Any symptoms experienced by participants were reported to a researcher not responsible for measuring pain thresholds to maintain blinding.

9.3.7 Objective cough monitoring

All participants underwent 24 hour objective cough monitoring at each of the randomised visits as described in section 7.2.1. The 24 hour digital sound recordings were also used to calculate the duration (minutes) of electrical stimulation of the pharynx, upper oesophagus and chest wall. The number of coughs was manually counted during each period of electrical stimulation and for 15 seconds after the period of stimulation had ended. Cough frequency during electrical stimulation was then expressed as cough sounds per minute (cs/min).

9.4 Justification for methodology

9.4.1 Ketamine

Ketamine is a mid-affinity, uncompetitive, highly selective and potent NMDA receptor antagonist, and has been a useful pharmacological tool[143]. For example, ketamine blocks and reverses the development of central sensitisation in somatic[80-84] and visceral[85] pain models. Furthermore, when administered intravenously, it has a rapid and prolonged pharmacodynamic effect by increasing pain thresholds for several hours in healthy volunteer models of central sensitisation[85] or for up to 6 months in patients
with chronic neuropathic pain[144]. Unfortunately, the clinical use of ketamine is limited by CNS side-effects and a narrow therapeutic window.

9.4.2 Measuring visceral pain sensitivity

Pain sensitivity of the oesophagus/pharynx can be measured in human subjects using mechanical, thermal or electrical stimulation. However, electrical stimulation provides the greatest degree of control over stimulus intensity/frequency and allows the measurement of highly reproducible pain thresholds[106, 145]. Electrical stimuli are delivered via a pair of silver-chloride electrodes connected to a constant current stimulator[105, 145, 146]. The frequency of electrical pulsations can be controlled and the intensity gradually increased until sensory or pain thresholds are reached. Therefore, in this study I measured oesophageal and pharyngeal pain thresholds to electrical stimulation.

It has been documented that the first measurement of oesophageal pain thresholds to electrical stimulation is likely to under-estimate pain sensitivity because of possible heightened arousal[145]. Therefore, an average of at least 2 pain thresholds is usually recorded. I recorded an average of 3 readings at baseline, and an average of 2 readings thereafter. The stimulus intensity was gradually increased until subjects reported pain.

9.5 Primary outcome

The change in capsaicin cough thresholds (C2/C5) after ketamine compared to placebo in healthy controls and chronic cough patients.

9.6 Secondary outcomes

- A comparison of baseline pain thresholds by group.
- The change in pain thresholds after ketamine compared to placebo
- The change in PASAT score after ketamine compared to placebo.
- 24 hour spontaneous cough frequency after ketamine compared to placebo in healthy controls and chronic cough patients
- Cough rates during electrical stimulation of the oesophagus, pharynx and chest wall.

9.7 Statistical Methods

Baseline characteristics were compared using parametric tests for normally distributed data, or non-parametric tests for non-normal data.

C5 and C2 were log transformed. A series of autoregressive, generalised estimating equations (GEE) were performed in SPSS Version 15.0 to explore the change in outcome variables over time following ketamine compared to placebo in chronic cough patients compared to healthy controls. Time was analysed as a categorical variable (reference category = baseline or time 0). Age was not included in the models because of total confounding by age (all HC were younger than the CC patients). The GEE models performed are summarised in Table 2.

9.7.1 Power calculations

It was unknown whether the placement of a naso-oesophageal catheter would alter the variability in capsaicin cough sensitivity. Therefore, there was no previous data upon which to perform sample size calculations. Based on the large number of physiological measures obtained during this study, and the time-consuming nature of the experiments, it was not thought to be feasible to recruit more than 12 subjects per group.
<table>
<thead>
<tr>
<th>Model</th>
<th>Subjects</th>
<th>Outcome variables</th>
<th>Between subject variables</th>
<th>Within subject variables</th>
<th>Main-Effects</th>
<th>Interaction Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline comparison by group</td>
<td>All subjects</td>
<td>• LogC5/C2</td>
<td>Group</td>
<td>Time Drug</td>
<td>Group</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pain thresholds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change over time following ketamine</td>
<td>All subjects</td>
<td>• LogC5/C2</td>
<td>Group</td>
<td>Time Drug</td>
<td>Time Drug</td>
<td>Time*drug</td>
</tr>
<tr>
<td>compared to placebo</td>
<td></td>
<td>• Pain thresholds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• PASAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC only</td>
<td>All subjects</td>
<td>• LogC5/C2</td>
<td>Group</td>
<td>Time Drug</td>
<td>Time Drug</td>
<td>Time*drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pain thresholds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC only</td>
<td>All subjects</td>
<td>• LogC5/C2</td>
<td>Group</td>
<td>Time Drug</td>
<td>Time Drug</td>
<td>Time*drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pain thresholds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect of ketamine compared by group</td>
<td>All subjects</td>
<td>• LogC5/C2</td>
<td>Group</td>
<td>Time Drug</td>
<td>Group</td>
<td>Group*drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pain thresholds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HC=healthy controls, CC=chronic cough, PASAT=Paced auditory serial attention test.
10 Results

10.1 Baseline Characteristics

Twelve healthy controls (HC) [6 male, mean (SD) age 22.50 (2.00) years] and 12 chronic cough (CC) patients [6 male, mean (SD) age 54.50 (18.25) years, mean (SD) duration cough 11.5 (8.82) years] were enrolled. Compared to HC, the CC patients were significantly older (p<0.001), and had larger body mass index (BMI) (p=0.029). Percentage predicted FEV1/FVC and pack year smoking history were not significantly different between the groups (see Table 3).

Table 3– Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>Chronic Cough</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>6:6</td>
<td>6:6</td>
<td></td>
</tr>
<tr>
<td>Age (years)*</td>
<td>22.50 (2.00)</td>
<td>54.50 (18.25)</td>
<td>&lt;0.001¥</td>
</tr>
<tr>
<td>BMI*</td>
<td>23.76 (2.67)</td>
<td>27.91 (5.4)</td>
<td>0.02¥</td>
</tr>
<tr>
<td>FEV1% *</td>
<td>95.01 (11.24)</td>
<td>102.77 (10.08)</td>
<td>0.089≠</td>
</tr>
<tr>
<td>FVC% *</td>
<td>101.77 (13.01)</td>
<td>100.14 (11.66)</td>
<td>0.749</td>
</tr>
<tr>
<td>Smoking (pack yrs)**</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.713</td>
</tr>
</tbody>
</table>

*Mean (SD), **Median (IQR), ¥indicates p-value ≤0.05, ≠indicates p-value ≤0.1

10.1.1 Screening capsaicin cough challenge

At screening, the CC patients had significantly lower capsaicin cough thresholds than the HC. The mean (SD) log C5 (µM) was 0.86 (0.50) in CC patients and 1.95 (0.45) in HC, p<0.001. The mean (SD) log C2 (µM) was 0.83 (0.44) in CC patients and 1.24 (0.25) in HC, p<0.001.

10.1.2 Gastro-intestinal investigations performed at screening

None of the HC had evidence of functional gastro-intestinal disorders (FGID) on screening with the ROME III questionnaire. One HC had abnormal manometry [Ineffective Oesophageal Motility (IEM)], and this subject was included in the final analysis. All remaining manometric traces in the HC were normal.

At least one FGID was identified in 7 of the CC patients with diagnoses including irritable bowel syndrome (n=1), functional constipation (n=2), functional bloating (n=2), functional
chest pain (n=2), unspecified excessive belching (n=1) and globus (n=1). In 4 CC patients, manometry was abnormal [IEM (n=2), low amplitude contractions (n=1) and low basal lower oesophageal sphincter pressure (n=1)].

In 3 CC patients, abnormal gastro-oesophageal reflux on impedance/pH testing was detected (>73 episodes of reflux over 24 hours). The mean (SD) number of acid and non-acid reflux events was 63.08 (27.48) over 24 hours (normal ≤ 73). The mean (SD) number of full column reflux events was 15.00 (10.22) over 24 hours.

10.2 Withdrawals and Adverse-Events

Two of the CC patients were unable to tolerate the ketamine infusion. Therefore, 10 CC patients and 12 HC completed the study, see Figure 8.

Adverse events during the ketamine infusion were lightheaded (100%), drunk (58.3%), drowsy (37.5%), blurred vision (29.2%), woozy (16.7%), paraesthesia/numb (12.5%), dizzy (8.3%), warm (4.2%), slow reactions (4.2%), vomiting (4.2%), hazy (4.2%), fuzzy (4.2%) and lack of co-ordination (4.2%). Lightheaded symptoms ranged from mild to severe in severity (severe in 8.3%, moderate in 50%, mild in 41.7%). Adverse events during the placebo (saline) infusion were headache (4.2%), sensation of butterflies in stomach (4.2%), drowsy (4.2%), lightheaded (4.2%) and blurred vision (4.2%).

Figure 8 - Consort diagram
10.3 Missing data

Given the large number of outcome measures and repeated time-points, there was some missing data. This is summarised in Table 4. The data was missing due to (i) subject unable to tolerate ketamine infusion therefore visit discontinued or (ii) outcome measure was not recordable. Where capsaicin cough thresholds (C5/C2) could not be recorded, the maximum dose of capsaicin was insufficient to induce at least 2/5 coughs. Where pharyngeal or oesophageal pain thresholds could not be recorded, this was due to a failure of the electrode pair to maintain good contact with the wall of the pharynx/oesophagus. It is important to note that a high proportion of the pharyngeal pain threshold measurements could not be recorded in the HC (40.8%).

Table 4 – Missing data

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Total No. repeated measures per group</th>
<th>Missing data in HC N (%)</th>
<th>Missing data in CC N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5</td>
<td>144</td>
<td>20 (13.9%)</td>
<td>11 (7.6%)</td>
</tr>
<tr>
<td>C2</td>
<td>144</td>
<td>0 (0%)</td>
<td>10 (6.9%)</td>
</tr>
<tr>
<td>PTo</td>
<td>120</td>
<td>10 (8.3%)</td>
<td>10 (8.3%)</td>
</tr>
<tr>
<td>PTp</td>
<td>120</td>
<td>49 (40.8%)</td>
<td>10 (8.3%)</td>
</tr>
<tr>
<td>PTc</td>
<td>144</td>
<td>1 (0.69%)</td>
<td>10 (6.9%)</td>
</tr>
</tbody>
</table>

PTo, PTp and PTc = pain thresholds in oesophagus, pharynx and chest wall
10.4 Localisation of pain sensations

The perceived location of the pain sensations evoked by electrical stimulation of the upper oesophagus, pharynx and chest wall are summarised in Table 5.

<table>
<thead>
<tr>
<th>Site of stimulation</th>
<th>Location</th>
<th>No. subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper Oesophagus</strong></td>
<td>Lower throat</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Upper chest</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Mid-throat</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24</td>
</tr>
<tr>
<td><strong>Pharynx</strong></td>
<td>Mid-throat</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Upper throat</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lower throat</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21</td>
</tr>
<tr>
<td><strong>Chest wall</strong></td>
<td>Upper Chest</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Centre chest</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24</td>
</tr>
</tbody>
</table>

NB: pharyngeal pain thresholds were not recordable in 3 of the healthy controls.

10.5 Effect of naso-oesophageal catheter on cough threshold to capsaicin

At each of the randomised visits, a naso-oesophageal catheter was positioned prior to the baseline cough challenge. Paired t-tests were used to compare average baseline C5/C2 (with naso-oesophageal catheter) to screening C5/C2 (without naso-oesophageal catheter). In CC patients, C5 and C2 were higher at baseline compared to screening (p=0.017 and p=0.099 respectively), suggesting the catheter desensitised cough. In HC, C5 and C2 were not significant different at baseline compared to screening (p=0.776 and p=0.373 respectively).
10.6 PASAT

There was no significant main effect of drug on PASAT score (p=0.604). However, there was a significant main effect of time (p<0.001) and a significant interaction of time*drug (p<0.001). Compared to placebo, there is a significant decrease in PASAT score 30 minutes (p=0.002) and 120 minutes (p=0.031) after ketamine, indicating a slight reduction in attention at these time-points, see Figure 9.

Figure 9 – PASAT score

Graph shows the change in model adjusted mean PASAT scores over time following ketamine (red) and placebo (black). Dotted area corresponds with the drug infusion. Compared to placebo, there is a significant (p≤0.05*) decrease in PASAT scores at 30 and 120 minutes after ketamine infusion, corresponding to a reduced level of attention.
10.7 Capsaicin cough threshold: Log C5

10.7.1 Baseline comparison Log C5 by group

At baseline, the CC patients had a significantly more sensitive cough reflex than the HC. The model adjusted mean Log C5 (µM) at baseline was 1.28 (95%CI 1.02 to 1.55) in CC patients compared to 1.86 (1.68 to 2.04) in HC, p<0.001, see Figure 10A.

10.7.2 Effect of ketamine over time on Log C5

Overall, there was no significant main effect of drug (p=0.293) or time (p=0.110) on Log C5, and no significant interaction between drug and time (p=0.691). This indicates a similar change in Log C5 over time following ketamine compared to placebo.

10.7.3 Effect of ketamine over time on Log C5 compared by group

There was no significant difference in the effect of ketamine in CC patients compared to HC (group*drug, p=0.383).

In HC, there was no significant main effect of drug (p=0.796) or time (p=0.313), and no significant interaction between drug and time (p=0.194), see Figure 10B. In CC, there was no significant main effect of drug (p=0.964) or time (p=0.169), and no significant interaction between drug and time (p=0.287). This indicates similar change in Log C5 over time following ketamine compared to placebo in the CC patients and HC, see Figure 10B. Mean values and 95% CI of the change in Log C5 over time in HC and CC are presented in Table 6.
Graphs compare Log C5 (µM) by group. **A.** Average Log C5 at baseline in healthy controls (HC) and chronic cough patients (CC). Model adjusted means and 95% CI are shown. Compared to HC, CC patients have significantly lower Log C5 at baseline, indicating a more sensitive cough reflex. **B.** Change in model adjusted mean (95% CI) Log C5 over time following ketamine (solid lines) and placebo (dashed lines) in HC (black circles) and CC patients (green squares). Dotted area corresponds to the drug infusion. There is no significant difference in the change in Log C5 over time following ketamine compared to placebo in HC or CC patients.
10.8 Capsaicin cough threshold: Log C2

10.8.1 Baseline comparison Log C2 by group

At baseline, there was no significant difference in C2 between groups. The model adjusted mean Log C2 (µM) at baseline was 1.02 (95%CI 0.87 to 1.17) in CC patients compared to 1.13 (0.96 to 1.30) in HC, p=0.333, see Figure 11A.

10.8.2 Effect of ketamine over time on Log C2

Overall, there was no significant main effect of drug (p=0.939) on Log C2. However, there was a significant main effect of time (p<0.001), with an increase in Log C2 at 135 minutes (p=0.070), 225 minutes (p=0.003), 315 minutes (p=0.070) and 1440 minutes (p=0.072) compared to baseline. This suggests a gradual desensitisation of cough over time. There was no significant interaction between drug and time (p=0.520), indicating a similar change in Log C2 following ketamine compared to placebo.

10.8.3 Effect of ketamine over time on Log C2 compared by group

There was no significant difference in the effect of ketamine in CC patients compared to HC (group*drug, p=0.448).

In HC, there was no significant main effect of drug (p=0.604). However, there was a significant main effect of time (p<0.001), with an increase in Log C2 at 135 minutes (p=0.067), 225 minutes (p=0.046), 315 minutes (p=0.067) and 1440 minutes (p=0.009) compared to baseline. This suggests a gradual desensitisation of the cough reflex over time, see Figure 11B. Overall, there was a significant interaction between drug and time (p<0.001), but the only individual time point reaching statistical significance was 315 minutes (p=0.043), when Log C2 increased to a greater extent after ketamine compared to placebo.

In CC patients, there was no significant main effect of drug (p=0.592). However, there was a significant main effect of time (p<0.001), with an increase in Log C2 at 225 minutes (p=0.011) compared to baseline. There was no significant interaction between drug and time (p=0.592), indicating a similar change in Log C2 following ketamine.
compared to placebo, see Figure 11B. Mean values and 95% CI of the change in Log C2 over time in HC and CC are presented in Table 6.
Figure 11 – Log C2

A

Graphs compare Log C2 (µM) by group. A. Average Log C2 at baseline in healthy controls (HC) and chronic cough patients (CC). Model adjusted means and 95% CI shown. Compared to HC, CC patients have no significant difference in Log C2 at baseline. B. Change in model adjusted mean (95% CI) Log C2 over time following ketamine (solid lines) and placebo (dashed lines) in HC (black circles) and CC patients (green squares). Dotted area corresponds to the drug infusion. Especially in HC, Log C2 tends to increase over time, indicating a gradual desensitisation of the cough reflex. Overall, ketamine has a similar effect on the change in Log C2 over time compared to placebo.
Table 6 – Model predicted mean (95%CI) Log C5/C2 and changes over time

**A. Model predicted Log C5 after ketamine (mean μM, 95% CI)**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Group</th>
<th>0</th>
<th>75</th>
<th>165</th>
<th>255</th>
<th>345</th>
<th>1440</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>1.87</td>
<td>(1.55, 2.18)</td>
<td>2.19</td>
<td>(1.84, 2.55)</td>
<td>2.30</td>
<td>(1.94, 2.65)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1.34</td>
<td>(1.09, 1.60)</td>
<td>1.31</td>
<td>(0.97, 1.66)</td>
<td>1.32</td>
<td>(1.02, 1.63)</td>
</tr>
</tbody>
</table>

**B. Model predicted Log C5 after placebo (mean μM, 95% CI)**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Group</th>
<th>0</th>
<th>75</th>
<th>165</th>
<th>255</th>
<th>345</th>
<th>1440</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>1.91</td>
<td>(1.77, 2.05)</td>
<td>2.08</td>
<td>(1.60, 2.55)</td>
<td>2.07</td>
<td>(1.80, 2.33)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1.22</td>
<td>(0.87, 1.56)</td>
<td>1.22</td>
<td>(0.83, 1.60)</td>
<td>1.34</td>
<td>(0.94, 1.75)</td>
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</table>

**C. Model predicted Log C2 after ketamine (mean μM, 95% CI)**

<table>
<thead>
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<th>Time (minutes)</th>
<th>Group</th>
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<th>75</th>
<th>165</th>
<th>255</th>
<th>345</th>
<th>1440</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>1.07</td>
<td>(0.83, 1.30)</td>
<td>1.34</td>
<td>(1.07, 1.62)</td>
<td>1.24</td>
<td>(1.00, 1.48)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1.07</td>
<td>(0.88, 1.26)</td>
<td>0.96</td>
<td>(0.65, 1.28)</td>
<td>1.05</td>
<td>(0.87, 1.24)</td>
</tr>
</tbody>
</table>

**D. Model predicted Log C2 after placebo (mean μM, 95% CI)**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Group</th>
<th>0</th>
<th>75</th>
<th>165</th>
<th>255</th>
<th>345</th>
<th>1440</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>1.19</td>
<td>(1.04, 1.35)</td>
<td>1.24</td>
<td>(0.91, 1.58)</td>
<td>1.39</td>
<td>(1.10, 1.68)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0.97</td>
<td>(0.75, 1.19)</td>
<td>0.87</td>
<td>(0.65, 1.08)</td>
<td>1.07</td>
<td>(0.80, 1.33)</td>
</tr>
</tbody>
</table>
10.9 Oesophageal pain thresholds (PTo)

10.9.1 Baseline comparison PTo by group

At baseline, there were no significant group differences in PTo. The model adjusted mean PTo (mA) at baseline was 54.24 (95%CI 43.70 to 64.78) in CC patients compared to 54.44 (44.15 to 64.73) in HC, p=0.979, see Figure 12A.

10.9.2 Effect of ketamine over time on PTo

Overall, there was no significant main effect of drug (p=0.460) or time (p=0.082) on PTo, and no significant interaction between drug and time (p=0.105). However, at 30 minutes post ketamine infusion there was a significant increase in PTo compared to placebo (p=0.008). This indicates reduced pain sensitivity in the upper oesophagus immediately after the ketamine infusion, see Figure 13A. Mean values and 95% CI of the change in PTo over time are presented in Table 7A.

10.9.3 Effect of ketamine on PTo compared by group

There was no significant difference in the effect of ketamine in CC patients compared to HC (group*drug, p=0.359).

In HC, there was no significant main effect of drug (p=0.908) or time (p=0.062), and no significant interaction between drug and time (p=0.103).

In CC, there was no significant main effect of drug (p=0.150) or time (p=0.111). However, there was a significant interaction between drug and time (p=0.007). At 30 minutes post ketamine infusion there was a significant increase in PTo compared to placebo (p=0.005), indicating reduced pain sensitivity.
10.10 Pharyngeal pain thresholds (PTp)

10.10.1 Baseline comparison PTp by group

At baseline, there were no significant group differences in PTp. The model adjusted mean PTp (mA) at baseline was 37.49 (95%CI 31.64 to 43.34) in CC patients compared to 32.49 (25.52 to 40.04) in HC, \( p=0.324 \), see Figure 12B.

10.10.2 Effect of ketamine over time on PTp

Overall, there was no significant main effect of time \( (p=0.546) \) on PTp. However, there was a significant main effect of drug \( (p=0.013) \) and a significant interaction between drug and time \( (p=0.001) \). At 30 minutes post ketamine infusion there was a significant increase in PTp compared to placebo \( (p=0.042) \). This indicates reduced pain sensitivity in the pharynx immediately after the ketamine infusion, see Figure 13B. Mean values and 95% CI of the change in PTp over time are presented in Table 7B.

10.10.3 Effect of ketamine over time on PTp compared by group

There was no significant difference in the effect of ketamine in CC patients compared to HC \( (\text{group*drug, } p=0.657) \).

In HC, there was no significant main effect of drug \( (p=0.182) \) or time \( (p=0.737) \). However, there was a suggestion of an interaction between drug and time \( (p=0.072) \). At 30 minutes post ketamine infusion there was an increase in PTp compared to placebo \( (p=0.022) \), indicating reduced pain sensitivity.

In CC patients, the average PTp was lower for the duration of the ketamine visit (34.55mA) compared to the placebo visit (38.70mA), \( p=0.037 \). However, there was no significant change in PTp over time \( (p=0.220) \). There was a significant interaction between drug and time \( (p<0.001) \), but this was largely driven by the difference in PTp between each of the randomised visits, rather than a particular effect of ketamine at any of the individual time-points.
10.11 Chest wall pain thresholds (PTc)

10.11.1 Baseline comparison PTc by group

At baseline, there were no significant group differences in PTc. The model adjusted mean PTc (mA) at baseline was 36.36 (95%CI 27.95 to 44.77) in CC patients compared to 44.36 (34.01 to 54.72) in HC, $p=0.240$, see Figure 12C.

10.11.2 Effect of ketamine over time on PTc

Overall, there was no significant main effect of drug ($p=0.631$) or time ($p=0.242$) on PTc. However, there was a suggestion of an interaction between time and drug ($p=0.056$), with a significant increase in PTc 30 minutes ($p=0.011$) and 90 minutes ($p=0.035$) post ketamine infusion compared to placebo, indicating reduced pain sensitivity, see Figure 13C. Mean values and 95% CI of the change in PTc over time are presented in Table 7C.

10.11.3 Effect of ketamine over time on PTc compared by group

There was no significant difference in the effect of ketamine in CC patients compared to HC (group*drug, $p=0.198$).

In HC, there was no significant main effect of drug ($p=0.316$) or time ($p=0.342$), and no significant interaction between drug and time ($p=0.208$).

In CC, there was no significant main effect of drug ($p=0.479$) or time ($p=0.107$). However, there was a significant interaction between drug and time ($p<0.001$). At 30 minutes ($p=0.041$), 120 minutes ($p=0.001$) and 210 minutes ($p=0.001$) post ketamine infusion there was a significant increase in PTc compared to placebo, indicating reduced pain sensitivity.
The graphs compare pain thresholds (mA) by group. The model adjusted means and 95% CI are shown. (A) Average baseline oesophageal pain thresholds (PTo); (B) average baseline pharyngeal pain thresholds and (C) average baseline chest wall pain thresholds (PTp) in healthy controls (HC) and chronic cough patients (CC). Compared to HC, CC patients have no significant difference in PTo, PTp or PTc at baseline.
Figure 13 – Change in pain thresholds over time

A. Oesophagus

The graphs show the change in the model adjusted mean pain thresholds over time after ketamine (red lines) compared to placebo (dashed grey lines). The error bars are 95% CI. The dotted area corresponds with the drug infusion. **A.** Oesophageal pain thresholds (PTo). Significant (p≤0.05*) increase in PTo at 30 minutes after ketamine compared to placebo. **B.** Pharyngeal pain thresholds (PTp). Significant (p≤0.05*) increase in PTp at 30 minutes after ketamine compared to placebo. **C.** Chest wall pain thresholds (PTc). Significant (p≤0.05*) increase in PTc at 30 minutes and 120 minutes after ketamine compared to placebo. Although the increases in PT reach statistical significance, the actual changes are small and may not be clinically significant.
### Table 7 - Model predicted mean (95% CI) pain thresholds and changes over time

#### A. Model predicted PT oesophagus (mean mA, 95% CI)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ketamine</td>
</tr>
<tr>
<td>0</td>
<td>52.65 (44.89, 60.42)</td>
</tr>
<tr>
<td>30</td>
<td>55.00 (47.97, 62.02)</td>
</tr>
<tr>
<td>120</td>
<td>51.74 (44.51, 58.96)</td>
</tr>
<tr>
<td>210</td>
<td>53.06 (45.69, 60.42)</td>
</tr>
<tr>
<td>300</td>
<td>54.01 (46.08, 61.94)</td>
</tr>
</tbody>
</table>

#### B. Model predicted PT pharynx (mean mA, 95% CI)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ketamine</td>
</tr>
<tr>
<td>0</td>
<td>33.55 (28.35, 38.77)</td>
</tr>
<tr>
<td>30</td>
<td>34.44 (27.81, 41.08)</td>
</tr>
<tr>
<td>120</td>
<td>32.47 (26.92, 38.02)</td>
</tr>
<tr>
<td>210</td>
<td>33.72 (28.44, 39.00)</td>
</tr>
<tr>
<td>300</td>
<td>35.67 (29.37, 41.97)</td>
</tr>
</tbody>
</table>

#### C. Model predicted PT chest wall (mean mA, 95% CI)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ketamine</td>
</tr>
<tr>
<td>0</td>
<td>39.81 (32.13, 47.48)</td>
</tr>
<tr>
<td>30</td>
<td>42.52 (34.73, 50.32)</td>
</tr>
<tr>
<td>120</td>
<td>41.34 (34.08, 48.59)</td>
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<tr>
<td>210</td>
<td>40.12 (33.09, 47.16)</td>
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<tr>
<td>300</td>
<td>40.32 (32.85, 47.79)</td>
</tr>
<tr>
<td>1440</td>
<td>41.84 (33.76, 49.92)</td>
</tr>
</tbody>
</table>
10.12 Cough frequency

Cough recordings failed on 2 of the visits therefore complete data is available for 9 CC patients and 11 HC. Spontaneous coughs and capsaicin-induced coughs were included in the data analysis.

Ketamine had no significant effect on cough frequency compared to placebo in either the HC or CC patients.

In HC, median (IQR) 24 hour cough frequency was 4.70 (2.75) coughs/hour after ketamine, compared to 4.40 (2.38) coughs/hour after placebo, p=0.444. In CC, median (IQR) 24 hour cough frequency was 13.00 (16.84) coughs/hour after ketamine, compared to 13.53 (14.85) coughs/hour after placebo, p=0.444.

In HC, median (IQR) cough frequency recorded for 5 hours and 30 minutes after the end of the infusion was 11.09 (6.00) coughs/hour after ketamine, compared to 10.54 (5.82) coughs/hour compared to after placebo, p=0.260. In CC, median (IQR) cough frequency recorded for 5 hours and 30 minutes after the end of the infusion was 25.27 (20.64) coughs/hour after ketamine, compared to 24.18 (21.82) coughs/hour after placebo, p=0.929.
10.13 Cough rates during electrical stimulation of the upper oesophagus, pharynx and chest wall

An unanticipated increase in cough frequency during and immediately after electrical stimulation of the oesophagus was observed. Therefore, an exploratory analysis was conducted to compare cough frequency during electrical stimulation of the oesophagus, pharynx and chest wall.

Cough rates during electrical stimulation were extremely negatively skewed due to a high proportion of 0 counts. Therefore the data could not be reliably modelled, even after transformation. Median values are compared within groups.

10.13.1 Average baseline cough rates during electrical stimulation

In HC, median (IQR) baseline cough rates during electrical stimulation of the oesophagus, pharynx and chest wall were in all cases 0.00 (0.00) coughs/minute.

In CC patients, average baseline cough rates were significantly higher during oesophageal stimulation compared to chest wall stimulation (p=0.002). There was no significant difference in cough rates during pharyngeal stimulation compared to chest wall stimulation (p=0.139).

Median (range) cough rates were 0.72 (0.00 to 10.86) coughs/minute during oesophageal stimulation, 0.04 (0.00 to 2.87) coughs/minute during pharyngeal stimulation and 0.00 (0.00 to 2.11) coughs/minute during chest wall stimulation, see Figure 14.

10.13.2 Effect of ketamine on cough rates during electrical stimulation of the oesophagus

In the CC patients, median (range) cough rates during electrical stimulation of the oesophagus changed from 0.31 (0.00 to 10.86) before ketamine to 0.56 (0.00 to 8.51) after ketamine, see Figure 15A. Median (range) cough rates changed from 0.89 (0.00 to 6.67) before saline to 0.26 (0.0 to 5.08) after saline, see Figure 15B.
Figure 14 – Baseline cough rates during electrical stimulation in chronic cough patients

This graph compares cough rates (coughs/minute) during electrical stimulation of the pharynx, oesophagus and chest wall in chronic cough patients. The median and range are shown. Each individual patient is labelled with a unique colour. Cough rates were significantly higher during electrical stimulation of the oesophagus compared to the chest wall. This suggests cough may be directly induced by oesophageal stimulation in CC patients.
Figure 15 – Effect of ketamine on cough induced by oesophageal stimulation

A

The graphs show the cough frequency during electrical stimulation of the oesophagus before and after ketamine (A) and placebo (B) in CC patients. Each line represents an individual patient. Note there are 2 missing subjects for ketamine (was not tolerated) and one missing subject for placebo (cough recording failed).
10.14 Summary of results

10.14.1 Effects of ketamine

- Compared to placebo, ketamine had no significant effect on capsaicin cough thresholds (C2/C5) or cough frequency in HC or CC patients
- Compared to placebo, ketamine had an acute analgesic effect on pain thresholds, with a similar effect in HC and CC patients.

10.14.2 Pain thresholds at baseline in CC patients compared to HC

- There were no significant differences in baseline pain thresholds between the CC patients and HC.

10.14.3 Cough rates during electrical stimulation

- In CC patients (but not HC), electrical stimulation of the oesophagus was associated with significantly higher cough frequencies than chest wall stimulation.
11 Discussion

Ketamine, a potent NMDA receptor antagonist, transiently raised pain thresholds and reduced attention (PASAT) scores, but did not suppress cough in CC patients or HC. CC patients demonstrated features of cough sensitisation including (i) lowered cough thresholds to inhaled capsaicin compared to HC, and (ii) increased cough frequency in response to electrical stimulation of the oesophagus. However, CC patients did not demonstrate increased sensitivity to pain, with no significant difference in baseline pain thresholds compared to HC. Taken together, these findings suggest that CC patients are selectively sensitised to cough, but not pain, and that cough sensitisation is independent of NMDA receptor-mediated mechanisms.

11.1 Ketamine had no significant anti-tussive effect compared to placebo

The finding that ketamine, a potent NMDA receptor antagonist, had no significant effect on capsaicin cough thresholds or spontaneous cough frequency in HC, suggests that the NMDA receptor does not regulate coughing. Furthermore, the same failure of ketamine to reduce cough in treatment-resistant CC patients suggests that pathological cough hypersensitivity is not maintained by the NMDA receptor. However, it is still possible that up-regulation of the NMDA receptor is involved in the initial induction of cough hypersensitivity, for example during acute upper respiratory tract infection.

Dextromethorphan, the active ingredient in many over-the-counter cough syrups, is also a weak NMDA receptor antagonist. In clinical trials, dextromethorphan consistently inhibits citric acid induced cough in healthy subjects[155-158] and significantly reduces cough in patients with acute viral cough[159]. Therefore, it is perhaps surprising that ketamine, a more potent NMDA receptor antagonist, failed to suppress cough in this study. However, dextromethorphan may reduce cough by alternative mechanisms e.g. sigma-1 agonist. Furthermore, in a large meta-analysis of 710 patients, dextromethorphan only reduced cough frequency by an average of 12-17%, an arguably small clinical effect. Thus, the present study of 24 subjects may be under-powered to detect a marginal antitussive effect. Indeed, based on the mean (SD) baseline Log C5 obtained in the CC patients, a sample size of at least 37 subjects would be required to detect a 20% change in cough sensitivity, with 90% power at the usual level of statistical significance (0.05), in a similar cross-over design.
There are several other possible explanations for the negative study findings. Firstly, the dose of ketamine could have been inadequate. This seems unlikely, however, since previous studies have shown that an identical dose of ketamine is sufficient to reverse central sensitisation of the oesophagus for at least 5 hours post-infusion[85]. Furthermore, in the present study, ketamine was administered at a pharmacologically active dose because all pain thresholds were transiently increased. This is consistent with other double-blind placebo-controlled trials confirming a short-lasting analgesic effect of low-dose ketamine[80-84]. Larger doses of ketamine could be more effective at suppressing cough, but because of the narrow therapeutic index would also increase the sedative side-effects, meaning the assessment of cough thresholds would be confounded.

Secondly, a single infusion of ketamine may not be sufficient to reduce cough sensitivity in CC. In the clinic, treatment trials are usually recommended for at least 2 months before assessing efficacy, therefore it is possible that prolonged NMDA receptor antagonism would be more effective. On the other hand, a single infusion of low dose ketamine can reduce neuropathic pain caused by trigeminal neuropathy for up to 24 hours[160], indicating that a benefit could have been observed within our study time period.

Thirdly, it is possible that ketamine has higher selectivity for NMDA receptors in the dorsal horn of the spinal cord rather than in the brainstem where cough is regulated, although the role of NMDA receptor sub-types in the regulation of cough is unknown. More selective NMDA receptor antagonists could demonstrate greater anti-tussive efficacy. Indeed, memantine a low-affinity NMDA receptor antagonist, almost completely abolished citric acid evoked cough in a conscious guinea-pig model, whereas ketamine had no significant effect (see appendix 1.3)[161].

Finally, in CC patients, cough sensitivity could have been reduced by the naso-oesophageal catheter that was positioned at the beginning of each of the randomised visits, and remained in place for the duration of the visit. Recent work in CC patients shows that placement of an oesophageal catheter reduces objectively quantified cough frequency by about one third, and therefore may desensitise cough[162].
explain the failure of ketamine to further suppress cough. The desensitising effect of a naso-oesophageal catheter on cough was unknown when the study was designed, but will be an important factor to consider for similar physiological studies in the future.

11.2 Chronic cough patients are sensitised to cough, but not pain

Pain sensitivity in the pharynx, upper oesophagus and chest wall was not significantly different in CC patients compared to HC. Although the study may have been underpowered to detect small differences in subjective pain thresholds, this suggests that CC patients are not sensitised to somatic/visceral pain. In contrast, in keeping with previous studies[17], CC patients had significantly lower cough thresholds to inhaled capsaicin compared to HC, indicating capsaicin cough hypersensitivity. Therefore, vagal afferent pathways mediating cough, but not spinal afferent pathways mediating pain, are likely to be selectively sensitised in these patients. Indeed, the neural pathways mediating cough are anatomically separate from those mediating pain.

11.2.1 Neural pathways mediating capsaicin-induced cough

Cough is mediated by the vagal nerve[163]. Spinal afferent fibres are unlikely to have a primary role[43]. Capsaicin initiates coughing by activating TRPV1 receptors located on bronchial c-fibres[45, 163], which terminate in the nTS in the brainstem (although the precise location is unknown[87]). From here, an urge-to-cough sensation is generated at a cortical level, to motivate a cough response[163].

11.2.2 Neural pathways mediating somatic pain

Noxious electrical stimulation of the chest wall activates high threshold polymodal somatic spinal afferent fibres (c-fibre and Aδ-fibres), which synapse in the thoracic dorsal horn of the spinal cord and project via the contra-lateral spino-thalamic tract to the cortex, where the intensity, duration and location of the noxious stimulus is encoded[67]. Therefore, central sensitisation of somatic pain pathways (spinally mediated) would be unlikely to influence cough (brainstem mediated) and vice versa.
11.2.3 Neural pathways mediating visceral pain

Processing of noxious visceral stimuli is more complex because there are 2 distinct afferent pathways including (i) vagal pathways projecting via the nodose and jugular ganglia to the nTS and (ii) spinal pathways projecting via the dorsal horn of the spinal cord[96]. It is generally believed that vagal afferents do not mediate the perception of pain, but rather contribute to the regulation of autonomic function, although there is some debate on this point[96]. Whilst peripheral sensitisation would non-specifically affect all afferent pathways, it could be predicted that sensitisation of nTS brainstem neurones would selectively up-regulate visceral vagal pathways, but not visceral spinal pathways.

The results of the present study support this notion of selective sensitisation because the oesophagus appeared to be sensitised to cough, but not pain, see Figure 16. Indeed, electrical stimulation of the oesophagus increased cough frequency in 5 of the 12 CC patients, but none of the HC. This suggests that a proportion of patients are centrally sensitised to vagal activation arising from either the oesophagus or bronchial airways, with convergence of vagal afferents onto the same sensitised nTS neurone. These findings do need to be interpreted with caution given that measurement of oesophageal sensitivity to cough was not an a priori hypothesis in the study design. Furthermore, an alternative explanation for these findings is that electrical stimulation of the oesophagus was directly transmitted to the trachea, where sensitised vagal terminals were activated to evoke cough.
Figure 16

A) Neural pathways in a healthy subject

This diagram shows the neural pathways mediating cough and pain in a healthy individual. Inhaled capsaicin activates vagal c-fibres which synapse in the brainstem and project ascending cortical pathways thereby generating an urge-to-cough sensation. Electrical stimulation of the oesophagus non-specifically activates spinal and vagal afferent fibres. Oesophageal spinal afferents synapse in the dorsal horn of the spinal cord, decussate and project ascending cortical pathways to generate an unpleasant pain sensation. Oesophageal vagal afferents provide sub-threshold input to nTS neurones in the brainstem, but do not elicit cough.
B) Peripheral sensitisation of oesophageal and airway afferent fibres

This diagram illustrates the up-regulation of cough and pain as a consequence of peripheral sensitisation in both the airways and oesophagus. In the airways, peripheral sensitisation lowers the threshold for activation of vagal afferent fibres by inhaled capsaicin. In the oesophagus, peripheral sensitisation lowers the threshold for activation of spinal and vagal afferent fibres by electrical stimulation. Sensitisation of oesophageal spinal afferents leads to lowered pain thresholds. Sensitisation of oesophageal vagal afferents, which converge on the same nTS neurones processing cough, means they may become capable of directly inducing cough.
This diagram illustrates the selective up-regulation of cough, but not pain, as a consequence of central sensitisation in the nTS in the brainstem. Normally sub-threshold input from airway and oesophageal vagal fibres activates hyper-excitable brainstem neurones. This manifests clinically as lowered thresholds for activation of cough following inhaled capsaicin or electrical stimulation of the oesophagus. However, spinally-mediated pain sensitivity remains normal.
11.3 Limitations of study design

1. Because of the small sample sizes in this pilot study, it may be under-powered (see above, section 4.1).
2. This was a complex study design, with multiple repeated measures within each subject, resulting in some missing data. In particular, 2 of the 12 CC patients were unable to tolerate the ketamine infusion and in 3 of the 12 HC, pharyngeal pain thresholds could not be recorded.
3. Because of the profound side-effects experienced by the majority of subjects during ketamine infusion, this study could not be adequately blinded.
4. The CC patients were significantly older than the HC. Although pain thresholds tend to increase with age[119, 164], I was unable to adjust for age in the models because all the CC patients were older than the HC. Age-matched groups would have been preferable.
5. At the time of study design, data showing that positioning of a naso-oesophageal catheter reduces cough frequency[162] was not known. In hindsight it would have been preferable to perform 2 separate studies in which (i) the effect of ketamine on cough sensitivity was investigated and (ii) visceral and somatic pain thresholds were compared by group.
6. The intensity of the electrical stimulation was gradually increased with no interspersed sham stimuli. This may have encouraged subjects to rate increasing pain intensities because of expectations of incremental intensity.

Given the limitations described above, future studies testing the effect of NMDA receptor antagonists in cough should be designed differently. Firstly, ketamine caused moderate levels of sedation meaning the study could not be adequately blinded. Therefore, it may be preferable to test other orally administered NMDA receptor antagonists with a better therapeutic index and less tendency to cause sedation e.g. memantine. Secondly, pharmacokinetic measurements could be taken to accurately determine the concentration-response relationship. Finally, repeated doses rather than single doses may show greater efficacy in patients. Objective measures of cough should be repeated after a suggested 2, 4 and 8 weeks of treatment.
12 Conclusion

This study provides further evidence of a central mechanism of cough sensitisation in CC patients, not mediated by the NMDA receptor.

Vagal afferent pathways from the airways and the oesophagus were sensitised in CC patients. This was demonstrated by:

(i) Lowered capsaicin cough thresholds in CC patients compared to HC
(ii) Increased cough frequency during oesophageal electrical stimulation in CC patients but not HC.

Conversely, spinal afferent pathways arising from the oesophagus, pharynx and anterior chest wall were not sensitised, with similar pain thresholds observed in CC patients and HC. The differential effect of oesophageal electrical stimulation on cough but not pain suggests a central rather than peripheral sensitisation mechanism. Further studies are required to identify pathways and receptor(s) involved in this central sensitisation.
CHAPTER 2

Comparing Capsaicin Dose-Response in Health and Disease
13 Introduction

13.1 Rationale

In chapter 1, it was demonstrated that capsaicin-induced cough is sensitised in chronic cough patients, most likely by central mechanisms. However, central sensitisation is only one possible mechanism by which patients may develop excessive cough, and other mechanisms may also play a role. For example, a failure of descending inhibitory pathways would increase the propensity for development of central sensitisation and could additionally amplify the encoding of cough within the CNS. A clinical test to differentiate one or more separate, but overlapping, mechanisms would be valuable in early stage drug development and in the clinical phenotyping of patients[165].

Inhaled tussive agents such as citric acid and capsaicin reproducibly evoke coughing in healthy volunteers and patients. In a standard cough challenge test, increasing doubling doses of tussive agent are inhaled up to a pre-determined cough threshold. The dose that induces at least 2 or 5 coughs is known as the C2 or C5. These end-points are reproducible[166], objective, quick and simple to perform[130, 131] and correlate with 24 hour cough frequency[15, 16]. However, they have several limitations. For example, although chronic cough patients have a lower C2/C5 on average, compared to healthy controls, there is substantial overlap of the 95% confidence intervals in health and disease[17]. Given the far higher 24-hour spontaneous cough rates observed in chronic cough patients, compared to healthy subjects, this is surprising, and may mean that C2/C5 measures are not capturing the most important patho-physiological mechanism(s).

In asthma, a research tool that has provided important mechanistic insights is methacholine challenge testing[167]. Increasing doses of methacholine (a broncho-constrictor agent) are inhaled, and the dose inducing a decrease in FEV1 of more than 20% is termed the PD20. Early in the development of this test, full methacholine dose-response curves were constructed to investigate the relationship between dose of methacholine and FEV1[168]. Airway hyper-responsiveness was characterized by 3 main features in asthmatics, compared to healthy controls (see Figure 17). Firstly, the curve was shifted to the left, indicating increased sensitivity. Secondly, the curve was steeper, indicating increased reactivity. Finally, asthmatics had a greater maximal
response. Subsequent attempts have been made to understand the mechanisms behind these observations. For example, the intensity of inflammation is associated with airway sensitivity, and airway wall thickness has been correlated with airway reactivity[169]. The plateau in maximum bronchial response is thought to be caused by mechanical forces that prevent complete bronchial closure[170].

13.2 Hypothesis

Therefore, it was hypothesized that capsaicin dose-response curves would provide insight into the neural mechanisms responsible for CC.

My predictions are shown in Figure 18. I predicted that compared to healthy controls, chronic cough patients would demonstrate:

(i) A left-shift of the dose-response curve, reflecting sensitisation of afferent cough pathways and lowering of cough thresholds
(ii) Increased steepness of the slope, reflecting an increased number of coughs or action potentials evoked by any given capsaicin dose
(iii) An increased maximal cough response, because of increased gain in the central encoding of cough and failed descending control pathways.

Previous studies have shown that mild/moderate asthmatics have increased capsaicin sensitivity compared to healthy controls[48], but lower spontaneous rates of cough frequency (2.6 cough seconds/hour)[50] than chronic cough patients (11.6 cough seconds/hour)[15]. Therefore, it was predicted that asthmatics would demonstrate a left-shift in the dose-response curve, but no change in the maximal cough response.

13.3 Aims

This study aimed to compare capsaicin dose-response curves in male and female chronic cough patients, asthmatics and healthy controls. To achieve this, novel capsaicin cough challenges were designed that extended beyond the usual cough thresholds (C2/C5), based on methodology by Morice et.al[171] and Davenport et.al. [59]. In the first capsaicin cough challenge, increasing doubling doses were sequentially administered, with no interspersed placebo doses. In the second capsaicin cough
challenge, the order of the doses was randomised in 4 blocks, and included interspersed placebo doses. For both challenges, each dose was inhaled 4 times.

**Figure 17** – Methacholine dose-response curves

Adapted from O’Byrne et.al.[168]. Methacholine dose-response curves plotting change in FEV1 with increasing doses of inhaled methacholine in moderate/severe and mild asthma compared to healthy controls. Asthmatics demonstrate a left-shift of the dose response curve, steeper curve and increased maximal bronchial response. Dotted lines indicate the PD20, the dose of methacholine inducing a decrease in FEV1 of at least 20%
Figure 18 – Predicted Capsaicin Dose-response Curves

Predicted capsaicin dose-response curves showing the change in number of coughs with increasing doses of capsaicin in healthy controls (A), asthmatics (B) and chronic cough patients (C). Asthmatics show a left-shift in the dose-response curve but no change in the maximal cough response. Chronic cough patients show a left-shift in the dose-response curve and an increase in maximal cough response.
14 Methodology

14.1 Subjects

Twenty healthy controls (HC), 20 asthmatics (A) and 20 chronic cough patients (CC) were enrolled. The eligibility criteria are described in section 7.1. The study was approved by a UK Research Ethics Committee (REC: 09/H1008/119) and registered at www.controlled-trials.com (ISRCTN65122210).

14.2 Study Design

All subjects attended 2 visits at least 24 hours apart. At visit 1 a series of questionnaires were completed including a Gastro-oesophageal reflux disease score (appendix 2.10), Sino-Nasal Outcome Test (appendix 2.2), Hospital Anxiety and Depression Scale (appendix 2.1) and the State-Trait Anxiety Index (appendix 2.6). Lung function (FEV1 and FVC), height and weight were measured.

At both visits a cough challenge test was performed using capsaicin that was inhaled in single-breaths through a dosimeter (Koko dosimeter, Ferraris Ltd, Hertford, UK) with inspiratory flow rate limitation. The number of coughs in the first 15 seconds after each inhalation was recorded, and urge-to-cough intensity was rated on a Modified Borg Scale (0-10). During both cough challenges subjects were attached to an ambulatory cough monitor (Vitalojak, UK) and instructed not to suppress coughing. The sound recordings were later used to verify number of coughs, defined as explosive sounds.

14.2.1 Challenge 1: Increasing doubling doses

At visit 1, increasing doubling doses of capsaicin were administered. Doses ranged from 0.48-1000µmol capsaicin, with 12 doses in total. Each inhalation was separated by 30 seconds and each dose of capsaicin was inhaled 4 times, see Figure 19. Subjects were told they would receive increasing doses of capsaicin during this challenge and that the challenge would continue to the maximum tolerated dose.
14.2.2 Challenge 2: Randomised cough challenge

At visit 2, 8 doses were individually determined from the first cough challenge to include the dose that induced an average of 5 coughs (or maximum tolerated dose), 6 doubling doses below and a placebo dose. These doses were administered in 4 blocks. The order of the doses was randomised to form a single presentation block, and then re-randomised for the 2nd, 3rd and 4th presentation blocks so that each dose was inhaled 4 times in a random order, see Figure 20. The inhalations were separated by 1 minute. Both subject and researcher were blinded to the randomisation codes.

Figure 19 – Design of Challenge 1

![Design of Challenge 1](image)

Figure 20 - Example of Challenge 2 design

![Example of Challenge 2 design](image)
14.2.3 Justification for method of inhalation

There are 2 methods by which tussive agents can be inhaled; tidal breathing and single-breath inhalation. Administration of a cumulative dose of tussive agent by tidal breath inhalation over pre-defined periods of time has shown large magnitudes of differences in average cough frequency in health and disease[172]. However, the dose administered is difficult to control because it varies with respiratory rate and depth[130, 131]. In contrast, single-breath inhalation through a dosimeter that has been modified to limit inspiratory flow rate delivers a more tightly controlled dose. Therefore to combine the advantages of both methods, each dose of capsaicin was administered by repeated single-breath inhalation over 4 occasions, to enable calculation of an average cough response at each dose.

14.2.4 Justification for use of placebo doses

Given that cough can be voluntarily initiated or suppressed and is known to be susceptible to placebo, the use of placebo doses in cough challenges has been recommended by the European Respiratory Society[131]. In challenge 1, however, there were no interspersed placebo doses. This is because in an incremental doubling dose challenge a placebo dose can be easily detected by patients, especially at the higher doses. In contrast, placebo doses were interspersed in challenge 2 in which the doses were presented in a randomised order to increase challenge blindness.

14.3 Statistical Methods

14.3.1 Generalised Estimating Equations

Repeated measures of cough frequency and urge-to-cough intensity were analysed for challenge 1 and challenge 2 using a series of generalized estimating equation (GEE) models, constructed in SPSS version 15.0. The data were classified and transformed as shown in Table 8. Both primary and secondary outcome variables (cough frequency and urge-to-cough intensity) were positively skewed because of a high proportion of 0 counts, therefore for the majority of models the outcome variables were log-transformed, or else a Poisson distribution was assumed. Predictor variables included dose of capsaicin (log-transformed), group (HC, A or CC) and gender. To investigate the relationship between urge-to-cough intensity and cough frequency, a separate series of
models were constructed using urge-to-cough as a predictor variable. The GEE models are summarized in Table 9.

**Table 8 - Classifying and transforming the data**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable description</th>
<th>Outcome or predictor?</th>
<th>Type of data</th>
<th>Transformed?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>Average number of coughs</td>
<td>Outcome</td>
<td>Scale</td>
<td>Poisson distribution or Log-transformed</td>
</tr>
<tr>
<td>Utc</td>
<td>Average urge-to-cough intensity</td>
<td>Outcome and predictor</td>
<td>Scale</td>
<td>Log transformed</td>
</tr>
<tr>
<td>Dose</td>
<td>Dose of capsaicin inhaled</td>
<td>Predictor</td>
<td>Ordinal</td>
<td>Log transformed</td>
</tr>
<tr>
<td>Group</td>
<td>1=HC, 2=A, 3=CC</td>
<td>Predictor</td>
<td>Nominal</td>
<td>No</td>
</tr>
<tr>
<td>Gender</td>
<td>0=female 1=male</td>
<td>Predictor</td>
<td>Nominal</td>
<td>No</td>
</tr>
</tbody>
</table>
Table 9 - GEE models

<table>
<thead>
<tr>
<th>GEE model</th>
<th>Outcome variables</th>
<th>Within subject variables</th>
<th>Between subject variables</th>
<th>Main effects</th>
<th>Interaction terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Cough Utc</td>
<td>Dose</td>
<td>Dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>Cough Utc</td>
<td>Dose</td>
<td>Group</td>
<td>Dose Group</td>
<td>Group*dose</td>
</tr>
<tr>
<td>Model 3</td>
<td>Cough Utc</td>
<td>Dose</td>
<td>Gender</td>
<td>Dose Gender</td>
<td>Gender*dose</td>
</tr>
<tr>
<td>Model 4</td>
<td>Cough Utc</td>
<td>Dose</td>
<td>Group Gender</td>
<td>Dose Group</td>
<td>Dose<em>group</em>gender</td>
</tr>
<tr>
<td>Model 5</td>
<td>Cough Utc</td>
<td>Dose</td>
<td>Group Gender</td>
<td>Dose Group</td>
<td>Gender*group</td>
</tr>
<tr>
<td>Model 6</td>
<td>Cough Utc</td>
<td>Utc</td>
<td>Utc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 7</td>
<td>Cough Utc</td>
<td>Utc</td>
<td>Group</td>
<td>Utc*group</td>
<td></td>
</tr>
<tr>
<td>Model 8</td>
<td>Cough Utc</td>
<td>Utc</td>
<td>Gender</td>
<td>Utc</td>
<td>Utc*gender</td>
</tr>
</tbody>
</table>

Utc=urge-to-cough

14.3.2 Comparing parameters between health and disease

The urge-to-cough threshold (dose that induced an average of at least 0.5 on the urge-to-cough scale across the 4 inhalations, corresponding to “very, very slight”) was compared by group and gender in challenge 1 and challenge 2.

In challenge 1, dose-response parameters were also compared by group and gender. These included the $C^{\text{max}}$ (maximal number of coughs induced at any dose during the challenge) and ED50 (dose that induces at least 50% of maximal cough response). The $C^{\text{max}}$ and ED50 are shown in Figure 18.

All doses were log transformed for analysis. Means and 95% confidence intervals were compared by group using independent t-tests.
15 Results

15.1 Subjects

Twenty HC (10 males), 18 A (9 males) and 20 CC patients (10 males) completed the study. Age, gender, body mass index, pack years smoking history and lung function are compared in Table 10. There was no significant difference in age (p=0.172), or FVC percent predicted (p=0.727) between groups, but CC and A had higher body mass index compared to HC (p=0.025). A patients had a significantly lower FEV1 percent predicted compared to HC/CC (p=0.032).

Table 10 – Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (HC)</th>
<th>Asthmatics (A)</th>
<th>Chronic Cough Patients (CC)</th>
<th>p-value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (years)*</td>
<td>57.05 (15.7)</td>
<td>51.17 (13.50)</td>
<td>58.83 (13.45)</td>
<td>0.172</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>10:10</td>
<td>9:9</td>
<td>10:10</td>
<td></td>
</tr>
<tr>
<td>BMI*</td>
<td>25.12 (3.93)</td>
<td>28.49 (3.93)</td>
<td>27.26 (3.42)</td>
<td>0.025¥</td>
</tr>
<tr>
<td>Pack years **</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.66)</td>
<td>0.00 (0.50)</td>
<td></td>
</tr>
<tr>
<td>FEV1 (L)*</td>
<td>3.2 (0.99)</td>
<td>2.96 (1.10)</td>
<td>3.02 (0.98)</td>
<td>0.727</td>
</tr>
<tr>
<td>FEV1%pred*</td>
<td>110.93 (14.83)</td>
<td>98.26 (18.86)</td>
<td>100.23 (12.68)</td>
<td>0.032¥</td>
</tr>
<tr>
<td>FVC (L)*</td>
<td>3.98 (1.12)</td>
<td>4.05 (1.36)</td>
<td>3.88 (1.15)</td>
<td>0.916</td>
</tr>
<tr>
<td>FVC%pred*</td>
<td>111.37 (17.69)</td>
<td>110.67 (18.14)</td>
<td>106.81 (11.75)</td>
<td>0.643</td>
</tr>
</tbody>
</table>

*mean (SD), **median (IQR), ¥p-value ≤0.05

15.1.1 Chronic Cough (CC) Patients

In CC patients the mean (SD) duration of cough was 15.25 (12.32) years. All patients had been investigated according to a routine clinical diagnostic protocol to identify underlying triggers for the cough; the results of these clinical investigations are presented in Table 11.

Six (30%) of the CC patients had a positive bronchial provocation test, but subsequently failed to respond to treatment with inhaled or oral corticosteroids, and a clinical diagnosis of asthma was excluded. Five (25%) of the CC patients had post-nasal discharge on nasendoscopy, but subsequently failed to respond to nasal steroids. Six (30%) of the CC patients had evidence of laryngo-pharyngeal reflux on nasendoscopy or abnormal 24 hour impedance/pH testing, but failed to respond to high dose proton pump inhibitor. In
all CC patients, the CT scan findings were not thought to be clinically significant, and did not sufficiently explain the cause of the cough. Therefore all CC patients were classified as “treatment-resistant” or “idiopathic” chronic cough.

**Table 11 - Clinical Investigations for Chronic Cough Patients**

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Number (%) patients</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial Provocation</td>
<td>13 (65%)</td>
<td>Positive = 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative = 7</td>
</tr>
<tr>
<td>Nasendoscopy</td>
<td>15 (75%)</td>
<td>Normal = 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PND* = 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LPR** = 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PND and LPR = 1</td>
</tr>
<tr>
<td>Bronchoscopy</td>
<td>18 (90%)</td>
<td>Normal = 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sticky secretions = 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TPO*** = 1</td>
</tr>
<tr>
<td>CT thorax</td>
<td>19 (95%)</td>
<td>Normal = 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild bronchiectasis = 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild scarring = 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild bronchial dilatation = 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild non-specific inflammatory change = 1</td>
</tr>
<tr>
<td>24 hour impedance/pH</td>
<td>9 (45%)</td>
<td>Normal = 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abnormal = 2</td>
</tr>
</tbody>
</table>

*PND=post-nasal drip, **LPR=laryngo-pharyngeal reflux, ***TPO=tracheopathia osteoplastica

15.1.2 Asthmatics (A)

Fifteen A patients (83.3%) had documented clinical evidence of bronchial hyper-reactivity and the remaining 3 patients (16.67%) had documented clinical evidence of significant reversibility in FEV1 (>12%). Fourteen (77.78%) were taking regular inhaled corticosteroids.
15.2 Questionnaires

The results of the questionnaires are presented in Table 12. Compared to HC and A, the CC patients had significantly higher Sino-Nasal Outcome Test (SNOT) (p<0.001) and reflux score (p<0.001). State anxiety scores, measured using the State-Trait Anxiety Index (STAI), were similar between groups (p=0.767). Depression scores, measured using the Hospital Anxiety and Depression (HADS) scale were also similar between groups (p=0.153). CC patients showed a trend towards higher STAI trait anxiety (p=0.075) and higher HADS anxiety (p=0.099) scores compared to A and HC.

Table 12 - Questionnaires

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Healthy Controls (HC)</th>
<th>Asthma (A)</th>
<th>Chronic Cough (CC)</th>
<th>p-value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNOT</td>
<td>0.18 (0.23)</td>
<td>0.58 (0.59)</td>
<td>1.87 (1.38)</td>
<td>&lt;0.001¥</td>
</tr>
<tr>
<td>Reflux Score</td>
<td>0.60 (1.19)</td>
<td>5.72 (10.04)</td>
<td>13.75 (9.90)</td>
<td>&lt;0.001¥</td>
</tr>
<tr>
<td>HADS-depression</td>
<td>2.70 (3.8)</td>
<td>2.11 (2.17)</td>
<td>4.0 (2.83)</td>
<td>0.153</td>
</tr>
<tr>
<td>HADS-anxiety</td>
<td>4.10 (3.02)</td>
<td>5.00 (3.36)</td>
<td>6.60 (4.38)</td>
<td>0.099≠</td>
</tr>
<tr>
<td>STAI-state</td>
<td>26.20 (7.66)</td>
<td>26.56 (7.21)</td>
<td>27.90 (8.26)</td>
<td>0.767</td>
</tr>
<tr>
<td>STAI-trait</td>
<td>30.05 (8.19)</td>
<td>35.28 (11.19)</td>
<td>37.25 (10.70)</td>
<td>0.075≠</td>
</tr>
</tbody>
</table>

Means (SD) shown. ¥p-value ≤0.05, ≠p-value ≤0.1. HADS=Hospital Anxiety and Depression scale. STAI=State-Trait Anxiety Scale. SNOT=Sino-Nasal Outcome Test.
15.3 Analysis Using Generalised Estimating Equations

15.3.1 Cough Frequency

The results of models 1-5 for cough frequency are summarized in Table 13.

As expected, cough frequency significantly increased with increasing dose of inhaled capsaicin (challenge 1, p<0.001; challenge 2, p<0.001), see Figure 21.

**Challenge 1**
CC patients had significantly higher cough frequencies than HC or A with increasing capsaicin dose as demonstrated in model 2 (dose*group, p=0.038), see Figure 22a.

Females had significantly higher cough frequencies than males with increasing capsaicin dose as demonstrated in model 3 (dose*gender, p=0.002), Figure 23a. As demonstrated in model 5, gender effects were not group specific, (gender*group, p=0.861) and within each disease category females coughed more frequently than males (model 4; gender, p<0.001), see Figure 24a.

**Challenge 2**
CC patients had significantly higher cough frequencies than HC or A with increasing capsaicin dose as demonstrated in model 2 (dose*group, p<0.001), see Figure 22b.

Females had significantly higher cough frequencies than males with increasing capsaicin dose as demonstrated in model 3 (dose*gender, p=0.001), see Figure 23b. As shown in model 5, gender effects were not group specific (gender *group, p=0.606) and within each group females coughed more frequently than males (gender, p=0.024), see Figure 24b, model 4.
Table 13 - Outcome Variable = Cough Frequency, Models 1-5

<table>
<thead>
<tr>
<th>Challenge 1</th>
<th>Challenge 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main Effects</strong></td>
<td><strong>Interaction Terms</strong></td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
<td>Dose</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td>Dose</td>
</tr>
<tr>
<td></td>
<td>Group</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td>Dose</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
</tr>
<tr>
<td><strong>Model 4</strong></td>
<td>Dose</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
</tr>
<tr>
<td></td>
<td>Group</td>
</tr>
<tr>
<td><strong>Model 5</strong></td>
<td>Dose</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
</tr>
<tr>
<td></td>
<td>Group</td>
</tr>
</tbody>
</table>

¥p-value ≤0.05
Figure 21 – Change in cough frequency with increasing capsaicin dose (model 1)

The means values and 95% CI predicted from GEE model 1 are shown for challenge 1 (a) and challenge 2 (b). The average number of coughs increases with increasing doses of inhaled capsaicin for all subjects. Group was not included in this model. Wald parameter estimates indicate where a given capsaicin dose significantly (p≤0.05*) increases the average number of coughs, compared to the lowest capsaicin dose. Note the ln scale for cough frequency in challenge 2.
The means values and 95% CI predicted from GEE model 2 are shown for challenge 1 (a) and challenge 2 (b). The average number of coughs increases with increasing doses of inhaled capsaicin for all subjects. However, CC patients had higher cough frequencies than HC/A, especially at higher doses. This reached statistical significance in challenge 1 (p=0.038) and challenge 2 (p<0.001). Wald parameter estimates indicate where the interaction term between CC group and any given dose is significant (*p≤0.05). Group differences appear to be greater in challenge 1, but note the Ln scale for cough frequency in challenge 2.
Figure 23 – Change in cough frequency with increasing capsaicin dose compared by gender (model 3)

The mean values and 95% CI predicted from GEE model 3 are shown. The average number of coughs increases with increasing doses of inhaled capsaicin for all subjects. However, females had higher cough frequencies than males, especially at higher doses. This reached statistical significance in challenge 1 ($p=0.002$), and challenge 2 ($p=0.001$). Wald parameter estimates indicate where the interaction term between gender and any given dose is significant ($^*p\leq0.05$). Note the Ln scale for cough frequency in challenge 2.
Figure 24 – Change in cough frequency with increasing capsaicin dose compared by group and gender (model 4)

The mean values predicted from GEE model 4 are shown. The average number of coughs increases with increasing doses of inhaled capsaicin for all subjects. As was also demonstrated by model 3, females had higher cough frequencies than males. This reached statistical significance in challenge 1 (p<0.001) and challenge 2 (p=0.024). The gender differences were not group specific and within each group females had higher cough frequencies than males. The gender differences appear greater in challenge 1, but note the Ln scale for cough frequency in challenge 2. As was also demonstrated by model 2, CC patients had higher cough frequency than HC/A. This reached statistical significance in challenge 1 (p<0.001) and challenge 2 (p<0.001).
15.3.2 Urge-to-Cough Intensity

The results of models 1-5 for urge-to-cough intensity are summarized in Table 14.

As expected, urge-to-cough intensity significantly increased with increasing dose of inhaled capsaicin (challenge 1, p<0.001; challenge 2, p<0.001), see Figure 25.

Challenge 1

CC patients reported significantly higher urge-to-cough intensity compared to HC or A with increasing capsaicin dose as demonstrated in model 2 (dose*group, p=0.002), Figure 26a.

Females reported significantly higher urge-to-cough intensity compared to males with increasing capsaicin dose as demonstrated in model 3 (dose*gender, p<0.001), Figure 27a. As shown in model 5, gender effects were not group specific (gender*group, p=0.114), and within each disease group females reported higher urge-to-cough than males (model 4, p=0.013), Figure 28a.

Challenge 2

CC patients reported significantly higher urge-to-cough intensity compared to HC or A with increasing capsaicin dose as demonstrated by model 2 (dose*group, p<0.001), see Figure 26b.

In females compared to males, there was no significant difference in reported urge-to-cough intensity with increasing capsaicin dose as demonstrated in model 3 (dose*gender, p=0.171), see Figure 28b. However, females reported a trend towards higher urge-to-cough intensity than males as shown in model 4 (gender, p=0.059), see Figure 28b. As demonstrated in model 5, gender effects are not group specific (gender*group, p=0.450).
Table 14 - Outcome Variable = Urge-to-cough Intensity, Models 1-5

<table>
<thead>
<tr>
<th>Challenge 1</th>
<th>Main Effects</th>
<th>Interaction Terms</th>
<th>Challenge 2</th>
<th>Main Effects</th>
<th>Interaction Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Dose p&lt;0.001¥</td>
<td></td>
<td>Dose</td>
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<td>Dose* Group p=0.001¥ p=0.003¥</td>
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<td>Dose* Gender p&lt;0.001¥</td>
<td>Dose Gender p&lt;0.001¥ p=0.203</td>
<td>Dose*Gender p=0.171</td>
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<td>Model 4</td>
<td>Dose Gender Group p&lt;0.001¥ p=0.013¥ p=0.017¥</td>
<td>Dose* Gender*Group p&lt;0.001¥</td>
<td>Dose Gender Group p&lt;0.001¥ p=0.059≠ p=0.002¥</td>
<td>Dose<em>Gender</em>Group P&lt;0.001¥</td>
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<td>Model 5</td>
<td>Dose Gender Group p&lt;0.001¥ p=0.041¥ p=0.111</td>
<td>Gender*Group p=0.114</td>
<td>Dose Gender Group p&lt;0.001¥ p=0.121 p=0.006¥</td>
<td>Gender*Group P=0.450</td>
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<td>p=0.041¥</td>
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¥p-value ≤0.05, ≠p-value ≤0.1
Figure 25 – Change in urge-to-cough intensity with increasing capsaicin dose (model 1)

The mean values and 95% CI predicted from GEE model 1 are shown. The average urge-to-cough intensity increases with increasing doses of inhaled capsaicin for all subjects. Group was not included in this model. Wald parameter estimates indicate where a given dose significantly (*p≤0.05) increases the average urge-to-cough, compared to the lowest dose.
The mean values and 95% CI predicted from GEE model 2 are shown. The average urge-to-cough intensity increases with increasing doses of inhaled capsaicin for all subjects. Compared to HC, CC patients reported a higher urge-to-cough intensity with increasing capsaicin dose. This reached statistical significance in challenge 1 ($p=0.002$) and challenge 2 ($p<0.001$). Wald parameter estimates indicate where the interaction term between CC group and any given dose is significant (*$p\leq0.05$).
The mean values and 95% CI predicted from GEE model 3 are shown. The average urge-to-cough intensity increases with increasing doses of inhaled capsaicin for all subjects. Compared to males, females reported a higher urge-to-cough intensity with increasing capsaicin dose. This reached statistical significance in challenge 1 (p<0.001) but not challenge 2 (p=0.171). Wald parameter estimates indicate where the interaction term between gender and any given dose is significant (*p≤0.05).

Figure 27 – Change in urge-to-cough intensity with increasing capsaicin dose compared by gender (model 3)
The mean values predicted from GEE model 4 are shown. The average urge-to-cough intensity increases with increasing doses of inhaled capsaicin for all subjects. Females reported higher urge-to-cough compared to males. This reached statistical significance in challenge 1 (p=0.013), but not in challenge 2 (p=0.059). As also demonstrated by model 2, CC patients tend to report higher urge-to-cough compared to HC/A. This reached statistical significance in challenge 1 (p=0.017) and challenge 2 (p=0.002).
15.3.3 Relationship between urge-to-cough intensity and cough frequency

As expected, cough frequency significantly increases with increasing urge-to-cough intensity, (p<0.001 for challenge 1 and p<0.001 for challenge 2, see Figure 29, model 6). However, for any given reported urge-to-cough intensity, CC patients coughed more frequently than A or HC in challenge 1 (Utc*group, p<0.001) and challenge 2 (Utc*Group, p<0.001), see Figure 30, model 7. Furthermore, for any given reported urge-to-cough intensity, females coughed more frequently than males in challenge 1 (Utc*gender, p=0.006) but not challenge 2 (Utc*gender, p=0.480), see Figure 31, model 8.
<table>
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<td>Utc* Gender</td>
<td>p&lt;0.001$\text{¥}$</td>
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<td>Utc* Gender</td>
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$\text{¥}p$-value ≤0.05
Figure 29 – Change in cough frequency with increasing urge-to-cough intensity (model 6)

The mean values predicted from GEE model 6 are shown. Average cough frequency increases with increasing reported urge-to-cough intensity. Group was not included in this model. Significant wald parameter estimates indicate where a given urge-to-cough intensity was associated with a significant (*p≤0.05) increase in cough frequency.
Figure 30 – Change in cough frequency with increasing urge-to-cough intensity compared by group (model 7)

The mean values predicted from GEE model 7 are shown. Cough frequency increases with increasing reported urge-to-cough intensity for all subjects. However, for any given reported urge-to-cough intensity, CC patients coughed more frequently than HC/A. This trend reached statistical significance in challenge 1 (p<0.001) and challenge 2 (p<0.001). Wald parameter estimates indicate where the interaction term between group and any given urge-to-cough intensity is significant (*p≤0.05).
**Figure 31** – Change in cough frequency with increasing urge-to-cough intensity compared by gender (model 8)

The mean values predicted from GEE model 8 are shown. The average cough frequency increases with increasing reported urge-to-cough for all subjects. However, for any given reported urge-to-cough intensity, females coughed more frequently than males. This reached statistical significance in challenge 1 ($p=0.006$) but not challenge 2 ($p=0.480$). Wald parameter estimates indicate where the interaction term between gender and any given urge-to-cough intensity is significant (*$p$*$\leq 0.05$).
15.3.4 Summary of Generalised Estimating Equations

With increasing doses of inhaled capsaicin, CC patients had significantly higher cough frequencies and reported significantly higher urge-to-cough intensity than HC or A. Furthermore, for any given reported urge-to-cough intensity, CC patients had significantly higher cough frequencies than HC or A.

With increasing doses of inhaled capsaicin, females had significantly higher cough frequencies than males. Females also reported higher urge-to-cough intensity than males with increasing doses, but this only reached statistical significance in challenge 1. Furthermore, for any given reported urge-to-cough intensity, females had significantly higher cough frequencies than males in challenge 1 but not challenge 2.
15.4 Urge-to-cough threshold

15.4.1 Comparison by group

In challenge 1, there were no significant differences in urge-to-cough threshold between HC and A (p=0.553) or between HC and CC (p=0.863). The mean (95% CI) Ln urge-to-cough threshold was 1.78 (0.94 to 2.61) in HC, 1.86 (1.31 to 2.41) in A and 1.49 (1.00 to 2.00) in CC, see Figure 32A.

In challenge 2, there were no significant differences in urge-to-cough threshold between HC and A (p=0.618). However, there was a trend towards lower urge-to-cough thresholds in CC compared to HC (p=0.074). The mean (95% CI) Ln urge-to-cough threshold was 3.16 (2.36 to 3.97) in HC, 2.94 (2.49 to 3.40) in A and 2.30 (1.73 to 2.87) in CC, see Figure 32B.

15.4.2 Comparison by gender

In challenge 1, females had significantly lower urge-to-cough threshold than males (p=0.011). The mean (95% CI) Ln urge-to-cough threshold was 1.26 (0.82 to 1.71) in females and 2.15 (1.62 to 2.68) in males, see Figure 33A.

In challenge 2, females had significantly lower urge-to-cough threshold than males (p=0.007). The mean (95% CI) Ln urge-to-cough threshold was 2.32 (1.85 to 2.78) in females and 3.24 (2.76 to 3.79) in males, see Figure 33B.
Figure 32 – Urge-to-cough threshold compared by group

A  Challenge 1

B  Challenge 2

Urge-to-cough threshold compared by group in challenge 1 (A) and challenge 2 (B). Bars represent means and 95% confidence intervals. Each data point represents the dose of capsaicin inducing an average urge-to-cough intensity of at least 0.5 on the urge-to-cough scale across 4 inhalations per individual.
Figure 33 – Urge-to-cough thresholds compared by gender

A  Challenge 1

B  Challenge 2

Urge-to-cough threshold compared by gender in challenge 1 (A) and challenge 2 (B). Bars represent means and 95% confidence intervals. Each data point represents the dose of capsaicin inducing an average urge-to-cough intensity of at least 0.5 on the urge-to-cough scale across 4 inhalations per individual. Compared to males, females have a significantly lower urge-to-cough threshold in challenge 1 and challenge 2.
15.5 Dose-response parameters in challenge 1

15.5.1 Comparison by group

CC patients had a significantly lower ED50 compared to HC (p=0.024). However, ED50 was not significantly different between A and HC (p=0.724). The mean (95% CI) Ln ED50 was 3.66 (3.13 to 4.20) in HC, 3.79 (3.26 to 4.32) in A and 2.78 (2.24 to 3.33) in CC, see Figure 34A.

CC patients had a significantly higher $C_{\text{max}}$ compared to HC (p<0.001). However, $C_{\text{max}}$ was not significantly difference between A and HC (p=0.138). The mean (95% CI) $C_{\text{max}}$ was 15.35 (11.53 to 19.17) in HC, 19.28 (15.43 to 23.13) in A and 30.2 (25.01 to 35.39) in CC, see Figure 34B.

15.5.2 Comparison by gender

There was no significant difference in ED50 in females compared to males (p=0.167). The mean (95% CI) Ln ED50 was 3.18 (2.80 to 3.55) in females and 3.62 (3.09 to 4.14) in males, see Figure 35A.

Females had a significantly higher $C_{\text{max}}$ compared to males (p=0.001). The mean (95% CI) $C_{\text{max}}$ was 26.28 (22.78 to 29.77) in females and 17.10 (12.92 to 21.29) in males, see Figure 35B.
Bars represent means and 95% confidence intervals. 

**A.** ED50 compared by group in challenge 1. Each data point represents the dose of capsaicin inducing at least 50% of maximal cough response per individual. 

**B.** C$^{\text{max}}$ compared by group in challenge 1. Each data point represents the maximal number of coughs induced at any dose across 4 inhalations per individual. CC patients have significantly lower ED50 and significantly higher C$^{\text{max}}$ compared to HC. There is no significant difference in ED50 or C$^{\text{max}}$ in A compared to HC.
Figure 35 – Dose-response parameters compared by gender

A. **ED50**

Bars represent means and 95% confidence intervals. A. ED50 compared by gender in challenge 1. Each data point represents the dose of capsaicin inducing at least 50% of maximal cough response per individual. B. **Cmax** compared by gender in challenge 1. Each data point represents the maximal number of coughs induced at any dose across 4 inhalations per individual. Compared to males, females have significantly higher Cmax, but no significant difference in ED50.
15.6 Correlation of dose-response parameters with 24 hour spontaneous cough frequency

In a separate study of 20 chronic cough patients and 20 healthy controls (see chapter 3), ED50 and $C^{\text{max}}$ were measured using challenge design 1, and as an exploratory analysis were correlated with 24 hour spontaneous cough frequency using Pearson's correlation. Twenty-four hour cough frequency and ED50 were Ln transformed to normalise the distributions. $C^{\text{max}}$ explained a greater proportion of the variability in 24 hour cough frequency (50%, $r=0.711$, $p<0.001$) than ED50 (10%, $r=-0.318$, $p=0.026$). After controlling for ED50, $C^{\text{max}}$ continued to explain 45% of the variability in 24 hour cough frequency ($r=0.672$, $p<0.001$) suggesting that $C^{\text{max}}$ and ED50 capture separate mechanisms. Scatterplots of the relationships between these variables are shown in Figure 40.

15.7 Summary of urge-to-cough threshold and dose response parameters

In females compared to males:
- Urge-to-cough thresholds are lower
- $C^{\text{max}}$ is higher
- ED50 is no different

In CC patients compared to HC:
- Urge-to-cough threshold is similar
- $C^{\text{max}}$ is higher
- ED50 is lower

In A patients compared to HC:
- No differences

$C^{\text{max}}$ shows a stronger correlation with 24 hour cough frequency than ED50.
Figure 40 – Scatterplots of ED50, $C_{\text{max}}$ and 24 hour cough frequency

A

Scatterplots show the relationship between 24 hour spontaneous cough frequency, $C_{\text{max}}$ and ED50. Note the Ln scales for ED50 and cough frequency. 

A. Higher 24 hour cough frequency is associated with a higher $C_{\text{max}}$.

B. Higher 24 hour cough frequency is associated with a lower ED50 (increased cough sensitivity). $C_{\text{max}}$ explains a greater proportion of the variability in cough frequency than ED50. There appears to be extensive overlap of ED50 between patient groups, but less overlap with $C_{\text{max}}$.  

B
16 Discussion

Using novel cough challenge designs, the relationship between inhaled capsaicin dose, cough frequency and urge-to-cough intensity has been explored in male and female chronic cough patients, asthmatics and healthy controls. The data were analysed using (i) generalised estimating equations (GEE) and (ii) unpaired t-tests of urge-to-cough threshold and dose-response parameters.

(i) Chronic cough patients demonstrate a left-shift in the capsaicin dose-response curve (decreased ED50) compared to healthy controls and asthmatics

(ii) Chronic cough patients demonstrate significantly higher cough frequencies in response to any given capsaicin dose compared to healthy controls and asthmatics

(iii) Chronic cough patients demonstrate an increased maximal cough response compared to healthy controls and asthmatics

(iv) Females demonstrate significantly higher cough frequencies in response to any given capsaicin dose than males, and have higher maximal cough response

(v) Asthmatics demonstrate no significant difference in capsaicin dose-response curves compared to healthy controls

(vi) Urge-to-cough intensity predicts cough frequency, but the relationship differs in chronic cough patients compared to healthy controls and asthmatics.

16.1 Chronic cough patients (CC) demonstrate a left-shift in the capsaicin dose-response curve compared to healthy controls (HC) and asthmatics (A)

As predicted, CC patients had a significant decrease in ED50 compared to healthy controls, indicating a left-shift in the capsaicin dose-response curve and a lowered threshold for activation of cough. Taken together, these findings suggest that CC patients have a hypersensitive cough response to capsaicin (cough hypersensitivity).

In support of these findings, several previous studies have shown that CC patients have significantly lower cough thresholds (increased cough sensitivity) compared to HC in
response to experimentally inhaled stimuli including capsaicin[17, 39, 173] and citric acid[127]. Furthermore, CC patients often describe lowered cough thresholds (increased cough sensitivity) in response to environmental stimuli/irritants. A retrospective review of 135 patients attending a specialist cough clinic found that two thirds of patients tend to cough in response to normally innocuous stimuli such as a change in air temperature and/or when talking, laughing and singing[140].

Lowered cough thresholds indicate that normally innocuous stimuli have now become capable of eliciting cough. This is analogous to allodynia, where normally innocuous stimuli become capable of eliciting pain e.g. pain in response to lightly stroking the skin after a burn injury to the hand. Pain and cough sensitisation may arise by one or more neurophysiological mechanisms at the level of the peripheral nerve terminal, axons, ganglia or central synapse.

Firstly, cough sensitisation may occur because of changes in peripheral nerve terminals:

(i) Local inflammatory mediators may phosphorylate TRP and voltage-gated channels, thereby lowering their threshold for activation[69], see figure 20. In support of this, there is some evidence for the presence of chronic airway inflammation in CC patients, with higher levels of inflammatory mediators in induced sputum compared to healthy controls[174, 175].

(ii) Trafficking of TRPV1 receptors to the cell surface may lead to an increase in expression on nerve terminals[65], see Figure 2. In this instance, the dose of inhaled capsaicin that is able to generate sufficient depolarization in the nerve terminal to produce an action potential may be lowered, and consequently the threshold for evoking cough would be reduced. Animal studies have shown an increased expression of TRPV1 on sensory afferents nerves in the airways under conditions of allergic inflammation[22, 176]. A single small study in humans has shown an increase in TRPV1-expressing nerves in CC patients in comparison to HC, despite there being no difference in total nerve density[177]. Furthermore, a significant correlation between capsaicin cough thresholds and density of TRPV1-expressing nerves was shown. However, the TRPV1 antibody may also have stained non-neuronal tissue, raising concerns over the validity and interpretation of these results.
Novel expression of TRPV1 could expand the population of neurons able to respond to capsaicin. This possibility was illustrated in a recent study by Lieu et al.[36]. Vagal sensory neurons originating from guinea-pigs trachea were identified by retrograde labelling techniques. Dye was injected into the tracheal wall, subsequently taken up by the nerve terminals and transported back to the cell bodies. Ganglions were then dissociated and neurons containing the dye studied using single neuron RT-PCR. TRPV1 was not normally expressed in nodose-derived Aδ-fibres innervating the trachea. However, direct tracheal application of Brain-Derived Neurotrophic Factors (BDNF) led to novel gene expression of TRPV1 in these same neurons, indicating that they had undergone a phenotypic switch. It is not known whether the increased TRPV1 gene expression translates into an up-regulation of functional TRPV1 proteins.

Secondly, increased nerve terminal density could sensitise cough. O'Connell et al.[178] showed that the density of nerves in the bronchial epithelium immune-reactive for PGP, substance P (SP) and calcitonin-gene related peptide (CGRP) was increased in CC compared to healthy controls, although the clinical significance of these very small changes in nerve density are uncertain. Indeed, PGP-immunoreactive nerve density showed no significant correlation with capsaicin cough sensitivity. In the somatic nervous system, “sprouting” of peripheral nerve fibres may arise following nerve crush injuries as a consequence of the release of neurotrophic factors[179]. Whether these mechanisms are important in the airways is unknown.

Thirdly, sensitization of cough could arise as a result of changes within the axons/ganglia. Indeed, in the pelvis, cross-excitation of spatially close neurons innervating two different organs has been proposed to occur in the ganglia or nerve bundles following local release of chemicals (e.g. neuropeptides or excitatory neurotransmitters) or as a result of electrical coupling[180]. Furthermore, so-called “dichotomizing fibres” represent a single neuron which projects two sensory endings innervating two different tissues e.g. the colon and bladder. In relation to cough, these proposed mechanisms of cross-organ sensitisation could explain why inflammation in the oesophagus leads to increased sensitivity of cough, particularly given that the
oesophagus and respiratory tract have shared embryological origins. However, only 2 CC patients in this study had evidence of abnormal gastro-oesophageal reflux.

Finally, sensitisation of cough could arise following changes within the central nervous system. Lowered cutaneous pain thresholds to mechanical stimuli have been demonstrated in established models of central sensitization in animals and humans[74, 181]. This is thought to arise as a result of increased central synaptic efficiency and/or an increase in the excitability of central neurons, and leads to the recruitment of previously sub-threshold afferent input[67]. These mechanisms could also apply to cough. To illustrate this possibility, bradykinin, a selective c-fibre stimulant, failed to evoke cough in anaesthetized guinea-pigs when applied topically to the trachea or when nebulised into the distal airways. However, bradykinin lowered the threshold for evoking cough by electrical stimulation of the trachea. This was replicated by micro-injection of capsaicin or substance P into the nucleus tractus solitarius (nTS) in the brainstem[89], suggesting that cough has the potential to be sensitised following c-fibre activation via central brainstem-mediated mechanisms.

In summary, an increased sensitivity of cough may arise through peripheral and/or central mechanisms, with lowered thresholds for activation operating within peripheral vagal afferent nerve terminals and/or within central integrating neurons located in the nTS in the brainstem.
16.2 Chronic cough (CC) patients demonstrate significantly higher cough frequencies than healthy controls (HC) and asthmatics (A)

For any given capsaicin dose, CC patients demonstrated a greater magnitude of cough frequency than HC/A, which could be described as an “up-shift” or amplification of the dose-response curve.

The differentiation of cough hypersensitivity (left-shift) and cough amplification (up-shift) in exploring underlying mechanisms in CC is crucial. As mentioned previously, cough hypersensitivity may arise as a result of lowered neuronal thresholds for action potential initiation, consequently allowing cough to be activated in response to normally innocuous stimuli. In contrast, cough amplification represents an increased cough response to noxious airway stimuli. This is analogous to the increased magnitude of pain in response to painful stimuli (hyperalgesia) reported by patients with chronic/neuropathic pain.

Underlying neurophysiological mechanisms responsible for hyperalgesia include an increase in the number, frequency and amplitude of action potentials generated by standard noxious stimuli[182]. For example, in humans, intra-dermal injection of capsaicin leads to a diffuse area of hyperalgesia in which painful stimuli provoke an increased magnitude of pain intensity when compared to control injection, and in animals, intra-dermal capsaicin injection correspondingly leads to an increase in the number and frequency of action potential discharges in central neurons[181]. In relation to the airways, firing frequency of afferent pathways has not yet been directly correlated with the number of coughs. However, the non-selective local anaesthetic agent mexilitine not only reduces the number of action potentials and peak frequency of activation in nodose ganglia neurons evoked by citric acid in vitro, but also markedly reduces cough frequency evoked by citric acid in anaesthetised guinea-pigs in vivo[183], see Figure 36. Furthermore, only high frequency electrical stimulation of the trachea is sufficient to elicit coughing in anaesthetised animals, with higher frequencies of stimulation eliciting a higher cumulative number of coughs[184]. However, neither of these animal models investigated c-fibre evoked cough. Therefore, this needs to be interpreted with caution in relation to this study in which the selective c-fibre stimulant capsaicin was used.
Figure 36 – Effect of mexiletine on citric acid evoked cough in guinea-pigs

Reproduced with permission. Canning 2007. [183, 185] Figures show the effect of mexiletine, a non-selective local anaesthetic agent, on the number of action potentials in vitro and cumulative number of coughs in vivo. A: In vitro study of nodose derived neurons projecting to the trachea. Mexiletine reduces the number of action potentials (shown) and the peak frequency of activation (not shown) evoked by citric acid. B: In vivo study of anaesthetized guinea-pigs. Mexiletine inhibits cough evoked by citric acid applied topically to the trachea. Mexiletine did not affect the neuronal thresholds for activation by acid, mechanical or electrical stimulation.
16.3 Chronic cough (CC) patients demonstrate an increase in maximal cough response ($C^{\text{max}}$) compared to healthy controls (HC) and asthmatics (A)

As predicted, CC patients demonstrated significantly higher Cmax (maximal cough response) when compared to HC and A. This observation is difficult to explain by peripheral mechanisms alone, because if all directly stimulated vagal afferents were firing at maximum action potential frequency/amplitude then the maximal response that could be evoked would be identical in both CC and HC/A. This would be the case even if the number of nerve terminals and/or peripheral receptors were increased, as the number of axons would be unchanged. Therefore, it is proposed that the greater maximal cough response observed in CC is more likely to be a result of amplification of neuronal responses within the central nervous system.

There is one exception to the conclusion that increased maximal cough responses must arise because of changes purely within the central nervous system. In theory, a phenotypic switch of airway afferents could also be responsible for the greater maximal cough response observed in this study. In somatic tissue, low threshold myelinated Aβ-fibres may undergo a phenotypic switch under conditions of tissue inflammation, and begin to express the neuropeptide substance P (SP)[186]. At central synapses, SP is released and binds to neurokinin receptors on the post-synaptic cell, causing sufficient depolarization to remove the voltage-gated magnesium block on NMDA receptors, leading to increased synaptic strength. Therefore, a phenotypic switch of Aβ-fibres may lead to the induction and maintenance of central sensitization, when this would not normally be possible. In the airways, low threshold Aβ-fibres, may also begin to synthesize neuropeptides following viral or allergen challenge[187]. Therefore, normally innocuous mechanical stimulation of airway Aβ-fibres would be capable of initiating and maintaining central sensitization. Recent work by Lieu et.al.[36] provides further evidence that neurons may undergo changes in gene expression following exposure to the neurotrophic factor BDNF (see section 5.1). In this study, nodose-derived Aδ-fibres begin to express TRPV1 after exposure to BDNF when they would not normally do so.
However, there is a larger body of evidence favouring central mechanisms. Indeed, changes within central synapses are thought to play a major role in the initiation and maintenance of chronic pain states[75]. One important characteristic of an up-regulated central nervous system is that receptive fields increase in size[74, 75] because of an unmasking of previously ineffective or sub-threshold central synapses. Central neurons receive input from “firing zones” which directly initiate action potential discharge, but also from “Low Probability Firing Fringes (LPFF)” which under normal circumstances evoke excitatory post-synaptic potentials (EPSPs) that are sub-threshold for action potential initiation. Woolf proposed that these LPFF represented a “reservoir of potential responsiveness” if the excitability of central neurons and/or synaptic efficiency were subsequently increased[74]. Under these latter circumstances, stimulation of afferent fibres distant from the original injury site may elicit pain, known as secondary hyperalgesia.

Similar mechanisms could also be applied to cough. In this study, capsaicin was directly inhaled into the airways, but may have been deposited in small amounts in the larynx/pharynx or even swallowed into the oesophagus. Therefore it is possible that a widening of receptive fields is partly responsible for the increased maximal cough response observed. For example, oesophageal vagal afferents converge on central integrating neurons in the brainstem that also receive input from bronchial vagal afferents[89]. If the receptive field of these central brainstem neurons is widened to include extra-pulmonary sites e.g. oesophagus, this could explain the greater maximal cough responses in CC.

A further possibility to explain the higher maximal cough responses in CC is a failure of endogenous inhibitory mechanisms. Endogenous inhibitory mechanisms play a major role in the regulation of pain. Sensory transmission of nociceptive information in the dorsal horn of the spinal cord is normally subject to profound tonic inhibition via pathways that originate in the cortex (especially in the limbic system), project to the mid-brain peri-aqueductal grey (PAG), and relay through the rostro-ventral medulla (RVM). In the 1970’s, it was shown that electrical stimulation of the PAG in patients with chronic intractable pain could exert a pronounced analgesic effect, superior to intra-muscular morphine injection[188]. A disturbance of these top-down inhibitory influences are now thought to be responsible for many chronic pain conditions such as irritable bowel
syndrome[132], fibromyalgia[116] and migraines[117]. These conditions are generally associated with minimal peripheral tissue pathology, most likely because the pain is actually generated within the central nervous system.

A similar disturbance of top-down inhibitory influences in cough would have a profound effect on the ability of the individual to control or terminate coughing bouts, leading to high cough frequencies and higher maximal cough responses than HC. If CC was a manifestation of disordered descending inhibitory control mechanisms, this would explain the minimal lung pathology identified in these patients[189]. It was previously shown that CC patients find it more difficult to voluntarily suppress cough evoked by citric acid inhalation[127], but whether endogenous inhibitory control mechanisms are additionally impaired has not been investigated. However, there is evidence from a study of anaesthetized cats that visceral reflexes, including cough, are regulated by descending inhibitory pathways arising in the PAG-RVM[190]. Conditioning stimulation of the PAG or RVM inhibited reflex neurons in the nucleus tractus solitarius (nTS) and thereby prevented cough evoked by mechanical stimulation of the larynx.

In summary, the increased maximal cough response in CC may arise following a phenotypic change in peripheral afferent nerves, amplification of central afferent pathways and/or a failure of descending inhibitory mechanisms.

16.4 Females do not demonstrate a left-shift in the capsaicin dose-response curve when compared to males, but have higher maximal cough response

Females had similar ED50 when compared to males. This finding is surprising, as it indicates that females do not demonstrate a left-shift in the dose-response curve, and have no difference in cough sensitivity as compared to males. This is contrary to established data using standard cough challenge designs which show that females have lower cough thresholds (C2/C5) when compared to males in HC[17, 40, 191] and CC patients[17, 56, 192, 193]. Given that the inter-individual variability of ED50 was unknown prior to the planning of this study, power calculations to determine sample size could not be performed. Therefore, it is possible that my study was under-powered to detect gender differences in ED50. However, these findings suggest that cough hypersensitivity is not the predominant mechanism that explains why females are more
likely to develop CC. In fact, it has been shown in a multiple regression analysis that the age, sex and C2/C5 only independently predict 38.8% of the variance in 24 hour cough frequency[56], suggesting that other unidentified factors may be important.

In contrast, $C_{\text{max}}$ was significantly higher in females compared to males. As described above (section 4.3) an increased maximal cough response probably reflects an amplification of sensory transmission within the central nervous system. This may arise because of a failure to activate descending inhibitory pathways.

In further support of this hypothesis, healthy, middle-aged females are known to have less effective endogenous inhibitory pain mechanisms than males[116, 119, 120]. Indeed, less effective pain inhibition is thought subsequently to pre-dispose middle-aged females to develop chronic pain conditions such as irritable bowel syndrome and fibromyalgia[194]. If middle-aged females also had less effective inhibitory cough mechanisms, they would be pre-disposed to develop CC. Given that inhibitory pathways tend to be more active during the night[113], a failure of cough inhibition could explain why females with CC and asthma have higher night time cough rates than males [50, 56].

Another possible explanation for the gender differences observed is that sex hormones modulate peripheral nerve terminal sensitivity and/or central synaptic transmission. There are no data to support this, and indeed cough thresholds are lower in post-menopausal women compared to pre-menopausal women[193]. Furthermore, cough rates tend gradually to increase with age[56].

Gender differences in lung function could cause varying deposition of capsaicin in the airways, and this could also explain the findings. However, previous studies show that lung function fails to predict or significantly to correlate with cough thresholds (C2/C5) to experimentally inhaled stimuli[192, 193].
16.5 Asthmatics (A) demonstrate no significant difference in capsaicin dose-response curves when compared to healthy controls (HC)

It was predicted that A would demonstrate a left-shift in the dose-response curve, but in this study I could find no significant differences between A and HC.

It has been documented that A have significantly lower C2/C5 to capsaicin compared to HC[49], although this was not consistently found by others[48]. Furthermore, although A have higher objective 24 hour cough frequencies than HC, C5/C2 correlate poorly[50]. Therefore, cough challenge testing, at least with capsaicin as the challenge agent, may not be a good predictive biomarker of cough in asthma.

16.6 The relationship between urge-to-cough and cough frequency

Consistent with the findings of Davenport et.al.[59], a linear loge-loge relationship between urge-to-cough intensity and cough frequency was demonstrated. This suggests that the urge-to-cough sensation directly motivates a cough response.

Several factors may influence reporting of urge-to-cough. In particular, CC patients tended to have higher trait anxiety and HADs anxiety scores than HC and A. These psychological confounders may partly explain the higher reported urge-to-cough in CC compared to HC, but it is more likely that CC patients have heightened awareness of their urge-to-cough sensation and therefore have a tendency to report higher intensities. Indeed, hyper-vigilance is recognized to be a confounder for which it is difficult to control in subjective reporting of pain sensations in chronic pain conditions[194]. Using a randomized challenge methodology I attempted to reduce the bias associated with anticipation of increasing capsaicin dose, but more objective measures of urge-to-cough (e.g. fMRI or EEG) would be required to investigate further and reconcile these issues.

Of greater interest is the finding that for any given reported urge-to-cough intensity, CC patients coughed more frequently than HC/A, and females coughed more than males. This suggests a possible disturbance in the relationship between the conscious sensation that motivates cough and the motor cough response in females and in CC patients. One possible explanation is that CC patients and/or females fail to inhibit coughing in response to the urge-to-cough sensation, and find coughing irresistible in response to the awareness of even low intensity airway irritation. It has been speculated
that voluntary cough, sensory-driven cough and reflex cough form a continuous spectrum[37], see appendix 1.2. As the strength of the tussive stimulus increases, the urge-to-cough sensation would increase also. At low intensity urge-to-cough sensations, cough can be controlled by voluntary suppression. However, as the stimulus becomes stronger, voluntary control weakens until cough becomes an irresistible reflex response. I propose that CC patients and/or females have less voluntary control even at low intensity urge-to-cough sensations, and more readily find coughing irresistible (see Figure 37). In females, this would pre-dispose to the development of CC.

16.7 Discussion of the study limitations

The challenge designs used in this study are not fully validated. Further work is needed to assess the repeatability of ED50 and C\textsuperscript{max} in patients and healthy subjects, and to understand the strength of their association with other cough measures such as objective cough frequency. The novel cough challenges were of longer duration (approximately 40 minutes) than a standard cough challenge (approximately 15 minutes) because there are repeated inhalations at each dose, and the challenge is continued up to the maximum tolerated dose of capsaicin. This may be more unpleasant for patients, and it may not be practical to employ routine use of these novel challenges in clinical practice. However, a larger sample size would allow simulation of the data to predict dose-response parameters in CC and HC. Therefore, I could further explore whether repeated inhalations at each dose and/or extension of the challenge to maximum tolerated dose is necessary to show significant group and gender differences. Furthermore, a larger sample size may demonstrate significant gender effects on ED50, which was not observed with the relatively small number of subjects in this study.
Figure 37 – Conscious control of cough

Suggested relationships between voluntary cough, sensory-driven cough and reflex cough in normal subjects (black/grey lines) and chronic cough patients (red lines). Adapted from Woodcock et.al. 2010[37].

Cough can be voluntarily initiated (voluntary cough) in the absence of any tussive stimulus or sensation in normal subjects and CC patients. In normal subjects, as the intensity of the sensation driving cough increases (sensory-driven cough), voluntary control correspondingly declines until cough can no longer be voluntarily controlled (“reflex” cough). In CC, the relationship between stimulus, urge-to-cough sensation and conscious control may be disturbed. At any given stimulus intensity, the reported urge-to-cough sensation is greater, and the urge-to-cough is less susceptible to voluntary control.
17 Conclusions

By constructing capsaicin dose-response curves, it has been demonstrated that when compared to healthy controls, chronic cough patients not only demonstrate cough hypersensitivity (decrease in ED50) but also show an amplification of cough response (increase in the number of coughs for any given capsaicin dose) and increased maximal cough response ($C_{\text{max}}$). This could be best described as cough hyper-responsiveness. There were no differences observed between asthmatics and healthy controls. Possible neuro-physiological mechanisms that explain these observations have been discussed.

In summary:
- cough hypersensitivity may represent lowered neuronal thresholds in the peripheral and central nervous system,
- increased cough frequency could arise because of increased frequency/amplitude of action potentials generated in peripheral and/or central neurons,
- increased maximal cough response most probably occurs because of heightened sensory transmission within the central nervous system, possibly as a result of failed descending inhibitory mechanisms.

Furthermore, chronic cough patients had higher cough frequencies for any given urge-to-cough intensity when compared to healthy controls. This suggests that urge-to-cough sensations may be less susceptible to voluntary control in these patients.

Finally, important gender differences have been identified that may explain why females are pre-disposed to the development of CC. Females demonstrated a greater maximal cough response, and tended to have higher cough frequencies for any given urge-to-cough intensity than males. This suggests that females may have an inherent underlying deficiency in cough inhibition.

In conclusion, a greater amount of information regarding the sensitivity, magnitude, and maximum cough response can be obtained from full dose-response curves when compared with a single arbitrary cough threshold such as $C_2/C_5$. Providing these new challenge designs can be validated, they may usefully employed in early phase drug development to investigate mechanisms of antitussive action.
CHAPTER 3

Mechanisms of Cough Inhibition
18 Introduction

18.1 Rationale

The over-representation of female patients in specialist cough clinics[11, 195-202] could be because of a greater tendency of females to report cough symptoms, or a more detrimental effect of cough on health-related quality of life[203]. However, there is now also convincing objective evidence that compared to males, females with chronic cough have increased cough sensitivity[17, 56, 192, 193] and higher 24 hour spontaneous cough frequency[56]. Furthermore, in chapter 2 it was demonstrated that regardless of disease status (healthy, asthmatic or chronic cough), females have significantly higher capsaicin-induced cough frequency when compared to males. This suggests that underlying patho-physiological mechanisms may pre-dispose females to the development of chronic cough.

Given the similarities between cough and pain outlined in the general introduction (section 3) it is interesting that the majority of chronic pain conditions also appear predominantly to affect females[204]. The reasons for these gender differences in pain disorders are being actively pursued, and one proposed explanation is that females have less effective endogenous inhibitory pain mechanisms[204]. Endogenous inhibition of pain can be measured experimentally using the “pain-inhibiting-pain” paradigm known as “Diffuse Noxious Inhibitory Controls” or DNIC[205]. Applying a tonic painful stimulus (conditioning stimulus) to one part of the body activates DNIC mechanisms which reduce the intensity of a phasic painful stimulus (test stimulus) elsewhere. DNIC mechanisms have been extensively investigated in pain research, and a systematic review has confirmed that compared to healthy males, healthy females have less effective DNIC-mechanisms[204]. A failure of endogenous inhibitory pain mechanisms, such as DNIC, may pre-dispose females to the development of chronic pain disorders[116]. Indeed, DNIC mechanisms are impaired in irritable bowel syndrome[118], fibromyalgia[206], osteoarthritis[207] and chronic headaches[208].
18.2 Hypothesis

I hypothesised that endogenous inhibition of cough would be less effective in:

(i) Chronic cough patients as compared to healthy controls
(ii) Females as compared to males

18.3 Aims

The aim of this study was to develop a model for measuring endogenous inhibition of cough using DNIC mechanisms. I investigated whether applying a tonic painful stimulus to the hand (conditioning stimulus) would activate DNIC-mechanisms to inhibit capsaicin-induced cough in male and female chronic cough (CC) patients and healthy controls (HC).
19 Methodology

19.1 Subjects

Twenty healthy controls (HC) and 20 chronic cough patients (CC) were enrolled. The eligibility criteria are described in section 7.1. The study was approved by a UK Research Ethics Committee (REC10/H1003/104) and registered at www.controlled-trials.com (ISRCTN31901405). All subjects gave written informed consent.

19.2 Study Design

All subjects attended 5 visits, including a screening visit and 4 randomised visits. The visits were separated by at least 48 hours.

19.2.1 Screening visit

Questionnaires

At the screening visit, a series of psychological questionnaires were completed by participants under guided supervision. These included:

(i) State-Trait Anxiety Index (STAI), see section 7.3 and appendix 2.6.
(ii) Hospital Anxiety and Depression Scale (HADS), see section 7.3 and appendix 2.1.
(iii) Perceived Stress Scale (PSS): validated to measure the degree to which situations are appraised as stressful, and assesses recent (<1 month) stress levels, see appendix 2.8.
(iv) Pain Catastrophising Scale (PCS): a validated self-report scale assessing the individual’s tendency towards pain catastrophising, see appendix 2.3. Pain catastrophising has been characterized as “the tendency to magnify the threat value of pain stimulus and to feel helpless in the context of pain, and by a relative inability to inhibit pain-related thoughts in anticipation of, during or following a painful encounter”[209]. Negative correlations between DNIC effect and pain catastrophising score have been shown in healthy subjects[210]
(v) Anxiety Sensitivity Index (ASI): measures the individual's fear of bodily sensations that are interpreted as having potentially harmful physical or psychological consequences, see appendix 2.9.

(vi) Body Vigilance Scale (BVS): measures the tendency to attend to or focus on internal body sensations, see appendix 2.4.

All subjects additionally completed the IBS sub-section of the ROME III questionnaire (see section 7.4.1 and appendix 2.7). Healthy subjects were excluded if they met the criteria for a diagnosis of IBS because DNIC are known to be impaired in IBS patients[118, 132].

All subjects completed a Sino-nasal Outcome Test (appendix 2.2) and Reflux Symptom questionnaire (appendix 2.10) to determine whether they were symptomatic of reflux disease or post-nasal drip syndrome.

CC patients completed a Cough-Specific Quality of Life questionnaire (appendix 2.5) to indicate the degree to which the cough was impacting on quality of life, as a marker of cough severity.

24-hour Cough Monitoring
All subjects then underwent a 24 hour period of objective cough monitoring, see section 7.2.1.

Screening Cough Challenge
After subjects returned their cough monitor (24 hours later), an ascending doubling dose cough challenge was performed as described in Chapter 2, section 14.2.1. The challenge was continued up the maximum tolerated capsaicin dose. The dose of capsaicin inducing at least half maximal cough response (ED50) was documented.

Practice with Cold Water Bath
All subjects were then instructed to place their non-dominant hand in a water bath (Clifton, stirred water-bath, NE4) in which the temperature was set to 10°C (accurate to +/-0.5C) for 1.5 minutes if possible, but the hand could be withdrawn if the pain became unbearable at any stage. All subjects practiced rating pain intensity using a numerical
scale ranging from 0 (no pain) to 10 (worst possible pain). Of note, all subjects had a clearly dominant hand.

19.2.2 Randomised Visits

At each of the subsequent 4 randomised visits, subjects completed a visual analogue scale of current stress and anxiety levels, ranging from 0 (no stress/anxiety) to 10 (severe stress/anxiety). An ambulatory cough monitor was worn throughout the visit.

At each visit, subjects were exposed to 2 blocks of interventions which were separated by 1 hour. Blood pressure and pulse rate were measured before and after each intervention block, see Table 20. During each block, 4 single-breaths of capsaicin were inhaled at the individually pre-determined ED50 dose. Each inhalation was separated by 15 seconds. After each inhalation the number of coughs evoked was counted, and urge-to-cough intensity was rated on a modified Borg Scale (0-10, no urge-to-cough to maximum urge-to-cough). The number of coughs was later verified using the sound recordings.

Simultaneous with each capsaicin cough challenge block, one of 4 possible interventions was administered including:

(i) Cold water; the non-dominant hand was placed in a painful cold water bath (10°C) and subjects coughed freely.

(ii) Warm water; the non-dominant hand was placed in a non-painful warm water bath (32°C) and subjects coughed freely

(iii) Warm water and cough suppression; the non-dominant hand was placed in a non-painful warm water bath (32°C) and subjects were told to try not to cough.

(iv) No intervention; no simultaneous intervention

Where a water bath was applied, the subject placed their non-dominant hand in the water bath 20 seconds before their first inhalation of capsaicin. Pain intensity was rated on a numerical scale (0-10, no pain to worst possible pain) immediately prior to the first inhalation of capsaicin and at the end of the block, see Figure 39. The interventions
administered at each visit, and their possible order, is summarised below and in Figure 38.

**Visit 1: Cold water visit:** during one of the blocks (block 1 or 2) the non-dominant hand was placed in a painful cold water bath ($10^\circ$C). During the other block (block 1 or 2) the non-dominant hand was placed in non-painful warm water bath ($32^\circ$C). The order of the cold and warm water blocks was randomised for each subject. The subject was instructed to cough freely throughout both blocks.

**Visit 2: Warm water visit:** during block 1 and block 2 the non-dominant hand was placed in a non-painful warm water bath ($32^\circ$C) and the subject was instructed to cough freely throughout both blocks.

**Visit 3: Cough suppression visit:** during block 1 and 2 the non-dominant hand was placed in a non-painful warm water bath ($32^\circ$C). However, during one of the blocks (block 1 or 2) the subject was instructed to “try not to cough”. During the other block (block 1 or 2) the subject was instructed to “cough freely”. The order of these blocks was randomised for each subject.

**Visit 4: No intervention visit:** during block 1 and block 2 no water bath was applied and the subject was instructed to cough freely throughout both blocks.
There are 4 randomised visits in total. At each visit, 2 short capsaicin cough challenges are separated by 1 hour (block 1 and block 2). At visits 1, 2 and 3, subjects place their hand in a water bath throughout each cough challenge. Where the block is shaded blue, a painful cold water bath is applied. Where the block is shaded orange, a non-painful water bath is applied. Subjects were instructed to cough freely throughout except where the block has a green boarder, when the instruction was to “try not to cough”. At visit 4, no water bath was applied.
A) Visit 4 (no water bath)

Visit 4

BP
PR

20s

utc
utc
utc
utc

15s

ED50
Capsaicin

B) Visit 1, 2 and 3 (cold or warm water bath)

Visit 1, 2 or 3

BP
PR

20s

utc
utc
utc
utc

15s

ED50
Capsaicin

Diagram illustrates a single intervention block. **A) Visit 4 (No Intervention)**, subjects do not place their hand in a water bath. Blood pressure (BP) and pulse rate (PR) are measured. After 20 seconds, the subject is instructed to take their first inhalation of capsaicin (individually determined ED50 dose). The subject inhales capsaicin 4 times, with 15 seconds between each inhalation. After each inhalation, the intensity of urge-to-cough sensation is rated, and the number of coughs is counted. BP and PR are repeated at the end of the block. **B) Visit 1, 2 and 3 (warm or cold water bath)** follow an identical protocol. However, during the entire cough challenge subjects place their hand in a warm or cold water bath. Pain intensity is rated after 20 seconds of immersion and at the end of the cough challenge. Note that the subject focuses on rating the urge-to-cough rather than pain for the majority of the duration of the block.
19.3 Justification for methodology

19.3.1 Modality of painful conditioning stimulus

In this study a cold water bath applied to the hand was utilised as the painful conditioning stimulus. In a study comparing the effectiveness of various painful conditioning stimuli to activate DNIC mechanisms (cold water, hot water of increasing temperatures, noxious pinch to the nasal septum and ischaemic arm pain) cold water was the most effective[114].

19.3.2 Positive control; voluntary cough suppression

A well-described mechanism of cough inhibition is voluntary cough suppression. I have shown that both HC and CC patients are significantly able to suppress citric acid-induced cough, but cough suppression was less effective in the CC patients[127], see appendix 1.4. I predicted that conscious cough suppression is a separate mechanism to endogenous cough inhibition. Therefore, voluntary cough suppression was used as a positive control.

19.4 Primary and Secondary Outcomes

19.4.1 Primary outcomes

Capsaicin-induced cough frequency and urge-to-cough intensity during the painful cold water intervention compared to warm water control.

19.4.2 Secondary outcomes

(i) Capsaicin-induced cough frequency and urge-to-cough intensity during cough suppression intervention compared to warm water alone.

(ii) Capsaicin-induced cough frequency and urge-to-cough intensity during the non-painful warm water intervention compared to no intervention.
19.5 Statistical Methods

19.5.1 Summary statistics

Where the data were normally distributed, parametric tests were applied to investigate group differences. Where the data were not normally distributed, equivalent non-parametric tests were applied to investigate between group differences.

The within block change in systolic blood pressure (sBP) and pulse rate (PR) was calculated for each individual as sBP/PR at the end of the block minus sBP/PR at the beginning of the block. Positive values represent an increase in sBP/PR, and negative values represent a decrease in sBP/PR. The within block change in sBP/PR was calculated for each intervention, and then compared by group using independent t-tests.

19.5.2 Power calculations

This was a pilot study. The within subject variability in cough frequency and urge-to-cough intensity during inhalation of an ED50 capsaicin dose is unknown. Therefore, sample size calculations could not be performed.

19.5.3 Generalised estimating equations

To normalise the data, the outcome variables of capsaicin-induced cough frequency and urge-to-cough intensity were transformed according to the following equation:
\[ Y_i = \ln(Y_i + 1) \]
where \( Y_i \) is the cough frequency or urge-to-cough intensity for the \( i \)th patient.
Because of a high number of 0 counts, the addition of 1 was first required in order to Log transform the data.

Generalised estimating equations were then performed to evaluate group, gender and intervention effects. All models were adjusted for the order of the interventions i.e. whether the interventions were administered during block 1 or during block 2 (intervention*block). A summary of the models is shown in Table 16. Results from visit 2 (warm water control visit) and visit 4 (no intervention visit) investigated the effects of applying a non-painful warm water bath to the hand compared to no intervention. Results from visit 1 (cold water visit) and visit 3 (suppression visit) investigated the
effects of applying a painful cold water bath to the hand and the effect of cough suppression, compared to non-painful warm water alone. The mean difference in transformed cough frequency and urge-to-cough between the interventions was calculated, and then exponentiated to estimate a ratio for the change.
Table 16 – Summary of Generalised Estimating Equations

<table>
<thead>
<tr>
<th>Model</th>
<th>Outcome variable</th>
<th>Intervention investigated</th>
<th>Reference intervention</th>
<th>Visits Analysed*</th>
<th>Main effects</th>
<th>Interaction Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group effects</strong></td>
<td>Cough frequency</td>
<td>Warm water</td>
<td>No intervention</td>
<td>2 and 4</td>
<td>Group Block Intervention</td>
<td>Group<em>intervention Block</em>intervention</td>
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<tr>
<td></td>
<td>Urge-to-cough</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cold water</td>
<td>Suppression</td>
<td>Warm water</td>
<td>1 and 3</td>
<td></td>
<td>Group Block Intervention</td>
<td>Group<em>intervention Block</em>intervention</td>
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<tr>
<td><strong>Gender effects</strong></td>
<td>Cough frequency</td>
<td>Warm water</td>
<td>No intervention</td>
<td>2 and 4</td>
<td>Gender Block Intervention</td>
<td>Gender<em>intervention Block</em>intervention</td>
</tr>
<tr>
<td></td>
<td>Urge-to-cough</td>
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</tr>
<tr>
<td>Cold water</td>
<td>Suppression</td>
<td>Warm water</td>
<td>1 and 3</td>
<td></td>
<td>Gender Block Intervention</td>
<td>Gender<em>intervention Block</em>intervention</td>
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<tr>
<td><strong>Group and gender</strong></td>
<td>Cough frequency</td>
<td>Warm water</td>
<td>No intervention</td>
<td>2 and 4</td>
<td>Gender Block Intervention</td>
<td>Gender<em>intervention Block</em>intervention</td>
</tr>
<tr>
<td>effects</td>
<td>Urge-to-cough</td>
<td></td>
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<tr>
<td>Cold water</td>
<td>Suppression</td>
<td>Warm water</td>
<td>1 and 3</td>
<td></td>
<td>Gender Block Intervention</td>
<td>Gender<em>intervention Block</em>intervention</td>
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</tbody>
</table>

*visit 1 = cold water visit, visit 2 = warm water control visits, visit 3 = cough suppression visit, visit 4 = no intervention visit
19.5.4 Co-variate analysis

The following co-variates were investigated:

(i) 24 hour cough frequency  
(ii) Stress, rated on a visual analogue scale at each visit  
(iii) Anxiety, rated on a visual analogue scale at each visit  
(iv) Pain intensity, rated on a numerical scale.

As an initial screening process, these co-variates were each separately included in the group models as an interaction term with intervention. For those co-variates with p-values ≤0.1, the relationships were further explored.

24 hour cough frequency and stress (rated on a visual analogue scale) were identified to demonstrate co-linearity with group. Therefore, a separate analysis was conducted in which 24 hour cough frequency and stress acted as a surrogate for group i.e. replaced group in the model. These co-variates were converted from continuous data to categorical data, and classified using quartiles.
20 Results

20.1 Subjects

Twenty healthy controls (10 males, 10 females) and 20 chronic cough patients (9 male, 11 female) completed the study. Age, gender, body mass index, pack years smoking history and lung function are compared in Table 17. There was no significant difference in age (p=0.217), FEV1 percentage predicted (p=0.157), FVC percentage predicted (p=0.309), pack years of previous smoking history (p=0.444) or body mass index (p=0.245) between the groups. However, chronic cough (CC) patients demonstrated significantly higher scores on the sino-nasal outcome test (SNOT) and gastro-oesophageal reflux disease (GORD) questionnaires compared to healthy controls (HC) (p<0.001 and p<0.001 respectively).

Table 17 – Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (HC)</th>
<th>Chronic Cough Patients (CC)</th>
<th>p-value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (years)*</td>
<td>55.05 (14.21)</td>
<td>60.50 (13.2)</td>
<td>0.217</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>10:10</td>
<td>9:11</td>
<td></td>
</tr>
<tr>
<td>BMI*</td>
<td>26.59 (4.36)</td>
<td>28.09 (3.59)</td>
<td>0.245</td>
</tr>
<tr>
<td>Pack years **</td>
<td>0.00 (0.00)</td>
<td>0.00 (1.81)</td>
<td>0.444</td>
</tr>
<tr>
<td>FEV1%pred*</td>
<td>105.72 (13.79)</td>
<td>98.78 (16.45)</td>
<td>0.157</td>
</tr>
<tr>
<td>FVC%pred*</td>
<td>107.78 (15.95)</td>
<td>102.65 (15.48)</td>
<td>0.309</td>
</tr>
<tr>
<td>SNOT</td>
<td>0.08 (0.46)</td>
<td>1.3 (1.44)</td>
<td>&lt;0.001¥</td>
</tr>
<tr>
<td>GORD score</td>
<td>0.00 (3.50)</td>
<td>7.00 (15.50)</td>
<td>&lt;0.001¥</td>
</tr>
</tbody>
</table>

Mean (SD)* or median (IQR)** shown, ¥p-value ≤0.05.

20.1.1 Chronic cough (CC) patients

In CC patients the mean (SD) duration of cough was 13.0 (9.8) years. All patients had been investigated according to a routine clinical diagnostic protocol to identify underlying triggers for the cough; the results of these clinical investigations are presented in Table 18.

Two (10%) of the CC patients had evidence of post-nasal drip on nasendoscopy, but subsequently failed to respond to nasal steroids +/- anti-histamines. Five (25%) of the CC patients had evidence of laryngo-pharyngeal reflux on nasendoscopy or abnormal 24
hour impedance/pH testing, but failed to respond to high dose proton pump inhibitor. In all CC patients, the CT scan findings were not thought to be clinically significant, and did not sufficiently explain the cause of the cough. Therefore all CC patients were classified as “treatment-resistant” or “idiopathic” chronic cough.

In CC patients, the mean (SD) total CQLQ score was 52.50 (12.83). The mean (SD) CQLQ score within each of the sub-domains was 12.15 (2.91) for psychosocial issues, 4.95 (1.61) for emotional impact, 18.15 (5.58) for physical complaints, 7.5 (2.84) for extreme physical complaints, 5.30 (1.42) for personal safety fears and 8.75 (4.19) for functional abilities.

**Table 18 – Clinical Investigations for Chronic Cough Patients**

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Number (%) patients</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nasendoscopy</strong></td>
<td>18 (90%)</td>
<td>Normal=14 PND* = 1 Chronic sinusitis = 1 PND and LPR** = 1 Deviated septum = 1</td>
</tr>
<tr>
<td><strong>Bronchoscopy</strong></td>
<td>18 (90%)</td>
<td>All normal</td>
</tr>
<tr>
<td><strong>CT thorax</strong></td>
<td>16 (80%)</td>
<td>Normal=10 Mild bronchiectasis=4 Mild bronchial dilatation=1 Mild non-specific inflammatory change=1</td>
</tr>
<tr>
<td><strong>24 hour impedance/pH</strong></td>
<td>10 (50%)</td>
<td>Normal= 5 Abnormal=5</td>
</tr>
</tbody>
</table>

*PND=post-nasal drip, **LPR=laryngo-pharyngeal reflux

20.1.2 Comparison of cough parameters by group

Consistent with the findings in chapter 2, CC patients had significantly lower ED50 to capsaicin (p=0.021) and significantly higher maximal cough response (p<0.001) when compared to healthy controls. The median (IQR) ED50 was 15.6 (23.50) μM in HC and 7.8 (11.70) μM in CC patients, a difference of 1 doubling dose. The mean (SD) maximal cough response (C_{max}) was 14.6 (4.78) coughs in HC was 28.95 (7.88) coughs and in CC patients.
As expected, spontaneous cough frequency was significantly higher in CC compared to HC. The median (IQR) 24 hour cough frequency in was 0.79 (1.09) coughs/hr in HC and 10.9 (15.40) coughs/hr in CC patients, p<0.001. The median (IQR) daytime cough frequency was 1.06 (1.54) coughs/hr in HC and 15.35 (14.43) coughs/hr in CC patients, p<0.001. The median (IQR) night time cough frequency was 0.13 (0.59) coughs/hour in HC and 1.80 (5.06) coughs/hour in CC patients, p<0.001.

20.2 Psychological Questionnaires

The results of the questionnaires are presented in Table 19.

Compared to HC, CC patients had significantly higher hospital anxiety and depression (HAD) scores for depression (p=0.001) and anxiety (p=0.002), state anxiety (p=0.022), trait anxiety (p=0.003) and anxiety sensitivity index (p=0.007). However, there were no significant group differences for perceived stress (p=0.086), body vigilance (p=0.057) or pain catastrophising (p=0.138) despite a trend towards higher scores in CC patients.

Table 19 - Psychological Questionnaires

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Healthy Controls (HC)</th>
<th>Chronic Cough (CC)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS-d*</td>
<td>1.90 (1.65)</td>
<td>4.9 (3.58)</td>
<td>0.001¥</td>
</tr>
<tr>
<td>HADS-a*</td>
<td>3.25 (3.19)</td>
<td>7.40 (4.42)</td>
<td>0.002¥</td>
</tr>
<tr>
<td>State anxiety**</td>
<td>23.00 (5.75)</td>
<td>28.50 (14.0)</td>
<td>0.022¥</td>
</tr>
<tr>
<td>Trait anxiety**</td>
<td>28.00 (6.75)</td>
<td>44.50 (20.75)</td>
<td>0.003¥</td>
</tr>
<tr>
<td>Perceived stress*</td>
<td>10.9 (6.08)</td>
<td>15.15 (8.86)</td>
<td>0.086≠</td>
</tr>
<tr>
<td>Body vigilance*</td>
<td>11.85 (6.86)</td>
<td>15.95 (6.31)</td>
<td>0.057≠</td>
</tr>
<tr>
<td>Pain catastrophising*</td>
<td>13.8 (11.26)</td>
<td>18.80 (9.55)</td>
<td>0.138</td>
</tr>
<tr>
<td>Anxiety sensitivity index**</td>
<td>9.50 (7.25)</td>
<td>18.50 (14.75)</td>
<td>0.007¥</td>
</tr>
</tbody>
</table>

HADSa = Hospital Anxiety and Depression scale for anxiety, HADSd for depression.
Mean (SD)* or median (IQR)** shown, ¥p-value ≤0.05, ≠p-value ≤0.1.
20.3 Blood pressure and pulse rate

Within block changes in sBP/PR were very small (mean of <+-3mmHg or <+-3bpm), and unlikely to be clinically important. There was no significance difference in the within block change in sBP/PR for CC patients compared to HC during any of the intervention blocks, see Table 20.

Table 20 – Within block change in blood pressure and pulse rate

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Healthy Controls (HC)</th>
<th>Chronic cough (CC)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD) within block change in systolic blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No intervention</td>
<td>-3.35 (5.41)</td>
<td>-1.65 (5.62)</td>
<td>0.336</td>
</tr>
<tr>
<td>Warm water</td>
<td>-0.85 (3.88)</td>
<td>1.40 (9.27)</td>
<td>0.326</td>
</tr>
<tr>
<td>Cold water</td>
<td>2.30 (9.95)</td>
<td>1.20 (6.41)</td>
<td>0.680</td>
</tr>
<tr>
<td>Suppression</td>
<td>-0.85 (9.37)</td>
<td>-2.10 (11.37)</td>
<td>0.706</td>
</tr>
<tr>
<td><strong>Mean (SD) within block change in pulse rate (beats per minute, bpm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No intervention</td>
<td>0.40 (4.12)</td>
<td>3.20 (8.02)</td>
<td>0.176</td>
</tr>
<tr>
<td>Warm water</td>
<td>0.40 (5.80)</td>
<td>1.10 (5.89)</td>
<td>0.707</td>
</tr>
<tr>
<td>Cold water</td>
<td>-0.60 (6.63)</td>
<td>-0.15 (6.00)</td>
<td>0.823</td>
</tr>
<tr>
<td>Suppression</td>
<td>-0.25 (5.50)</td>
<td>0.70 (9.34)</td>
<td>0.697</td>
</tr>
</tbody>
</table>
20.4 Anxiety and stress

Overall, CC patients reported higher levels of anxiety and stress compared to HC, consistent with the psychological questionnaire scores. The median anxiety and stress levels rated using a visual analogue scale at each visit are compared by group and presented in Table 21.

Table 21 – Anxiety and stress levels at each visit

<table>
<thead>
<tr>
<th>Visit</th>
<th>Healthy Controls (HC)</th>
<th>Chronic cough (CC)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (IQR) stress</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No intervention</td>
<td>8.00 (16.00)</td>
<td>14.50 (32.00)</td>
<td>0.099≠</td>
</tr>
<tr>
<td>Warm water</td>
<td>8.50 (17.50)</td>
<td>17.50 (41.75)</td>
<td>0.074≠</td>
</tr>
<tr>
<td>Cold water</td>
<td>8.50 (12.00)</td>
<td>18.00 (25.00)</td>
<td>0.004¥</td>
</tr>
<tr>
<td>Suppression</td>
<td>8.50 (13.75)</td>
<td>14.50 (20.25)</td>
<td>0.040¥</td>
</tr>
<tr>
<td><strong>Median (IQR) anxiety</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No intervention</td>
<td>8.00 (16.00)</td>
<td>14.50 (32.00)</td>
<td>0.099≠</td>
</tr>
<tr>
<td>Warm water</td>
<td>8.50 (17.50)</td>
<td>17.50 (41.75)</td>
<td>0.074≠</td>
</tr>
<tr>
<td>Cold water</td>
<td>8.50 (12.00)</td>
<td>18.00 (25.00)</td>
<td>0.004¥</td>
</tr>
<tr>
<td>Suppression</td>
<td>8.50 (13.75)</td>
<td>14.50 (20.25)</td>
<td>0.040¥</td>
</tr>
</tbody>
</table>

¥p-value ≤0.05, ≠p-value ≤0.1

20.5 Pain intensity

Although there were no significant differences in reported pain intensity between the groups, there was a trend for CC patients to perceive greater levels of pain. The mean (SD) reported pain intensity after 20 seconds of hand immersion in the cold water bath was 3.7 (2.60) in HC and 5.05 (1.85) in CC patients, p=0.066. The mean (SD) reported pain intensity at the end of the painful cold water intervention was 5.8 (2.56) in HC and 7.00 (1.49) in CC patients, p=0.092. During warm water control interventions none of the subjects reported any pain (rated as 0 on the scale).
20.6 Order effect

The change in cough frequency and urge-to-cough intensity between blocks 1 and 2 was explored using data from visit 1 (warm water control visit) and visit 4 (no intervention visit), because at these visits the interventions administered were identical for both blocks, thereby allowing any “order-effect” to be observed, see Figure 41.

At the no intervention visit, there was a significant reduction in cough frequency during block 2 compared to block 1 (p=0.002). At the warm water control visit, this same trend did not reach statistical significance (p=0.060).

At the no intervention visit, there was a significant reduction in urge-to-cough intensity during block 2 compared to block 1 (p=0.029). However, at the warm water control visit, there was no significant between-block change in urge-to-cough (p=0.603).
Figure 41 – Order-effect

A

The graphs show the GEE model predicted mean change (95% CI) in capsaicin-induced cough frequency (A) and urge-to-cough intensity (B) from block 1 to block 2 at the warm water control visit (black lines) and at the no intervention visit (red lines). At the no intervention visit, there is a significant reduction in cough frequency (p=0.002) and urge-to-cough intensity (p=0.029) during block 2 compared to block 1. In contrast, at the warm water control visit there were no significant change in cough frequency (p=0.060) or urge-to-cough intensity (p=0.606).
20.7 Group Effects

20.7.1 Effect of non-painful warm water compared by group

Overall, compared to no intervention, applying a warm water bath did not significantly change capsaicin-induced cough frequency (p=0.179), see Table 22 and Figure 42. On average, warm water increased cough frequency by ~29% in HC, p=0.078, and slightly decreased cough frequency by ~4% in CC patients, p=0.817, (NB: percentage change magnitudes appear larger in HC due to a lower baseline number of coughs evoked). There was no significant difference in the effect of warm water on cough frequency in HC compared to CC patients (p=0.184).

Overall, compared to no intervention, applying a warm water bath did not significantly change capsaicin-induced urge-to-cough intensity (p=0.606). Warm water slightly decreased urge-to-cough intensity on average in both groups; by ~2% in HC (p=0.838) and by ~5% in CC patients (p=0.508). There was no significant difference in the effect of warm water on urge-to-cough in HC compared to CC patients (p=0.824).
Table 22 – Summary of the effect of warm water on capsaicin-induced cough, compared by group

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Group</th>
<th>Mean difference*(Δ)</th>
<th>Exp(Δ)</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value group*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough frequency</td>
<td>HC</td>
<td>0.25</td>
<td>1.28</td>
<td>+29%</td>
<td>0.078≠</td>
<td>0.179</td>
<td>0.184</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>-0.04</td>
<td>0.96</td>
<td>-4%</td>
<td>0.817</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urge-to-cough intensity</td>
<td>HC</td>
<td>-0.02</td>
<td>0.98</td>
<td>-2%</td>
<td>0.838</td>
<td>0.606</td>
<td>0.824</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>-0.05</td>
<td>0.95</td>
<td>-5%</td>
<td>0.508</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean difference is model predicted mean change in outcome variables at warm water intervention visit (visit 2) compared to no intervention visit (visit 4). ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

Figure 42 – Effect of non-painful warm water, compared by group

The mean values (95% CI) are predicted from the GEE model and back-transformed. The graph shows the change in capsaicin-induced cough frequency (A) and urge-to-cough (B) after applying a warm water bath compared to no intervention in healthy controls (black lines) and chronic cough patients (green lines). There is no significant change in cough frequency or urge-to-cough intensity after applying warm water for either group. However, in HC there is a trend towards an increase in cough frequency (p=0.078) when applying warm water. Note that the overall mean cough frequency is higher in CC patients compared to HC (p=0.002), whereas urge-to-cough is similar in both groups (p=0.403).
20.7.2 Effect of painful cold water compared by group

Overall, compared to the warm water control, applying a painful cold water bath to the hand significantly reduced capsaicin-induced cough frequency (p=0.023), see Table 23 and Figure 43. On average, cold water reduced cough frequency by ~50% in HC (p<0.001) and by ~37% in CC patients (p=0.005). There was no significant difference in the effect of cold water on cough frequency in HC compared to CC patients (p=0.266).

Overall, compared to the warm water control, applying a painful cold water bath to the hand did not significantly reduce urge-to-cough intensity (p=0.104), see Table 24 and Figure 44. However, on average, cold water reduced urge-to-cough by ~27% in HC (p=0.001) and by ~16% in CC patients (p=0.010). There was no significant difference in the effect of cold water on urge-to-cough in HC compared to CC patients (p=0.219).

There was no significant difference in the effect of the cold water on cough frequency or urge-to-cough whether it was applied during block 1 or block 2 (p=0.209 and p=0.417 respectively).

20.7.3 Effect of cough suppression compared by group

Overall, compared to warm water control, cough suppression significantly reduced capsaicin-induced cough frequency (p<0.001), see Table 23 and Figure 43. On average, cough suppression reduced cough frequency by ~63% in HC (p<0.001) and by ~40% in CC patients (p=0.029). There was a suggestion that cough suppression was less effective at reducing cough frequency in CC patients compared to HC (p=0.077).

Overall, compared to warm water control, suppressing cough had no significant effect on urge-to-cough intensity (p=0.456), see Table 24 and Figure 44. On average, suppression changed urge-to-cough intensity by an increase of ~8% in HC (p=0.203) and by ~0% in CC patients (p=1.000). There was no significant difference in the effect of cough suppression on urge-to-cough in HC compared to CC patients (p=0.425).

There was no significant difference in the effect of cough suppression on cough frequency or urge-to-cough whether it was applied during block 1 or block 2 (p=0.687 and p=0.962 respectively).
20.7.4 Comparison of cold water and cough suppression by group

In CC patients and HC, cold water and cough suppression reduced cough frequency to a similar extent (p=0.839 and p=0.141 respectively).

In HC, cold water and cough suppression had a significantly different effect on urge-to-cough intensity (p=0.004); whereas cold water tended to reduce urge-to-cough intensity, cough suppression did not. However, in CC patients there was no similar trend (p=0.122).
Table 23 – Summary of the effect of cold water and cough suppression on capsaicin-induced cough frequency, compared by group

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Intervention</th>
<th>Group</th>
<th>Mean difference* (Δ)</th>
<th>Exp(Δ)</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value group*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough frequency</td>
<td>Cold water</td>
<td>HC</td>
<td>-0.70</td>
<td>0.50</td>
<td>-50%</td>
<td>&lt;0.001 ¥</td>
<td>0.023 ¥</td>
<td>0.266</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>-0.46</td>
<td>0.63</td>
<td>-37%</td>
<td>0.005 ¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cough Suppression</td>
<td>HC</td>
<td>-0.99</td>
<td>0.37</td>
<td>-63%</td>
<td>&lt;0.001 ¥</td>
<td>&lt;0.001 ¥</td>
<td>0.077 ≠</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>-0.51</td>
<td>0.60</td>
<td>-40%</td>
<td>0.029 ¥</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean difference is model predicted mean change in outcome variables after cold water application or cough suppression compared to warm water control. ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

Figure 43 – Effect of cold water and cough suppression on capsaicin-induced cough frequency, compared by group

The mean values (95% CI) are predicted from the GEE model and back-transformed. Graph shows that compared to warm water control, there is a significant (*p≤0.05) reduction in capsaicin-induced cough frequency after applying a painful cold water bath to the hand, and after instructing subjects to “try not to cough” in HC (black lines) and CC patients (green lines). Although cold water has a similar effect in HC and CC (p=0.266), cough suppression appears to be more effective in HC compared to CC patients (p=0.077).
Table 24 - Summary of the effect of cold water and cough suppression on capsaicin-induced urge-to-cough, compared by group

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Intervention</th>
<th>Group</th>
<th>Mean difference*(Δ)</th>
<th>Exp(Δ)</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value group*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urge-to-cough</td>
<td>Cold water</td>
<td>HC</td>
<td>-0.32</td>
<td>0.73</td>
<td>-27%</td>
<td>0.001¥</td>
<td>0.104</td>
<td>0.219</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>-0.18</td>
<td>0.84</td>
<td>-16%</td>
<td>0.010¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cough Suppression</td>
<td>HC</td>
<td>0.08</td>
<td>1.08</td>
<td>+8%</td>
<td>0.203</td>
<td>0.456</td>
<td>0.425</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean difference is model predicted mean change in outcome variables after cold water application or cough suppression compared to warm water control. ¥ indicates p-value ≤0.05 and ≠ indicates p-value ≤0.1.

Figure 44 – Effect of cold water and cough suppression on urge-to-cough intensity, compared by group

The mean values (95% CI) are predicted from the GEE model and back-transformed. Graph shows that compared to warm water control, capsaicin-induced urge-to-cough intensity significantly (*p≤0.05) decreases after applying a painful cold water bath to the hand, whereas there is no change in urge-to-cough during cough suppression in both HC (black lines) and CC patients (green lines).
20.7.5 Co-variate analysis

The results of the models in which several co-variates were systematically included are summarised in Table 25. None of the co-variates significantly influenced the effect of painful cold water or cough suppression on capsaicin-induced urge-to-cough. However, the effect of painful cold water and cough suppression on capsaicin-induced cough frequency was significantly influenced by stress (p=0.018) and 24 hour spontaneous cough frequency (p<0.001). Indeed, an increase in stress levels was associated with more effective cough suppression (p=0.006), and an increase in 24 hour spontaneous cough frequency was associated with an increase in cough inhibition with cold water (p<0.001). However, since the CC patients have significantly higher 24 hour cough frequency and stress levels compared to HC, it was deduced that these co-variates may be acting as a “proxy” for group. Therefore, to investigate whether these relationships were accurately describing the data, a further analysis was conducted whereby group was removed from the model, and replaced by either 24 hour spontaneous cough frequency or stress.

24 hour spontaneous cough frequency

Twenty-four hour spontaneous cough frequency was stratified in quartiles for all subjects (HC and CC) where quartile 1 represented subjects with the lowest cough frequencies and quartile 4 represented subjects with the highest cough frequencies, see Table 26. Cold water and cough suppression significantly reduced capsaicin-induced cough frequency in quartiles 1, 2 and 4 (p≤0.05), but not quartile 3 (p>0.1). Compared to quartile 1, quartiles 2 and 4 were similar in their response to both the interventions (p>0.1), see Table 27 and Figure 45. However, compared to quartile 1, quartile 3 had less effective inhibition of capsaicin-induced cough during cough suppression (p=0.038). These findings may be due to a small number of subjects within each quartile and should be interpreted with caution.

Stress

Stress, rated on a visual analogue scale at each visit, was also stratified in quartiles for all subjects (HC and CC) where quartile 1 represented subjects with the lowest stress levels, and quartile 4 represented subjects with the highest stress levels, see Table 26.
Capsaicin-induced cough frequency was significantly reduced by cold water and cough suppression in all quartiles (p≤0.05) except for quartile 4 who were significantly able to suppress cough (p=0.006), but only showed a trend towards a reduction in cough with cold water (p=0.062), see Table 28 and Figure 46. Compared to quartile 1, quartiles 2, 3 and 4 had a similar response to cold water and cough suppression (p>0.1).

Table 25 – Summary of co-variate analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Outcome variable</th>
<th>Co-variate</th>
<th>Main effect</th>
<th>Interaction with intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block Intervention Group</td>
<td>Capsaicin-induced cough frequency</td>
<td>Anxiety VAS</td>
<td>0.614</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stress VAS*</td>
<td>0.439</td>
<td>0.018†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain intensity</td>
<td>0.655</td>
<td>0.227</td>
</tr>
<tr>
<td></td>
<td>24 hour spontaneous cough frequency*</td>
<td>0.893</td>
<td>&lt;0.001‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urge-to-cough</td>
<td>Anxiety VAS</td>
<td>0.322</td>
<td>0.771</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stress VAS</td>
<td>0.637</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain intensity</td>
<td>0.122</td>
<td>0.805</td>
</tr>
<tr>
<td></td>
<td>24 hour spontaneous cough frequency</td>
<td>0.844</td>
<td>0.246</td>
<td></td>
</tr>
</tbody>
</table>

*co-variates that interact significantly with the effect of painful cold water and cough suppression compared to warm water control, but show co-linearity with group. †indicates p-value ≤0.05 and ‡indicates p-value ≤0.1.

Table 26 – Defined range of co-variate quartiles

<table>
<thead>
<tr>
<th>Co-variate</th>
<th>Quartile number</th>
<th>Defined range of values</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour spontaneous cough frequency (coughs/hour)</td>
<td>1</td>
<td>&lt;0.70</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.70 to &lt;2.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.46 to &lt;10.30</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>≥10.30</td>
</tr>
<tr>
<td>Stress VAS (mm)</td>
<td>1</td>
<td>&lt;2.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.25 to &lt;11.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.00 to &lt;19.75</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>≥19.75</td>
</tr>
</tbody>
</table>
**Table 27** – 24 hour spontaneous cough frequency as a surrogate for group

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Quartile</th>
<th>Mean difference(Δ)</th>
<th>ExpΔ</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value quartile*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cold Water</strong></td>
<td>1</td>
<td>-0.63</td>
<td>0.53</td>
<td>-47%</td>
<td>0.006¥</td>
<td>0.166</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.73</td>
<td>0.48</td>
<td>-52%</td>
<td>0.002¥</td>
<td>0.750</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.25</td>
<td>0.78</td>
<td>-22%</td>
<td>0.146</td>
<td>0.197</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-0.77</td>
<td>0.46</td>
<td>-54%</td>
<td>0.004¥</td>
<td>0.678</td>
<td></td>
</tr>
<tr>
<td><strong>Suppression</strong></td>
<td>1</td>
<td>-0.96</td>
<td>0.38</td>
<td>-62%</td>
<td>0.001¥</td>
<td>0.002¥</td>
<td>0.862</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.90</td>
<td>0.41</td>
<td>-59%</td>
<td>&lt;0.001¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.25</td>
<td>0.78</td>
<td>-22%</td>
<td>0.290</td>
<td>0.038¥</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-0.73</td>
<td>0.48</td>
<td>-52%</td>
<td>0.052¥</td>
<td></td>
<td>0.631</td>
</tr>
</tbody>
</table>

*Mean difference is model predicted mean change in outcome variables after cold water application or cough suppression compared to warm water control. ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

**Figure 45** – Inhibition of capsaicin-induced cough frequency, stratified by quartiles of 24 hour spontaneous cough frequency

The mean values are predicted from the GEE model, and back-transformed. The graph shows that compared to warm water control, there is a reduction in capsaicin-induced cough frequency after applying a cold water bath to the hand and after instructing subjects to “try not to cough” in all quartiles, defined by 24 hour spontaneous cough frequency. This reached statistical significance (p≤0.05) in all quartiles for both interventions except quartile 3.
Table 28 – Self-reported stress as a surrogate for group

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Quartile</th>
<th>Mean difference(Δ)</th>
<th>ExpΔ</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>quartile*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Water</td>
<td>1</td>
<td>-0.57</td>
<td>0.57</td>
<td>-43%</td>
<td>0.007¥</td>
<td>0.173</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.66</td>
<td>0.52</td>
<td>-48%</td>
<td>0.006¥</td>
<td>0.771</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.57</td>
<td>0.57</td>
<td>-43%</td>
<td>0.001¥</td>
<td>0.993</td>
<td>0.842</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-0.50</td>
<td>0.61</td>
<td>-39%</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppression</td>
<td>1</td>
<td>-0.52</td>
<td>0.59</td>
<td>-41%</td>
<td>0.010¥</td>
<td>0.048¥</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.97</td>
<td>0.38</td>
<td>-62%</td>
<td>0.003¥</td>
<td></td>
<td>0.252</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.50</td>
<td>0.61</td>
<td>-39%</td>
<td>0.014¥</td>
<td></td>
<td>0.951</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-1.14</td>
<td>0.32</td>
<td>-68%</td>
<td>0.006¥</td>
<td></td>
<td>0.176</td>
</tr>
</tbody>
</table>

*Mean difference is model predicted mean change in outcome variables after cold water application or cough suppression compared to warm water control. ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

Figure 46 - Inhibition of capsaicin-induced cough frequency, stratified by quartiles of self-reported stress levels

The mean values are predicted from the GEE model, and back-transformed. Graph shows that compared to warm water control, there is a reduction in capsaicin-induced cough frequency after applying a cold water bath to the hand and after instructing subjects to “try not to cough” in all quartiles, defined by self-reported stress. This reached statistical significance (p≤0.05) in all quartiles for both interventions except quartile 4 for cold water (p=0.062). Although it appears that quartile 4 had higher capsaicin-induced cough frequency compared to quartile 1, this did not reach statistical significance (p=0.258).
20.8 Gender effects

20.8.1 Effect of warm water, compared by gender

Overall, when compared to no intervention, applying a warm water bath did not significantly change capsaicin-induced cough frequency (p=0.839), see Table 29 and Figure 47. On average, warm water increased cough frequency by ~26% in females (p=0.111) and slightly decreased cough frequency by ~3% in males (p=0.839). There was no significant difference in the effect of warm water on cough frequency in males as compared to females (p=0.224).

Overall, when compared to no intervention, applying a warm water bath did not significantly change capsaicin-induced urge-to-cough intensity (p=0.789), see Table 29 and Figure 47. On average, warm water slightly decreased urge-to-cough intensity by ~2% in males (p=0.322) and increased urge-to-cough by ~1% in females (p=0.899). There was no significant difference in the effect of warm water on urge-to-cough in males compared to females (p=0.435).
Table 29 – Summary of the effect of warm water on capsaicin-induced cough, compared by gender

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Gender</th>
<th>Mean difference*(Δ)</th>
<th>Exp(Δ)</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value gender*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough frequency</td>
<td>Males</td>
<td>-0.03</td>
<td>0.97</td>
<td>-3%</td>
<td>0.839</td>
<td>0.248</td>
<td>0.224</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.23</td>
<td>1.26</td>
<td>+26%</td>
<td>0.111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urge-to-cough intensity</td>
<td>Males</td>
<td>-0.09</td>
<td>0.92</td>
<td>-8%</td>
<td>0.322</td>
<td>0.789</td>
<td>0.435</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.01</td>
<td>1.01</td>
<td>+1%</td>
<td>0.899</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean difference is model predicted mean change in outcome variables at warm water intervention visit (visit 2) compared to no intervention visit (visit 4). ¥ indicates p-value ≤0.05 and ≠ indicates p-value ≤0.1.

Figure 47 – Effect of non-painful warm water in males and females

A

B

The mean values (95% CI) are predicted from the GEE model and back-transformed. The graph shows the change in capsaicin-induced cough frequency (A) and urge-to-cough (B) after applying a warm water bath compared to no intervention in males (blue lines) and females (pink lines). There is no significant change in cough frequency or urge-to-cough intensity after applying warm water for either group.
20.8.2 Effect of painful cold water, compared by gender

Overall, when compared to a warm water control, applying a painful cold water bath to the hand significantly reduced capsaicin-induced cough frequency ($p=0.025$), see Table 30 and Figure 48. On average, cold water reduced cough frequency by ~34% in males ($p=0.001$) and by ~52% in females ($p<0.001$). There was no significant difference in the effect of cold water on cough frequency in males as compared to females ($p=0.137$).

Overall, when compared to a warm water control, applying a painful cold water bath to the hand showed a trend towards a reduction in urge-to-cough intensity ($p=0.091$), see Table 31 and Figure 49. On average, cold water reduced urge-to-cough by ~16% in males ($p=0.009$) and by ~28% in females ($p=0.001$). There was no significant difference in the effect of cold water on urge-to-cough in males as compared to females ($p=0.179$).

20.8.3 Effect of cough suppression, compared by gender

Overall, when compared to a warm water control, cough suppression significantly reduced capsaicin-induced cough frequency ($p<0.001$), see Table 30 and Figure 48. On average, cough suppression reduced cough frequency by ~41% in males ($p=0.004$) and by ~62% in females ($p<0.001$). There was no significant difference in the effect of cough suppression on cough frequency in males compared to females ($p=0.128$).

Overall, when compared to a warm water control, suppressing cough had no significant effect on urge-to-cough intensity ($p=0.741$), see Table 31 and Figure 49. On average, suppression changed urge-to-cough intensity by an increase of ~12% in males ($p=0.142$) and by a decrease of ~3% in females ($p=0.693$). There was no significant difference in the effect of cough suppression on urge-to-cough in males as compared to females ($p=0.193$).

20.8.4 Comparison of painful cold water and cough suppression by gender

Cold water and cough suppression reduced cough frequency to a similar extent in males ($p=0.650$) and females ($p=0.310$).
In contrast, cold water and cough suppression had a significantly different effect on urge-to-cough intensity in both males (p=0.025) and females (p=0.046), with cough suppression tending not to change urge-to-cough (or if anything increase it in males) whereas cold water tends to decrease urge-to-cough.

### 20.9 Group and gender effects

#### 20.9.1 Effect of painful cold water in male and female healthy controls and chronic cough patients

Compared to a warm water control, applying a cold water bath to the hand significantly reduced capsaicin-induced cough frequency (p=0.007), see Table 32 and Figure 50. In HC, the average reduction in capsaicin-induced cough frequency was ~36% in males (p=0.004), and ~61% in females (p<0.001). In CC patients, the average reduction in capsaicin-induced cough frequency was ~30% in males (p=0.078) and ~41% in females (p=0.032). Overall, cold water had a similar effect in male and female CC patients and HC (p=0.447).

Compared to a warm water control, applying a cold water bath to the hand significantly reduced capsaicin-induced urge-to-cough (p=0.056), see Table 33 and Figure 51. In HC, the average reduction in capsaicin-induced urge-to-cough was ~16% in males (p=0.002), and ~37% in females (p=0.005). In CC patients, the average reduction in capsaicin-induced urge-to-cough was ~15% in males (p=0.183) and ~18% in females (p=0.035). Overall, cold water had a similar effect in male and female CC patients and HC (p=0.283).

#### 20.9.2 Effect of cough suppression in male and female healthy controls and chronic cough patients

Compared to a warm water control, capsaicin-induced cough frequency was significantly reduced by cough suppression (p<0.001), see Table 32 and Figure 50. However, there is a suggestion of a different effect of cough suppression in male and female CC patients and HC (p=0.071). In HC, the average reduction in capsaicin-induced cough frequency was ~60% in males (p<0.001), and ~63% in females (p<0.001). In CC patients, the average reduction in capsaicin-induced cough frequency was 60% in females (p=0.009) but only in ~3% in males (p=0.892). Therefore, the male CC patients do not appear to
be significantly able to suppress cough, although only 9 male CC patients were studied, so this should be interpreted with caution.

Compared to a warm water control, capsaicin-induced urge-to-cough was not significantly changed by cough suppression (p=0.283), see Table 33 and Figure 51. However, there is a suggestion of a different effect of cough suppression in male and female CC patients and HC (p=0.087). In HC, capsaicin-induced urge-to-cough increased by an average of ~8% in males (p=0.402), and ~11% in females (p=0.317). In male CC patients, capsaicin-induced urge-to-cough increased by an average of ~20% in males (p=0.168), but in female CC patients average urge-to-cough intensity decreased by ~15% (p=0.067). Given that only 11 female chronic cough patients were included in the study, this finding needs to be interpreted with caution.
Table 30 – Summary of the effect of cold water and cough suppression on capsaicin-induced cough frequency, compared by gender

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Intervention</th>
<th>Group</th>
<th>Mean difference*(Δ)</th>
<th>Exp(Δ)</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value gender*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough frequency</td>
<td>Cold water</td>
<td>Males</td>
<td>-0.41</td>
<td>0.66</td>
<td>-34%</td>
<td>0.001¥</td>
<td>0.025¥</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>-0.73</td>
<td>0.48</td>
<td>-52%</td>
<td>&lt;0.001¥</td>
<td>¥</td>
<td>¥</td>
</tr>
<tr>
<td>Cough Suppression</td>
<td>Cold water</td>
<td>Males</td>
<td>-0.52</td>
<td>0.59</td>
<td>-41%</td>
<td>0.004¥</td>
<td>0.001¥</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>-0.96</td>
<td>0.38</td>
<td>-62%</td>
<td>&lt;0.001¥</td>
<td>¥</td>
<td>¥</td>
</tr>
</tbody>
</table>

*Mean difference is the model predicted mean change in outcome variables after cold water application or cough suppression compared to warm water control. ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

Figure 48 – Effect of cold water and cough suppression on capsaicin-induced cough frequency, compared by gender

The mean values are predicted from the GEE model and back-transformed. The graph shows that compared to a warm water control, there is a significant (*≤0.05) reduction in capsaicin-induced cough frequency after applying a painful cold water bath to the hand, and after instructing subjects to “try not to cough” in males (blue lines) and females (pink lines).
Table 31- Summary of the effect of cold water and cough suppression on capsaicin-induced urge-to-cough, compared by gender

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Intervention</th>
<th>Gender</th>
<th>Mean difference*($\Delta$)</th>
<th>Exp($\Delta$)</th>
<th>Estimated % change</th>
<th>p-value for $\Delta$</th>
<th>p-value intervention</th>
<th>p-value group*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urge-to-cough</td>
<td>Cold water</td>
<td>Males</td>
<td>-0.17</td>
<td>0.84</td>
<td>-16%</td>
<td>0.009¥</td>
<td>0.091≠</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>-0.33</td>
<td>0.72</td>
<td>-28%</td>
<td>0.001¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cough Suppression</td>
<td>Males</td>
<td>0.12</td>
<td>1.12</td>
<td>+12%</td>
<td>0.142</td>
<td>0.741</td>
<td>0.193</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>-0.03</td>
<td>0.97</td>
<td>-3%</td>
<td>0.693</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean difference is the model predicted mean change in outcome variables after cold water application or cough suppression compared to warm water control. ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

Figure 49 – Effect of cold water and cough suppression on urge-to-cough, compared by gender

Mean values are predicted from GEE model and back-transformed. The graph shows that compared to a warm water control, capsaicin-induced urge-to-cough intensity significantly (*≤0.05) decreases after applying a painful cold water bath to the hand, whereas there is no change in urge-to-cough during cough suppression in both males (blue lines) and females (pink lines).
Table 32 – Summary of the effects of cold water and cough suppression on capsaicin-induced cough frequency in male and female CC patients and HC

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Intervention</th>
<th>Group</th>
<th>Gender</th>
<th>Mean difference*(Δ)</th>
<th>Exp(Δ)</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value group*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough frequency</td>
<td>Cold water</td>
<td>HC</td>
<td>Males</td>
<td>-0.44</td>
<td>0.64</td>
<td>-36%</td>
<td>0.004¥</td>
<td>0.007¥</td>
<td>0.447</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>-0.95</td>
<td>0.39</td>
<td>-61%</td>
<td>&lt;0.001¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>Males</td>
<td>-0.36</td>
<td>0.70</td>
<td>-30%</td>
<td>0.078#</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>-0.53</td>
<td>0.59</td>
<td>-41%</td>
<td>0.032¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>Males</td>
<td>-0.97</td>
<td>0.40</td>
<td>-60%</td>
<td>&lt;0.001¥</td>
<td>&lt;0.001¥</td>
<td>0.071#</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>-0.99</td>
<td>0.37</td>
<td>-63%</td>
<td>&lt;0.001¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>Males</td>
<td>-0.03</td>
<td>0.97</td>
<td>-3%</td>
<td>0.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>-0.92</td>
<td>0.40</td>
<td>-60%</td>
<td>0.009¥</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean difference is the model predicted mean change in outcome variables after cold water application or cough suppression compared to a warm water control. ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

Figure 50 – Effects of cold water and cough suppression on capsaicin-induced cough frequency in male and female CC and HC

The mean values (95% CI) are predicted from the GEE model, and back-transformed. The graph shows that compared to a warm water control, there is a reduction in capsaicin-induced cough frequency after applying a cold water bath to the hand and after instructing subjects to “try not to cough” in male and female HC (black) and in female CC patients (green inverted triangles). However, male CC patients (green triangles) show a reduction in cough frequency with cold water, but not cough suppression.
Table 33 – Summary of the effects of cold water and cough suppression on capsaicin-induced urge-to-cough in male and female CC patients and HC

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Intervention</th>
<th>Group</th>
<th>Gender</th>
<th>Mean difference*(Δ)</th>
<th>Exp(Δ)</th>
<th>Estimated % change</th>
<th>p-value for Δ</th>
<th>p-value intervention</th>
<th>p-value group*intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough frequency</td>
<td>Cold water</td>
<td>HC</td>
<td>Males</td>
<td>-0.17</td>
<td>0.84</td>
<td>-16%</td>
<td>0.002¥</td>
<td>0.056≠</td>
<td>0.283</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>-0.47</td>
<td>0.63</td>
<td>-37%</td>
<td>0.005¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>Males</td>
<td>-0.16</td>
<td>0.85</td>
<td>-15%</td>
<td>0.183</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>-0.20</td>
<td>0.82</td>
<td>-18%</td>
<td>0.035¥</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HC</td>
<td>Males</td>
<td>0.08</td>
<td>1.08</td>
<td>+8%</td>
<td>0.402</td>
<td>0.366</td>
<td>0.087≠</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>0.10</td>
<td>1.11</td>
<td>+11%</td>
<td>0.317</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>Males</td>
<td>0.18</td>
<td>1.20</td>
<td>+20%</td>
<td>0.168</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Females</td>
<td>-0.16</td>
<td>0.85</td>
<td>-15%</td>
<td>0.067≠</td>
<td></td>
<td></td>
</tr>
</tbody>
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*Mean difference is the model predicted mean change in outcome variables after cold water application or cough suppression compared to warm water control. ¥indicates p-value ≤0.05 and ≠indicates p-value ≤0.1.

Figure 51 – Effects of cold water and cough suppression on capsaicin-induced urge-to-cough in male and female CC and HC

The mean values (95% CI) are predicted from the GEE model and back-transformed. The graph shows that compared to a warm water control, capsaicin-induced urge-to-cough intensity decreases after applying a painful cold water bath to the hand, whereas there is no change in urge-to-cough during cough suppression in males and female HC (black lines) and male CC patients (green triangles). However, female CC (green inverted triangles) show a reduction in urge-to-cough with cold water and cough suppression.
20.10 Summary of main results

1. Compared to no intervention, applying a non-painful warm water bath to the hand had no significant effect on capsaicin-induced cough frequency or urge-to-cough intensity:
   a. In HC and CC patients although:
      i. HC tend to show an increase in cough frequency with warm water
   b. In males and females.

2. Compared to a warm water control, applying a painful cold water bath to the hand significantly reduces capsaicin-induced cough frequency and urge-to-cough intensity:
   a. In HC and CC patients
   b. In males and females.

3. Compared to a warm water control, cough suppression significantly reduces capsaicin-induced cough frequency, but does not affect urge-to-cough intensity:
   a. In HC and CC patients although:
      i. CC patients tend to find suppression of cough frequency more difficult compared to HC
   b. In males and females except:
      i. In male CC patients, suppression of cough frequency is less effective than in other groups.
      ii. In female CC patients, the urge-to-cough tends to decrease with cough suppression.

4. There is no apparent relationship between 24 hour spontaneous cough frequency and the effectiveness of a painful cold water bath or cough suppression to reduce capsaicin-induced cough frequency.

5. There is no apparent relationship between stress and the effectiveness of a painful cold water bath or cough suppression to reduce capsaicin-induced cough frequency.

6. Anxiety and pain intensity did not influence the effects of the interventions.
21 Discussion

The results suggest that there are at least 2 mechanisms by which cough can be inhibited in HC and CC patients. Firstly, applying a painful cold water bath to the hand inhibits capsaicin-induced urge-to-cough intensity and cough frequency. Secondly, capsaicin-induced cough frequency (but not urge-to-cough) can be voluntarily inhibited following direct instruction to “try not to cough”. These mechanisms appear similar in their effectiveness in HC, but CC patients find it more difficult to voluntarily inhibit coughing. There were no significant gender effects, with females tending to show similar cough inhibition after applying painful cold water compared to males, contrary to the original predictions.

21.1 Applying a painful cold water bath to the hand inhibits capsaicin-induced cough and urge-to-cough in chronic cough patients and healthy controls

The endogenous inhibition of cough, as measured using a DNIC-paradigm, appears to be intact in CC patients. Applying a painful cold water bath to the hand inhibited capsaicin-induced cough and urge-to-cough by a similar magnitude in HC and CC patients. Furthermore, there was no significant correlation between 24 hour spontaneous cough frequency and the magnitude of cough inhibition with cold water.

Two important limitations should be recognised when interpreting these findings. Firstly, DNIC-independent mechanisms of endogenous inhibition were not tested, and therefore it is still possible that other inhibitory mechanisms are impaired in CC. Secondly, although the DNIC-effect has been thoroughly characterised in relation to the endogenous inhibition of pain, this has not been applied to cough. Therefore, it cannot be concluded that applying a painful conditioning stimulus inhibits cough by activating DNIC mechanisms.

21.1.1 The proposed neural pathways of DNIC mechanisms

The neuro-anatomy of DNIC, in relation to inhibition of pain, is important in understanding how these mechanisms may also apply to cough. Bouhassira and colleagues carefully delineated ascending and descending DNIC pathways in a series of
experiments using anesthetised rats[211-216], see Figure 52. DNIC are activated by noxious somatic stimulation of c-fibres or Aδ-fibres, which synapse on central neurones in the dorsal horn of the spinal cord. Central neurones immediately decussate[211, 213], ascend in the ventro-lateral quadrant of the spino-reticular tract[213], and terminate in the sub-nucleus reticularis dorsalis[212, 214, 215] in the most caudal part of the medulla. Descending inhibitory pathways then project ipsilateral to the test pain site[211, 216] in the dorso-lateral funniculus[216], and inhibit the transmission of other nociceptive information in the dorsal horn[114]. By testing DNIC mechanisms in healthy subjects and patients with well-defined neurological deficits (e.g. Brown-Sequards syndrome[211], thalamic lesions[212], Wallenburgs syndrome[212], quadriplegic patients[217]), the neuroanatomy of DNIC was found to translate well into humans.

**Figure 52 – Neuro-anatomy of Diffuse Noxious Inhibitory Controls (DNIC)**

![Diagram of DNIC neuroanatomy](image)

The neuroanatomical pathway of DNIC. DNIC is one mechanism of endogenous analgesia. Nociceptive afferent fibres enter the dorsal horn of the spinal cord and synapse on central neurons which decussate and project to the dorsal reticular nucleus (DRt) in the medulla. Descending inhibitory pathways travel ipsilateral to the test pain site to inhibit further pain transmission in the dorsal horn. Adapted from Ossipov[218].

It is possible that DNIC mechanisms also inhibit cough. Although the exact neural pathways cannot be confirmed from this study, there are some clues. Applying a painful stimulus to the hand inhibited the consciously perceived urge-to-cough sensation as well as the subsequently evoked motor cough response. Therefore, it is reasonable to
assume that inhibition of cough occurred on the afferent arm of the cough reflex, at a level before signals reach the cortex. The most likely site for cough inhibition by DNIC mechanisms is in an area of the brainstem known to be responsible for central afferent processing of cough, the nucleus tractus solitarius (nTS)[184].

21.1.2 The complementary role of psychological mediators in DNIC

Clinical studies have shown that DNIC mechanisms and psychological factors can be difficult to tease apart[205]. Therefore, in this study it is entirely possible that psychological factors may be additionally or alternatively acting to reduce cough, see Figure 53.

Distraction
Applying a painful cold stimulus to the hand may have shifted the focus of attention away from the capsaicin-induced urge-to-cough, and thereby reduced cough frequency. In support of this possibility, a recent study showed that the analgesic effect of distraction using a demanding visual computer task was similar in magnitude to the analgesic effect of DNIC mechanisms[219]. However, when distraction was combined with DNIC, an additive inhibitory effect was observed compared to DNIC alone, suggesting that distraction and DNIC are complementary, but independent, mechanisms.

To address the possible confounding issue of distraction, the following features were incorporated into the study design:

(i) Subjects were requested to focus their attention on rating urge-to-cough intensity throughout the intervention block, rather than focusing on the pain. This method has been used by others to minimise the effects of distraction[116].
(ii) The effect of the painful cold water was compared to non-painful warm water, to attempt to control for the effects of distraction.

However, there was no evidence that the warm water bath had a distracting effect on capsaicin-induced cough. In fact, in HC, applying a warm water bath showed a trend in the opposite direction with an increase in cough frequency. In summary, a painful
stimulus is likely to be more “distracting” than a non-painful stimulus, and therefore it is intrinsically difficult to separate distraction from DNIC mechanisms.

Expectations
Expectation of analgesia is one mechanism by which placebo-analgesia exerts its effect[220], and expectation of reduced cough when applying the cold water bath could similarly evoke a placebo response. Conversely, expectations of increased pain are completely able to block DNIC mechanisms[124]. In the present study, we were careful to avoid raising any expectations as to the effect of the painful conditioning stimulus. All subjects were told that the cold water might increase, decrease or have no effect on coughing. However, perhaps it would have been useful to ask subjects to rate their own pre-conceived expectations prior to the experiment in order to further control for this.

Stress
Stress-induced analgesia was first documented in severely wounded soldiers in combat situations who reported only mild-moderate pain levels[218], and has since been demonstrated in experimental human models (e.g. white noise with difficult mental arithmetic[221]) and in animals (e.g. brief foot-shock of forepaw)[218] where the analgesic response is blocked by naloxone, an opiate receptor antagonist.

Similarly, it is possible that stress activates opiate sensitive inhibitory mechanisms which reduce cough. However, I would argue that stress is unlikely to be an important inhibitory mechanism in the present study because:

(i) Subjects were asked to remove their hand from the cold water bath if at any time the pain became “intolerable”. All subjects tolerated the cold water bath for the duration of the intervention block and none rated the pain as 10/10 (maximum pain).
(ii) There was no significant change in pulse rate or blood pressure across the intervention blocks in either HC or CC patients.
(iii) Stress levels, rated on a VAS scale at the beginning of each randomised visit, did not significantly influence the inhibitory effectiveness of the cold water intervention.
Other psychological factors
Overall, the CC patients had higher levels of anxiety, depression, body vigilance, stress and a greater tendency towards pain catastrophising than the HC. Although there was no statistical evidence to suggest an influence of these psychological factors on cough inhibition, the study may be under-powered to detect subtle effects of these subjective psychological scores.

Figure 53 – Proposed mechanisms of cough inhibition after applying a painful stimulus

Figure shows the possible inhibitory cough mechanisms activated by applying a painful cold water bath to the hand. Excitatory pathways are shown in red, inhibitory pathways are shown in green. Inhibitory mechanisms include (i) The “DNIC-loop”, which could directly inhibit the afferent cough pathway, most likely in the brainstem and (ii) other psychological mediators such as distraction, expectation of reduced cough or stress. Whereas the DNIC loop is a “bottom-up” mechanism of inhibition, the psychological mediators are potential “top-down” regulators of cough.
21.1.3 Inhibitory mechanisms not directly tested in a DNIC paradigm which may be impaired in CC

Another pain modulating system that is distinct from DNIC mechanisms arises in the mid-brain peri-aqueductal grey (PAG) and projects through the rostro-ventral medulla (RVM) to inhibit or facilitate nociceptive transmission in the dorsal horn[113]. Facilitation of pain is mediated by RVM “on-cells” which are activated by prolonged tissue inflammation or nerve injury to maintain a hyperalgesic state. In contrast, inhibition of pain is mediated by RVM “off-cells” which are activated by exogenous administration of morphine (systemic or micro-injected into the PAG) or stress-inducing noxious stimuli. It has been proposed that a disturbance of the balance between pain facilitation and inhibition may be responsible for the development of chronic pain disorders[115].

This system is thought to be distinct from DNIC mechanisms, because lesions of the PAG[222] or RVM[223] in rats had no significant effect on the endogenous analgesic effect of a painful conditioning stimulus compared to control animals with sham lesions. However, subsequent studies suggested that systemic administration of morphine activates inhibitory neurons in the PAG, which subsequently block DNIC, suggesting a complex interaction between the two mechanisms[224]. Although the PAG-RVM pathway was not directly tested in the present study, there is a single animal study suggesting that the PAG-RVM modulating system may have relevance to cough. In healthy anaesthetised cats, conditioning stimulation of the PAG or RVM neurons inhibited cough evoked by mechanical stimulation of the larynx[190].

The role of the PAG-RVM system in regulating cough in humans needs to be further investigated. Of interest, RVM “off-cells” are known to be more active during sleep[113]. Therefore, if these cells have a tonic inhibitory effect on cough, this inhibitory effect should be more pronounced during the night. In support of this, spontaneous cough frequency declines substantially at night in chronic cough patients[56]. However, female CC patients have higher average night time cough rates compared to males[56]. A future study of cough during sleep in females could be revealing; if coughing were occurring during sleep, rather than as a consequence of frequent nocturnal waking, this would suggest a failure of tonic inhibitory mechanisms.
21.1.4 The role of cold temperature in the DNIC effect

Cold water was applied to induce pain rather than hot water. This is because cold water has been shown to consistently activate DNIC mechanisms[114], and reproducibly elicits pain in all subjects. In contrast, heat pain detection thresholds can vary from individual to individual[116]. Furthermore, with cold pain there is no risk of causing burn injury to the hand.

Ligand-gated ion channels that may transduce noxious cold stimuli and thereby mediate cold pain include TRPA1 and TRPM8[225]. TRPA1 activation has been shown to paradoxically desensitise TRPV1 co-localised on the same peripheral nerve terminal[226]. However, TRPA1 activation by a cold stimulus applied to the hand (i.e. somatic nervous system) is unable to directly desensitise peripheral TRPV1 receptors in the airways (i.e. autonomic nervous system). Thus this latter mechanism is unlikely to be responsible for the inhibition of capsaicin induced cough by cold water. Whether the thermal quality of the stimulus used to evoke the effects of DNIC on cough response is of importance is not known and requires further study.

21.2 Applying a painful cold water bath to the hand inhibits capsaicin-induced cough and urge-to-cough in males and females

Further data on gender differences in cough inhibition are needed, but the preliminary results of this first study suggest that DNIC mechanisms do not play a major role in explaining the predominance of females with chronic cough. Regardless of gender, applying a painful cold water bath to the hand inhibited capsaicin-induced cough and urge-to-cough by a similar magnitude. No significant gender differences could be identified in the analysis.

21.2.1 Previous studies of DNIC mechanisms in females

Females have increased cough sensitivity and a greater tendency to develop chronic cough[56]. Likewise, females also report higher pain intensity to experimentally induced pain and have a greater tendency to develop chronic pain[204]. Given these clinical similarities, chronic cough and chronic pain may arise in females as a result of common underlying mechanism including (i) sensitisation of afferent pathways or (ii) a failure of descending inhibitory mechanisms or (iii) both[204].
DNICs are one inhibitory mechanism that has been extensively tested in humans to clarify whether a failure of inhibition explains the female predominance in chronic pain. If a failure of DNIC pre-disposes females to develop chronic pain, then healthy females should demonstrate less effective DNIC than healthy males[116]. A comprehensive systematic review highlighted inconsistent data on this point, with females demonstrating less effective DNIC mechanisms in some studies, but similar or more effective DNIC mechanisms in others[204]. Some of these inconsistencies may be a consequence of varying DNIC protocols (pain stimulus intensity, modality, duration etc.)[204, 227]. Furthermore, only one study compared DNIC mechanisms in females of a particular hormonal status (on the contraceptive pill or in the follicular phase of their menstrual cycle) and showed no significant differences between the groups[228]. It is interesting that the greatest gender differences were observed in studies using subjective outcome measures of pain intensity, rather than objective neurophysiological measures of pain[204]. Perhaps psychological confounders play a greater role in females, making it more difficult to assess DNIC mechanisms in isolation. In support of this, a recent study showed that the failure of DNIC mechanisms in females is partly mediated by higher pain catastrophising scores[229]. Further data assessing gender differences in endogenous inhibition are needed to reach a firm conclusion in both pain and cough.

21.3 Voluntary cough suppression in CC patients and HC

Compared to HC, CC patients had poorer levels of conscious control over capsaicin-induced cough. Voluntary cough suppression of an individually determined ED50 capsaicin dose significantly reduced cough frequency in CC patients and HC. However, compared to HC, CC patients showed a trend towards less effective cough suppression.

In support of these findings, I have also shown that compared to HC, CC patients have poorer conscious control over citric acid-induced cough. HC instructed to suppress cough were significantly able to raise the C5 by 3 doubling doses, whereas CC patients given the same instruction only increased C5 by 1 doubling dose[127], see appendix 1.4.
21.3.1 The proposed neural pathways of cough suppression

The exact neural pathways involved in voluntary cough suppression cannot be determined from the results of this study. However, it is clear that cold water and cough suppression inhibit cough by different mechanisms. Instructing subjects to “try not to cough” had no significant effect on the urge-to-cough sensation. Thus, voluntary cough suppression must inhibit cough at a level after afferent signals reach the cortex, see Figure 54. This is in contrast to the inhibitory effect of a painful stimulus, which reduced urge-to-cough and cough frequency, therefore must inhibit cough at a level before afferent signals reach the cortex, see Figure 53.

Only one study has attempted to describe the cortical areas activated during cough suppression using functional magnetic resonance imaging (fMRI)[230]. Nineteen healthy subjects were provided with visual cues to inhale deeply on a nebuliser delivering either saline or a threshold dose of capsaicin. Cough suppression after capsaicin inhalation was compared to 3 other conditions including (i) capsaicin-induced cough (ii) voluntary cough after saline inhalation and (iii) normal breathing after saline inhalation. Activation of the anterior mid-cingulate cortex, the primary somato-sensory cortex and the supplementary area were correlated with urge-to-cough intensity[60] whereas activation of the inferior frontal gyrus, anterior insula and prefrontal cortex were associated with cough suppression[230]. The role of the PAG-RVM system could not be delineated, because a higher spatial resolution is required for accurate analysis[194]. The medulla was activated during capsaicin evoked cough and cough suppression, but not during voluntary coughing[230].

Anecdotally, patients suppress cough using a variety of strategies including breath-holding, swallowing or by general distraction e.g. counting. Breath-holding may lead to a diversion of focus towards the need to breathe rather than the urge-to-cough, possibly because hypoxia would be of greater threat to the individual than airways irritation. In support of this, exercise and voluntary isocapnic hyperventilation down-regulate fog-evoked urge-to-cough in healthy subjects[231]. Mechanisms by which cough can be suppressed need further investigation, and could be useful therapeutically if better understood.
21.3.2 Explanation for poor conscious control of the urge-to-cough in CC

CC patients have not yet been studied using fMRI. However, suppression of the urge-to-cough may be similar to suppression of other biological urges such as the urge-to-void. Brain responses to high volume bladder infusion (inducing a strong urge-to-void) were compared in healthy controls and patients with urge incontinence or overactive bladder. At high bladder volumes, healthy subjects showed slightly increased cortical activation patterns. In contrast, patients with bladder disorders showed widespread intense cortical activation, especially in the ACC, suggesting a very different higher-order processing of the same stimulus[232]. Similar disordered cortical responses to the urge-to-cough could occur in idiopathic CC, and could explain why such patients find cough suppression more difficult.
Figure 54 – Proposed neural pathways in cough suppression

Figure shows the proposed neural pathways involved in voluntary cough suppression. Excitatory pathways are shown in red, inhibitory pathways are shown in green. Capsaicin-induced cough must be inhibited at a level after afferent signals reach the cortex to generate an “urge-to-cough”, because voluntary suppression had no effect on urge-to-cough intensity.
21.4 Voluntary cough suppression in males and females

There were no significant gender differences in the effectiveness of cough suppression to reduce capsaicin-induced cough. However, on average, male CC patients were unable to suppress cough. In view of the small sub-group sample size (n=9), this finding should be further investigated before drawing any firm conclusions.

21.5 Study limitations

Some of the study limitations have already been discussed and include the following:

(i) A painful cold stimulus may be inhibiting cough by acting as a distraction. In future studies it would be preferable to include a non-painful visual distraction intervention as a control.

(ii) The study could not be blinded because of the nature of the interventions. This means that a placebo-effect after applying the cold water cannot be excluded.

(iii) There were small numbers of males and females within each group, so conclusions regarding within-group gender differences cannot be confidently made. A larger sample size is needed to explore further.
22 Conclusions

Capsaicin-induced cough can be inhibited by:

(i) Applying a painful cold water bath to the hand
(ii) Cough suppression

Both interventions reduced cough frequency. In contrast, the interventions had significantly different effects on the urge-to-cough, with no change in urge-to-cough during cough suppression, but a decrease in urge-to-cough during application of painful cold water. This suggests distinct underlying inhibitory mechanisms.

A comparison of HC and CC showed that the effect of the interventions was not significantly different between the groups. However, there was a trend towards less effective cough suppression in CC patients.

A comparison of females and males showed that the effect of the interventions was not significantly different between genders.

In summary, this study provides evidence that:

(i) Painful DNIC stimuli are capable of inhibiting capsaicin-induced cough responses
(ii) A failure of DNIC mechanisms does not contribute to the pathogenesis of chronic cough
(iii) A failure of DNIC mechanisms does not pre-dispose females to the development of chronic cough.
Final Discussion and Conclusions
23 Final Discussion

23.1 Summary of main findings

23.1.1 Hyper-responsive afferent pathways in chronic cough

This thesis provides new evidence that afferent pathways are hyper-responsive in patients with chronic cough (CC), at least partly as a result of up-regulation within the central nervous system (CNS). Firstly, capsaicin cough thresholds are lower in CC patients when compared to HC, as measured by standard cough thresholds (C2/C5, chapter 1) and pharmacodynamic parameters (ED50, chapter 2). Thus, normally innocuous doses of capsaicin are able to elicit coughing in CC patients, probably because of lowered neuronal activation thresholds in peripheral and/or central neurones. Secondly, CC patients have higher maximal cough frequency induced by inhaled capsaicin as compared to HC (C\text{max}, chapter 2). Thus, either the number of afferent fibres recruited by capsaicin is increased because of a phenotypic switch and/or widening of receptive fields, or else central encoding of cough is amplified as a result of failed inhibitory mechanisms. Finally, electrical stimulation of the upper oesophagus directly evokes coughing in CC patients, but not HC, suggesting that cough can be activated by normally innocuous stimuli from areas outside of the usual receptive fields, a phenomenon analogous to secondary allodynia which is known to be mediated by central sensitisation (chapter 1).

23.1.2 Conscious control of coughing is less effective in chronic cough

I have also provided new evidence that the conscious control of coughing is disordered in patients with CC. Firstly, cough suppression of an individually determined ED50 capsaicin dose was less effective in CC patients when compared to HC (chapter 3). Furthermore, in a study of mindfulness meditation, voluntary suppression of coughing after citric acid inhalation was less effective in CC patients compared to HC, see appendix 1.4. Secondly, CC patients show a disproportionate cough response to any given magnitude of reported urge-to-cough sensation (chapter 2). Finally, although CC patients have high levels of self-reported psychological co-morbidity including depression, anxiety, stress and hyper-vigilance (chapter 2 and 3), these factors did not seem to influence the ability to suppress coughing (chapter 3).
23.1.3 Gender differences in cough

In addition, this thesis provides new data that may further explain the predominance of females with CC. Firstly, females have lower urge-to-cough thresholds to inhaled capsaicin when compared to males (chapter 2), suggesting a sensitisation of sensory afferent cough pathways. Secondly, within all disease groups studied (HC, asthmatics and CC), females demonstrated higher maximal cough frequency induced by inhaled capsaicin as compared to males ($C^{max}$, chapter 2), indicating underlying cough hyper-responsiveness that may pre-dispose females to the development of CC. Finally, although the ability to voluntarily suppress cough was similar in male and female CC patients and HC (chapter 3), females demonstrated a disproportionate cough response to any given magnitude of reported urge-to-cough sensation (chapter 2). This suggests that females may have disordered cortical control mechanisms.

23.1.4 Application of pain models to investigate mechanisms in cough

DNIC

I have designed a method for measuring the inhibition of cough based on an experimental pain model known as Diffuse Noxious Inhibitory Controls (DNIC). Applying a painful stimulus to the hand activated inhibitory cough mechanisms to reduce capsaicin-induced cough responses in male and female HC and CC patients (chapter 3). Although these results suggest that DNIC mechanisms are not impaired in CC patients, the question as to whether other types of inhibitory mechanisms are dysfunctional remains unanswered. However, I have demonstrated that, in some circumstances, pain models can be usefully applied to develop an improved understanding of mechanisms in cough.

Ketamine

Ketamine, a potent NMDA receptor antagonist, had no significant anti-tussive effect in either HC or CC patients (chapter 1). Given that dextromethorphan (the active ingredient in several “over-the-counter” cough syrups) is also a weak NMDA receptor antagonist, these findings could have important clinical implications. However, ketamine was shown to transiently increase pain thresholds in HC and CC patients immediately after the infusion. Thus, the ability of ketamine to suppress pain but not cough is of interest,
and suggests the possibility of distinct NMDA receptor sub-types mediating cough and pain, discussed further below.

23.2 Pathogenetic mechanisms in treatment-resistant chronic cough

23.2.1 A CNS mediated imbalance between stimulus and cough

It is suggested that CC arises as a consequence of a CNS mediated imbalance between stimulus and cough. Not only is the threshold for eliciting cough reduced, indicating cough hypersensitivity, but the magnitude of cough response to any given stimulus is greatly increased, indicating a general cough hyper-responsiveness, see Figure 55. Indeed, it is the magnitude of cough response ($C_{\text{max}}$) rather than the cough threshold ($ED_{50}$) which best explains the variability in 24 hour spontaneous cough frequency (chapter 3). Therefore it will be critical to understand more precisely how this magnification of cough arises and is maintained in CC.

I have studied patients with treatment-resistant CC. Such patients are likely to represent an extreme example of the neural mechanisms that can lead to excessive coughing. However, one or more of these mechanisms may be directly relevant in other respiratory conditions. For example, healthy subjects with acute viral upper respiratory tract infection and patients with idiopathic pulmonary fibrosis have similarly high spontaneous cough frequencies[68, 233], with the potential for similar underlying mechanisms. This requires further investigation.
Figure 55 - Central regulation of the balance between stimulus and cough

A

\[\text{STIMULUS} \rightarrow \text{COUGH} \]

B

\[\text{STIMULUS} \rightarrow \text{COUGH} \]

C

\[\text{STIMULUS} \rightarrow \text{COUGH} \]

Figures show the CNS mediated balance between airway sensory stimulus and motor cough response. **A.** In healthy subjects, a supra-threshold stimulus evokes a proportional amount of coughing. **B.** In chronic cough patients, a normally sub-threshold stimulus evokes a disproportionate amount of coughing. **C.** In patients with suppressed cough e.g. from cerebro-vascular disease, even supra-threshold stimuli are insufficient to evoke normal amounts of coughing. An ideal cough suppressant would reset the balance between stimulus and cough to a normal level as in A, but not suppress cough below a normal level as in C.
23.2.2 Hyper-responsive sensory afferent pathways and impaired descending control

The urge-to-cough is defined as a sensation of airway irritation that is perceived prior to coughing. In human studies, the magnitude of urge-to-cough sensation can be subjectively recorded. This is useful because it provides information regarding the sensitivity and response characteristics of sensory afferent cough pathways. In a recent review article[37] (appendix 1.2) it was proposed that at low intensity urge-to-cough sensations, coughing can be consciously controlled. Conversely, at high intensity sensations, coughing becomes an irresistible reflex response.

The findings in chapter 2 suggest that the urge-to-cough sensation could be abnormally up-regulated in CC patients. It is possible that a heightened urge-to-cough sensation would also be more persistent, leading to severe coughing bouts that are difficult to terminate. In addition, the findings in chapter 3 and appendix 1.4 implicate impaired descending cortical control mechanisms (see Figure 56). A combination of prolonged, excessive urge-to-cough sensations that are difficult to relieve and impaired voluntary control could easily begin to explain the high spontaneous cough frequencies observed in these patients. The respective role of hyper-responsive sensory afferent pathways and impaired descending inhibitory control mechanisms could be further investigated using more objective techniques such as fMRI or EEG. What are the temporal relationships between urge-to-cough and cough? Which cortical areas are involved? Which emotional, cognitive and behavioural factors influence the process of engaging descending control mechanisms?

23.2.3 Clinical phenotyping to aid drug development

Improved clinical phenotyping of patients is needed to maximise success in drug development, and this will only arise by a more detailed understanding of underlying mechanisms. I have shown that capsaicin dose-response curves allow the measurement of key pharmacodynamic parameters providing far more mechanistic information than standard cough challenge end-points (C2/C5). Furthermore, it would be interesting to discover whether those CC patients with high cough frequencies during electrical stimulation of the oesophagus also demonstrate a positive temporal association between distal reflux and cough. Oesophageal sensitisation to cough could provide clues to underlying central mechanisms in distinct patient sub-groups.
Figure 56 – Descending inhibitory cortical control of cough

A. In healthy subjects, a supra-threshold stimulus activates sensory afferent cough pathways to generate an urge-to-cough sensation (red arrow). In this instance, the urge-to-cough sensation can be completely suppressed by descending cortical control mechanisms (green arrow).

B. In chronic cough patients, a normally sub-threshold stimulus activates hyper-responsive sensory afferent cough pathways to generate a magnified urge-to-cough sensation (red arrow). The ability to suppress cough is additionally impaired (green arrow), and this leads to an excessive cough response.
23.3 Gender differences in cough

Compared to males, females have greater prevalence of cough in epidemiological studies[7], are over-represented in specialist cough clinics[197], report worse cough-related quality of life[203], demonstrate lower cough thresholds to inhaled tussive agents[17] and have higher objective 24 hour cough frequency, especially overnight[56].

The reason for this female predominance in cough is unknown. One theory is that sex hormones increase peripheral ion channel sensitivity e.g. TRPV1[234], although this does not explain why the mean age of the female CC patients studied in this thesis was >50 years (i.e. post-menopausal), nor why cough frequency tends to increase gradually with age[56], and does not show a post-menopausal decline (personal communication, Dr Jaclyn Smith).

This thesis provides evidence that females have underlying cough hyper-responsiveness that predisposes them to the development of CC. This hyper-responsiveness may be partly mediated by sensitisation of afferent pathways, but could also be related to impairment of descending control mechanisms, as discussed above.

23.4 Is pain a useful paradigm for investigating cough mechanisms?

It has been proposed that pain provides a useful scientific model for investigating mechanisms in cough[235]. For example, urge-to-cough and pain are both unpleasant sensations that are subjectively experienced by the individual and serve as a warning to protect the body from harm. Although neural pain pathways are spinally-mediated whereas cough pathways are brainstem-mediated, both pain and urge-to-cough represent a continuous spectrum of unpleasant sensations from innocuous to intensely noxious. Therefore it is feasible that the central gating of cough and pain share certain similarities. Furthermore, the clinical features of patients with chronic pain are similar to chronic cough e.g. lowered thresholds for evoking pain/cough (allodynia/allotussia) and increased pain/cough in response to noxious stimuli (hyperalgesia/hypertussia).

One pharmacological treatment that is well-documented to reduce both cough[128, 129] and pain is morphine. One mechanism by which morphine reduces pain is via activation of endogenous inhibitory pain mechanisms, to reduce the afferent transmission of
nociceptive information in the dorsal horn of the spinal cord[113]. The mechanism by which morphine reduces cough is less clearly documented, but in humans, it reduces cough only when administered systemically, not when inhaled[129]. Therefore, it is plausible that morphine may also reduce cough by activating endogenous inhibitory mechanisms in the CNS. In chapter 3, the inhibition of cough was measured by applying DNIC mechanisms. DNIC is thought to reflect the individual ability to activate endogenous inhibitory mechanisms, and is known to be impaired in several chronic pain states[205]. Applying these same concepts to cough, I found that a painful DNIC stimulus activated inhibitory cough mechanisms, and that these were also intact in CC. Therefore, a failure of DNIC mechanisms cannot explain the pathogenesis of CC. Other inhibitory mechanisms could still be defective and this requires further work. For example, a failure of the opiate-mediated PAG-RVM descending inhibitory pathways is possible. In this case, exogenous administration of morphine could be predicted to re-activate these endogenous inhibitory control mechanisms, see Figure 57. An improved understanding of the mechanisms by which opiates suppress cough would provide a scientific rationale for the development of novel, better-tolerated, selective opiate receptor agonists.

Another therapeutic strategy that has not translated as well from pain to cough is antagonism of the NMDA receptor. Dextromethorphan is a weak NMDA receptor antagonist with modest anti-tussive efficacy in acute viral cough[159], and evidence of analgesic effect in experimental pain models[236], but has other mechanisms of action e.g. sigma-1 agonist[237]. In chapter 1, I showed that ketamine, a more selective and potent NMDA receptor antagonist, raised somatic and visceral pain thresholds, but had no observed effect on experimentally induced cough. Whilst ketamine appears to be an effective analgesic, but not an anti-tussive, another NMDA receptor antagonist, memantine, may have anti-tussive[161], but not analgesic, efficacy[238], see appendix 1.3. This raises the possibility of selective NMDA receptor sub-types regulating cough, but not pain, and highlights the difficulties with assuming that all pain mechanisms have direct relevance to cough.
This figure demonstrates the endogenous inhibition of cough in chronic cough patients before (A) and after (B) administration of morphine. **A.** In CC patients, endogenous inhibitory mechanisms (blue arrow) exhibit an insufficient level of tonic level of control on the CNS processing of sensory afferent cough pathways in the brainstem.  **B.** It is hypothesised that endogenous inhibitory mechanisms (e.g. PAG-RVM pathways) are re-activated by the administration of morphine to inhibit CNS processing of afferent cough pathways in the brainstem, and therefore reduce cough.
24 Final Conclusion

It is increasingly recognised that a greater understanding of the heterogeneity of mechanisms responsible for disease is of instrumental importance to the future successful development of drugs. These concepts are especially relevant to the development of cough suppressants. For example, cough in COPD is unlikely to arise as a result of the same underlying mechanisms as cough in pulmonary fibrosis, or idiopathic chronic cough. Even within disease categories, cough could develop by one or more different mechanisms. Carefully defining these patho-physiological processes provides a clear scientific rationale for testing of novel cough therapeutics in specific, well-defined patient groups.

This thesis has made significant progress towards these goals. I have designed and tested novel capsaicin cough challenges, which may prove to be a valuable drug development tool. I have provided evidence for a process analogous to central sensitisation in treatment-resistant chronic cough patients, although the specific molecular pathways responsible require further investigation. I have tested two separate inhibitory cough mechanisms, and shown that chronic cough patients may have impaired descending cortical control of cough. Finally, I have demonstrated that utilising pain as a scientific framework for better understanding mechanisms in cough is, within limits, a useful strategy.

In conclusion, an improved understanding of mechanisms in cough will provide a logical route to the development of badly needed novel therapeutics.
References


Appendices

1 Publications

1.1 Quality of life in Patients with Chronic Cough
Quality of life in patients with chronic cough

Emma C. Young and Jacyn A. Smith

Abstract: Chronic cough is a relatively common symptom for which effective, acceptable treatments are lacking. Many patients suffer frequent coughing over several years without resolution and this has significant physical, social and psychological consequences. The recent development of cough-specific quality-of-life tools now allows quantification of the burden of coughing both in patients specifically presenting with chronic cough and also in common respiratory conditions.

Keywords: cough, quality of life

Introduction

Cough is a common and distressing symptom for many patients with pulmonary disease. There is little convincing evidence for the effectiveness of any currently available anti-tussive treatments, therefore cough represents a significant health problem.

In healthy individuals, the cough reflex is triggered by noxious airway stimuli that generate a consciously perceived urge-to-cough sensation. As the intensity of the urge-to-cough increases, coughing becomes irresistible. The motor component of the cough reflex is divided into three phases including (1) inspiration (2) forced expiration against a closed glottis and (3) opening of the glottis with rapid expiration generating the characteristic cough sound. In patients suffering from chronic coughing, the sensitivity to noxious stimuli often becomes heightened, resulting in disproportionate coughing.

Chronic cough is simply defined as a cough lasting longer than 2 months irrespective of underlying cause, but is also a commonly used term to describe a group of patients primarily complaining of coughing despite normal chest X-ray and lung function [Morice et al. 2006; Pratter et al. 2006]. Such patients are commonly subsequently found to have evidence of cough-variant asthma, nasal disease and gastro-oesophageal reflux disease, but a significant proportion do not respond to treatment of these conditions [Haque et al. 2005].

This review will outline the impact of persistent coughing on health-related quality of life in this latter patient group, and highlight the importance of cough symptoms in relation to other chronic pulmonary diseases including asthma, chronic obstructive pulmonary disease (COPD) and interstitial lung disease.

Impact of cough

A recent focus group study has suggested that chronic cough patients perceive the severity of their cough in terms of the cough frequency, intensity, and disruptiveness [Vernon et al. 2009]. Clearly these factors are inter-related as more frequent and intense coughing is likely to cause the most disruption (Figure 1). Patients with chronic cough are known experimentally to have hypersensitivity of their cough reflex to noxious agents [Choudry et al. 1992] and, clinically, patients describe coughing triggered by trivial environmental exposures such as perfumes, aerosols, temperature changes and during talking or laughing [McGarvey et al. 2009]. The consequence is very frequent coughing and objective measurements have demonstrated average cough frequencies of 13.9 coughs/h with many patients coughing hundreds of times per day [Kelsall et al. 2009]. The intensity of coughing has rarely been studied and objective measurements are currently lacking however more violent coughing is probably responsible for the more extreme physical complications. The complications and disruption caused by excessive coughing often motivate the individual to seek medical attention [French et al. 1998]. A UK postal survey found that the majority of people reporting cough were distressed, angry, anxious and depressed, and 64% felt the cough interfered with their social life [Everett et al. 2007].
**Figure 1.** Dimensions of cough severity [Vennet et al. 2009].

**Physical complications**
Coughing generates very high intra-thoracic [Lavietes et al. 1998] and intra-abdominal pressures [Man et al. 2003] in order to remove harmful irritants from the respiratory tract. Vigorous coughing can directly result in tissue trauma causing symptoms including chest pains, hoarse voice, and more rarely rib fractures and hernia. Many patients also report retching and vomiting as a result of violent coughing, light-headedness, stress incontinence, headaches and physical exhaustion which may be due to both the physical exertion of prolonged coughing and sleep deprivation if nocturnal coughing is a problem. Urinary incontinence is particularly distressing for females; in our clinic 50% report incontinence related to coughing. Cough syncope is an uncommon complication, occurring in males more frequently than females [Bonekat et al. 1987; Sharpey-Schafer, 1953]. Apart from the risk of injury associated with sudden loss of consciousness, cough syncope has profound implications for drivers who lose their licence until the cough is treated.

**Social complications**
Many patients are driven to seek help for chronic cough as a result of social disruption; a recent study found interference with lifestyle and leisure was the commonest complaint in relation to coughing (80% of subjects) [Kuzniar et al. 2007] and a third of patients under 65yrs complained that partners were forced sleep in another room due to coughing overnight. The embarrassment of coughing in public places and the workplace environment can be compounded by friends or colleagues who wrongly assume the patient has a contagious condition or is a heavy smoker. Difficulties occur for those who have to speak on the telephone for long periods and speak in public. The inability to suppress coughing paroxysms in quiet environments such as at the theatre or in church can significantly disrupt attendance at social gatherings. Embarrassment and social withdrawal can also occur as a result of the physical complications of coughing, especially urinary incontinence and retching/vomiting in public places. In addition, frequent doctor visits whilst complex diagnostic algorithms are explored are time consuming and with access to specialist cough clinics poor, travel and prescription costs may be significant.

**Psychological complications**
A prospective study recruiting patients with chronic cough from a specialist cough clinic in North America found that 53% met diagnostic criteria for depression using the Centre for Epidemiologic Studies Depression scale (CES-D) [Ripinigiatitis et al. 2006]. The prevalence of depression and anxiety in patients attending a cough clinic in Belfast, UK was 15.8% and 33.3%, respectively using the Hospital Anxiety and Depression Scale (HADS) [McGarvey et al. 2006]. The authors’ experience in Manchester, UK is similar [Decatalo et al., 2007]. However, specialist cough clinic patient samples may not be representative of chronic cough in the general population since those with psychological distress may be more likely to seek specialist medical attention. Furthermore, although both the CES-D and HADS are validated in a number of medical settings they have not been specifically validated for chronic cough. It is possible that patient responses could be influenced directly by their cough symptoms, leading to over-reporting of psychiatric co-morbidity, e.g. social withdrawal due to the embarrassment of coughing rather than altered mood. Evidence suggests that patients with medically unexplained cough, described as ‘idiopathic chronic cough’, are no more likely to have depression or anxiety than patients with treatable cough [McGarvey et al. 2006]. Unsurprisingly, increased levels of anxiety and depression are associated with a worse quality of life both before and after successful treatment of the cough [Kalpakoglou et al. 2005].

**Measuring cough-specific quality of life**
Health-related quality of life refers to an individual’s emotional, social, and physical well-being, and their ability to function in the ordinary tasks of living [Fallowfield, 2009]. Three validated,
repeatable and responsive questionnaires have been designed to capture the impact of cough symptoms on quality-of-life, including the Leicester Cough Questionnaire (LCQ) [Birring et al. 2003], the Cough-Specific Quality of Life Questionnaire (CQLQ) [French et al. 2002], and the Chronic Cough Impact Questionnaire (CCIQ) [Bairdini et al. 2005]. All three questionnaires are self-administered, easily used clinically and have been validated against generic quality-of-life scales. Many of the items included are common to all three questionnaires, confirming consistent themes in quality-of-life impairments, despite development in three different countries (UK, US and Italy). The CQLQ and LCQ have been most widely published upon and hence will be discussed in detail in this review.

**Leicester Cough Questionnaire**

The LCQ contains 19 items and three health domains (physical, psychological and social) rated on a 7-point Likert scale. A low LCQ score represents poorer quality of life. It was developed by studying patients presenting with unexplained chronic cough (defined as >3 weeks' duration). In addition to chronic cough patients [Kelsall et al. 2009; Kelsall et al. 2008; Decalmer et al. 2007; Morice et al. 2007; Birring et al. 2006] the LCQ has also been applied in patients with bronchiectasis [Murray et al. 2009a, 2009b; Muzalitash et al. 2008], cystic fibrosis [Fathi et al. 2009], asthma [Marsden et al. 2008] and idiopathic pulmonary fibrosis [Hamilton et al. 2008; Holt et al. 2008].

**Cough Quality of Life Questionnaire**

The CQLQ contains 28 items and six health domains (physical complaints, extreme physical complaints, psychosocial issues, emotional well-being, personal safety fears and functional abilities) rated on a 4-point Likert scale. A high CQLQ score represents poorer quality of life. An earlier iteration, the Adverse Cough Outcome Questionnaire (ACOQ), was developed in chronic cough patients (>8 weeks) although the CQLQ was validated in a broader patient group including acute cough (<3 weeks) and smokers with cough in addition to chronic cough subjects [French et al. 2002]. This questionnaire has also been used to compare different management strategies in chronic cough [Field et al. 2009] and for comparison with objective measures in cystic fibrosis [Smith, 2006] and COPD [Smith et al. 2006].

**Comparative studies**

Two studies comparing the LCQ and CQLQ have been performed. Polley et al. compared these questionnaires in patients with asthma, bronchiectasis, COPD and chronic cough, finding moderate-to-strong correlations between scores in all patient groups [Polley et al. 2008], although the correlations were weakest for chronic cough and COPD subjects. A second study, only in chronic cough patients again found correlations were moderate both before and after treatment [Kalpaklioglu et al. 2005].

**Gender differences in quality of life**

Patients attending specialist cough clinics are predominantly middle-aged females. Females are known to have a more sensitive cough reflex to inhaled irritants such as capsaicin in both health and in chronic cough [Kastelik et al. 2002; Fujimura et al. 1996]. It has also been proposed that coughing has a greater impact on health-related quality of life in women and therefore more women seek medical attention for their cough [French et al. 2004]. Urinary incontinence is a common physical complication of coughing affecting older women, and our own data suggest that patients with urinary incontinence are more likely to be depressed (unpublished). CQLQ scores suggest that women with chronic cough have a significantly worse quality of life than men, especially in relation to physical complaints (including urinary incontinence), extreme physical complaints and psychosocial issues [French et al. 2005; 2004]. In contrast, the authors have been unable to demonstrate gender differences in quality of life with the LCQ, despite women having almost double objectively measured 24-hour cough frequency in comparison to men [Kelsall et al. 2009]. However, Polley et al. found that gender differences in quality of life were detected using both the CQLQ and LCQ in a mixed group of respiratory patients [Polley et al. 2008]. The authors' findings suggest that women have more frequent coughing, independent of the gender differences in cough reflex sensitivity, which could contribute to the high prevalence of females with chronic cough and its greater impact on health-related quality of life.

**Impact of cough in chronic respiratory diseases**

A cross-sectional survey of cough-related quality of life in outpatients with chronic cough, COPD, asthma and bronchiectasis found no significant difference in LCQ or CQLQ total scores between
these patient groups [Polley et al. 2008], implying that cough symptoms impair quality of life to a significant extent in many chronic respiratory conditions.

Cough can now be objectively quantified using ambulatory cough monitors recording cough frequency over 24 hours [Decalmer et al. 2007]. Cough frequency is measured as the number of explosive cough sounds per hour (cough sounds/h) or the number of seconds containing at least one explosive cough sound (cough seconds/h) and these two measures are interchangeable [Kelsall et al. 2008]. Increasing cough frequency is generally associated with worsening quality of life in chronic cough [Kelsall et al. 2008; Decalmer et al. 2007; Birring et al. 2006], classical asthma [Marsden et al. 2008], COPD [Smith et al. 2006] and idiopathic pulmonary fibrosis (IPF) [Hamilton et al. 2008]. Table 1 summaries the relationships between cough-specific quality of life and objective cough rates across different pulmonary diseases. For a particular cough frequency a wide range of QOL scores are found, hence the correlations are generally moderate in most conditions. Cough quality of life may be significantly influenced by factors such as mood, the attention paid to coughing and the presence of additional symptoms, e.g. breathlessness which may make coughing more unpleasant and distressing. Furthermore the cough intensity (in addition to frequency) may be an important independent predictor of quality of life in particular in relation to physical complications which would be expected to be related to the most vigorous coughing.

**Cough in classical asthma**

Cough is a common symptom in classical asthma that is clinically associated with poor disease control. Yet remarkably, objective data on the relationship between cough and asthma severity or change in cough symptoms in response to standard treatments is lacking. A conjoint analysis performed on 273 unslected adult patients with moderately severe asthma confirmed that daytime cough was rated by patients as twice as important compared to wheeze, chest tightness or sleep disturbances [Ouanan et al. 2001]. In 56 patients with mild-to-severe asthma not selected on the basis of cough symptoms, 24-hour objective cough frequency showed that, although most patients had relatively low cough frequencies (Table 1), a proportion of patients had cough frequencies comparable to chronic cough patients. Moreover, LCQ in asthma patients overlapped considerably with scores collected in chronic cough subjects in a separate study [Kelsall et al. 2009] (Figure 2). Cough frequency moderately correlated with LCQ scores.

**Table 1. Comparison of objective cough frequency and cough-specific quality of life in chronic cough and common respiratory conditions.**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>Cough-specific quality of life</th>
<th>Objective cough frequency</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LCQ score</td>
<td>24-hour</td>
<td></td>
</tr>
<tr>
<td>Chronic cough</td>
<td>n = 62</td>
<td>12.8 (±3.7)</td>
<td>11.4 (1.1–46.5)*</td>
<td>2.9</td>
</tr>
<tr>
<td>[Decalmer et al. 2007]</td>
<td></td>
<td>(2–74.8)*</td>
<td>(0–27.7)*</td>
<td>r = -0.42</td>
</tr>
<tr>
<td>Kelsall et al. 2008</td>
<td>n = 70</td>
<td>12.3 (±3.8)</td>
<td>15.9 (8.6–23.0)*</td>
<td>19.3</td>
</tr>
<tr>
<td>Birring et al. 2006</td>
<td>n = 20</td>
<td>12.1 (±1.2)</td>
<td>11.4 (11.4–30.6)*</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N/a</td>
<td>(1.2–7.4)*</td>
<td>r = 0.03</td>
</tr>
<tr>
<td>Asthma</td>
<td>n = 56</td>
<td>17.8 (±8.7–21.0)</td>
<td>2.6 (2.0–14.2)*</td>
<td>0.3</td>
</tr>
<tr>
<td>Marsden et al. 2008</td>
<td></td>
<td>(0.0–18.5)*</td>
<td>(0.0–8.7)*</td>
<td>r = 0.56</td>
</tr>
<tr>
<td>COPD</td>
<td>n = 26</td>
<td>16.4 (±5.5)</td>
<td>7.5 (2.7–23.1)*</td>
<td>12.4</td>
</tr>
<tr>
<td>Smith et al. 2006</td>
<td></td>
<td>(29–76)</td>
<td>(3.3–40.4)*</td>
<td>1.9</td>
</tr>
<tr>
<td>LCQ</td>
<td>n = 10</td>
<td>15.4 (±3.7)</td>
<td>11.2 (1.8–36.7)*</td>
<td>14.1</td>
</tr>
<tr>
<td>Hamilton et al. 2008</td>
<td></td>
<td>(1.9–47.9)*</td>
<td>(0–19.7)*</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*Cough quantified in *cough seconds/h, *cough sounds/h, values quoted are appropriate means (±SD), or medians (range). Note: A lower LCQ score indicates a worse quality of life. LCQ: Leicester Cough Questionnaire; LCQ-SQ: Cough-Specific Quality of Life Questionnaire.*

http://ar.sagepub.com
indicating that particularly in patients with higher cough frequencies, coughing reduces quality of life for patients with asthma [Marsden et al. 2008]. This correlation was significant for all sub-domains of the LCQ score (physical, psychological and social). There were no significant correlations between objective cough frequency and percent predicted FEV1 or exhaled nitric oxide and only weak correlations with subjective cough measures, suggesting these commonly used end-points do not adequately reflect severity of coughing in asthma.

Cough in COPD

Although cough is a common symptom in COPD, many patients probably attribute cough to their smoking habits and may therefore be less inclined to complain about coughing. In a primary care setting in Spain, 84.7% of patients with stable, moderate COPD admitted to cough symptoms with a trend towards a worse quality of life (measured using the St Georges Respiratory Questionnaire – SGRQ) in those patients with cough [Miravitlles et al. 2007]. Nonetheless, in 20 patients with mild to moderate stable COPD selected for the presence of cough, increasing cough frequency was associated with worsening CQoL total score, especially for nocturnal coughing [Smith et al. 2008].

Cough in interstitial lung disease

Patients with interstitial lung disease (ILD) typically present with breathlessness and dry cough. A single qualitative study suggested patients find coughing to have significant physical and social impacts in these patients [Swigris et al. 2005]. We have recently studied cough in a small number of patients with ILD not selected on the basis of cough symptoms; very high daytime cough rates and quality-of-life impairments comparable to patients with chronic cough have been observed [Hamilton et al. 2008]. This group of patients seem to have the strongest correlations between objectively measured cough frequency and LCQ score (Table 1), particularly during the daytime. Cough rates were unrelated to pulmonary function (FEV1, FVC, DLco or Kco) and the mechanism of coughing in IPF is currently unknown, but may be due to the observed high prevalence of gastro-oesophageal reflux disease [Raghu et al. 2006].

Conclusions

Chronic coughing is clearly associated with significant physical, social and psychological impacts. This is especially true in patients presenting with chronic cough and high cough frequencies but the impact for some patients with chronic respiratory diseases is probably underestimated. These impacts can now be captured using quality-of-life tools which are well-validated, responsive to treatment effects and have been applied in a variety of conditions associated with cough.

For comprehensive assessment of coughing, objective measures of cough are also important. Cough frequency and quality of life, although related, capture different aspects of the symptoms. In future cough studies the ideal endpoints are a combination of objective measures of 24-hour cough frequency and validated quality-of-life questionnaires.

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Conflict of interest statement

No financial conflicts of interest exist for either of the authors, but JS is an inventor on a patent owned by the University Hospital of South Manchester, on novel cough detection methods. She also has a collaboration with Vitalograph UK Ltd.

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1.2 New Insights in Cough
New insights in cough

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Chronic cough is common, blights patients' lives and is hard to treat. Chronic cough patients demonstrate high objective cough rates and as a group have increased cough reflex sensitivity. However, conventional cough challenge techniques show substantial overlap with normal subjects. This suggests that other important mechanisms have yet to be determined. For the last two decades, chronic cough has been considered to be caused by gastro-esophageal reflux, post-nasal drip or Asthma. However, many patients with these conditions do not have cough, and in those with cough, the response to specific treatments is unpredictable at best. In addition, many chronic cough patients do not have an identifiable cause. This raises questions about the concept of a triad of treatable causes for chronic cough. Our current understanding of the neurophysiology of the cough reflex is largely derived from animal work with limited data in humans. By analogy with chronic pain syndromes, both peripheral and central sensitization may be important mechanisms in chronic cough, and are under active investigation. We need to understand the mechanisms underlying sensitization, how they interact with cough triggers and their relationship with the sensations that drive the urge to cough, and the subsequent motor cough response in chronic cough. Only then will we develop effective interventions.

Keywords: chronic cough/neurophysiology/urge to cough/peripheral and central sensitization

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Chronic cough—a typical patient

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Chronic cough is very common, with a prevalence of up to 12% in community surveys. A typical patient with chronic cough is most commonly a post-menopausal female, who has a cough for >5 years. She has a sensation of an irritation located at the back of the throat associated with an urge to cough. The cough may be an exaggerated response to irritants, changes in temperature and chemical sprays. It can also occur doing normal activities such as eating, even stretching her neck.
into a particular position. She has cough-induced stress incontinence, and will be taking an anti-depressant. She is socially isolated and sleeps separately from her partner. She has consulted many physicians in different specialities, been extensively investigated and irradiated, and tried many treatments, all to no avail. She carries a bottle of water at all times, because swallowing water gives temporary relief.

It is interesting to speculate why this chronic cough syndrome has not received more attention. Does it suffer from lack of a specific disease name? Or is it because individual physicians and pharmaceutical company executives have dismissed this as comparable with their own transient coughs that come and go with a URTI? On the contrary, this is an area of a major unmet need.

The cause of cough—the conventional view

Published guidelines on the management of chronic cough\textsuperscript{1–3} recommend the investigation and systematic treatment of a triad of underlying triggers—asthma, gastro-oesophageal reflux disease (GORD) and post-nasal drip syndrome (PNDS). There are case series in the last 20 years attributing virtually all cough to these conditions, and describing a >98% response to specific treatments of these causes.\textsuperscript{4} However, more recent case series have described increasing numbers of patients with unexplained cough which is resistant to treatment.\textsuperscript{5–7} In our specialist cough clinic, by very detailed investigation we have been able to identify specific triggers (often more than one) in most patients, but in two-thirds of patients the cough is completely or partially resistant to specific treatment of diagnosed triggers. Conversely, the large majority of patients with GORD, asthma and PNDS do not complain of cough at all.

Why do our chronic cough patients cough in response to often minor triggers? And why do not they improve when we treat them? The simplistic view that there are three treatable causes of chronic cough has held sway on both sides of the Atlantic for two decades and needs revisiting.

The neurophysiology of cough—parallels with pain

First a word of caution; most of what we know about the structure and function of the cough reflex comes from experiments in healthy animals, and the data needs careful interpretation in the context of patients with chronic cough. This article draws parallels with neuropathic pain, and especially examine(s) whether the concepts of peripheral and central sensitization could explain the persistence of cough in patients with chronic cough.
Airway nerves and cough

Based on animal work, there are at least five vagal afferent nerve sub-types that can be characterized by their electrophysiological, morphological and immuno-histochemical properties. Airway nerves differ in their patterns of response to different stimuli, consequent on differences in ion channels. These are broadly described below:

- A putative ‘cough receptor’ has been identified in the central airways of guinea pigs which is responsive to punctate mechanical stimuli and rapidly changing pH (the latter probably via activation of ASIC3 ion channels). This cough receptor likely mediates the immediate protective cough reflex to aspiration of foreign material and especially gastric contents into the airways.

- C-fibres are subdivided into two types. Bronchial and pulmonary C-fibres arise from jugular and nodose ganglia, respectively. Bronchial C-fibres when activated strongly evoke coughing in conscious humans and animals, but not in anaesthetized animals, suggesting they mediate a conscious perception of airway irritation that is suppressed during anaesthesia. They are activated by a family of ligand-gated ion channels of which the best characterized is the transient receptor potential vanilloid 1 (TRPV1) receptor which responds to noxious heat (42–53°C), low pH and capsaicin. At low tissue pH, TRPV1 is activated at physiological body temperatures. One study suggested increased expression of TRPV1 receptors in bronchial biopsies of patients with chronic cough. However, TRPV1 receptor immunostaining may be non-specific and not limited to nerves and this finding has not been replicated. The TRPA1 receptor is co-expressed with TRPV1 on C-fibre terminals, and may be sensitized by increased TRPV1 activity. TRPA1 responds to cold air and is activated by aldehydes in cigarette smoke, and 4-hydroxy-2-nonenal produced in response to oxidative stress at sites of tissue inflammation. Cinnamaldehyde activates TRPA1 receptors and induces cough when inhaled by humans.

- Rapidly adapting receptors (RARs) and slowly adapting receptors (SARs) are intra-pulmonary mechanically sensitive afferent nerves; SARs are activated during inspiration; RARs activate with bronchial constriction. They may facilitate cough but are considered less important in cough in human disease.

The vagus nerve

The vagus nerve transmits sensory information from the lungs to the brainstem, where vagal afferent nerves synapse on second-order relay neurones in the nucleus tractus solitarius (nTS). Vagotomy or vagal block with local anaesthesia abolishes cough in human subjects.
while the cough reflex is preserved in patients with cervical spinal cord injury.\textsuperscript{18}

**Central pathways**

Activation of the 'cough receptors' releases glutamate centrally since injection of an N-methyl-D-aspartic acid (NMDA) receptor antagonist into a discrete region of the nTS where cough receptors terminate blocks coughing evoked by citric acid in anaesthetized guinea pigs.\textsuperscript{19} C-Fibres release neuropeptides, such as substance P, calcitonin gene-related peptide and neurokinin A, which bind to neurokinin receptors on the post-synaptic cell. C-fibres and RAR fibres are thought to converge onto the same second-order neurone in the nTS as co-activation of RAR fibres and C-fibres has a synergistic effect on cough responses in animal models.\textsuperscript{20} Neurokinins temporarily sensitize the post-synaptic cell by increasing depolarization and allowing NMDA receptors to open.\textsuperscript{21} This central up-regulation could become more permanent following prolonged sensory stimulation (central sensitization). Centrally acting neurokinin antagonists can produce antitussive activity in patients particularly if they are centrally sensitized.

**Central pathways and the 'urge to cough'**

The difficulty with animal cough data is that it lacks an essential qualitative component of cough in human disease. Most patients with chronic cough have a sensation or irritation in the throat and/or upper chest that induces an urge to cough, which is to some degree under conscious control, (unless the sensation is overwhelming) and then results in the motor action of coughing.\textsuperscript{22} This urge to cough may be C-fibre mediated. Intravenous injection of lobeline evokes sensations of burning, tickling, irritation and suffocation in the throat, larynx and upper chest, followed by coughing bouts, in healthy subjects, asthmatics and patients with chronic bronchitis, probably via bronchial C-fibre stimulation.\textsuperscript{23} During a capsaicin cough challenge, an increasing urge to cough correlates with increased cough frequency and cough intensity.\textsuperscript{24} An urge to cough can normally be suppressed, which would be advantageous when coughing would cause social disruption, e.g. in an important meeting. We have shown that while normal subjects can suppress the cough threshold by three doubling doses, patients with chronic cough could only suppress the cough threshold by one doubling dose.\textsuperscript{25} This suggests that disordered conscious inhibition of coughing may contribute to chronic cough.
Recently there have been attempts to map central pathways in man with functional magnetic resonance imaging. Mazzone et al.\textsuperscript{26} performed a study in healthy subjects to measure cortical activation during a capsaicin cough challenge. Unfortunately, inhalation of capsaicin causes significant side effects with burning mouth and watering eyes. This may have been responsible for the widespread activation of the cortex (including the primary somatosensory cortex, inferior parietal lobe, primary motor cortex, orbito-frontal cortex, inferior frontal gyrus, anterior cingulate cortex, anterior insula and cerebellar regions). More recent data with titration of citric acid at low doses suggest a much narrower field of activation, with more clearly defined sensory and motor centre involvement.\textsuperscript{27}

**Peripheral sensitization and cough**

Peripheral sensitization is the up-regulation of peripheral nerve sensitivity by local factors, for example the extreme sensitivity to touch with burn injuries. In chronic cough, conditions for peripheral sensitization are present, with elevated levels of inflammatory mediators (histamine, prostaglandin D2 and prostaglandin E2) in the airways of all patients with chronic cough compared to healthy controls.\textsuperscript{28,29} Inflammatory mediators sensitize peripheral vagal nerve terminals by binding to G-protein coupled receptors and activating a variety of intracellular mechanisms, including phosphorylation of ion channels, which subsequently lower the threshold for initiation of an action potential.\textsuperscript{30} This phenomenon has been confirmed in humans by a study in which prior inhalation of prostaglandin E2 increased the cough response to subsequently inhaled capsaicin.\textsuperscript{31}

**Central sensitization and cough**

Central sensitization is an enhanced central nervous system (CNS) response to peripheral nerve stimulation initially described in the study of pain. Chronic cough patients describe excessive coughing in response to minor exposures to inhaled irritants such as perfumes or cold air,\textsuperscript{26} which are the clinical features of a lowered threshold in response to cough-provoking stimuli; this could be due to central or peripheral sensitization. However, patients also describe coughing in response to normally innocuous stimuli such as talking on the telephone, laughing or singing.\textsuperscript{32} These stimuli are usually insufficient to evoke coughing and must indicate a change in the way the CNS responds to this sensory information. This is analogous to the pain perceived in response to an
innocuous stimulus surrounding an injury site (secondary allodynia) typical of central sensitization.\textsuperscript{33}

The visceral nervous system displays extensive central convergence of sensory nerves in the brain stem.\textsuperscript{34} Visceral hypersensitivity as a result of central sensitization could explain why coughing is provoked from extra-pulmonary sites such as the oesophagus (see below).

The role of GORD as a trigger in chronic cough

Gastro-oesophageal reflux disease

What is the relationship between GORD and chronic cough? Between a third and a half of chronic cough patients are reported in large series as having GORD.\textsuperscript{35–37} But the definition of GORD in these series is unclear. Is GORD proximal or distal oesophageal reflux? Acid or non-acid? With or without oesophagitis? Occurring with or without reflux symptoms? Responding to reflux therapy? And what is the mechanism?

Most patients (~75\%) do not complain of classical reflux symptoms.\textsuperscript{38} With oesophageal impedance/pH studies, the majority of chronic cough patients have reflux events that fall within the normal range.\textsuperscript{39} This relationship is confounded by the fact that sometimes cough causes reflux and so reflux rates may be higher in patients with chronic cough (by reverse causation), even if reflux did not cause the cough.

The evidence does not support a major role for micro-aspiration. First, there is very little proximal reflux in chronic cough patients.\textsuperscript{40} Secondly, pepsin levels in broncho-alveolar lavage fluid (as a marker of gastric aspiration) were not elevated in chronic cough patients with or without GORD, compared to healthy controls.\textsuperscript{41}

A temporal relationship between individual cough and distal reflux episodes would provide some evidence for a causal relationship. Studies that count cough in conjunction with an oesophageal catheter are complicated by the fact that having the catheter in place reduces cough rates on average by about a third,\textsuperscript{42} presumably through a local effect. However, in spite of this proviso, it is clear that the majority of patients with chronic cough have significant temporal relationships between cough and distal oesophageal reflux (i.e. occurring within a specified time window).\textsuperscript{35,43,44} In our experience, just under half of chronic cough patients have significant association, where reflux precedes coughing, accounting for approximately 40\% of coughing bouts. Just over half of patients have the reverse association, i.e. cough preceding reflux, with a third of patients exhibiting both processes.\textsuperscript{39}
Since reflux rates are not generally increased, this close temporal relationship implies that even physiological levels of reflux can stimulate cough in patients. Vagal afferent nerves innervating both the respiratory tract and the oesophagus may converge on the same relay neurones in the brainstem, meaning that altered sensory input from the oesophagus could up-regulate the response of the CNS to airway irritants. This concept is supported by studies showing that distal oesophageal acid infusion increases cough reflex sensitivity in asthmatics and cough frequency/reflex sensitivity in patients with chronic cough. In this context, physiological levels of distal reflux could act as a trigger to coughing.

Treatment of GORD and cough

Occasional patients improve their cough on anti-reflux treatment. However, double-blind placebo-controlled trials of proton pump inhibitor’s are negative. There have not been any controlled trials of augmented anti-acid therapy, alginates or motility agents. Anecdotal data also suggest that occasional patients respond well with laparoscopic fundoplication, but many patients do not, and this is a potentially hazardous procedure, with long-term complications. The scientific data would suggest that we may have to abolish even physiological levels of reflux if we are to have a major impact on cough. At present, we have no predictors of clinical response to medical or surgical intervention; surgery should not be contemplated until we do.

Post-nasal drip and asthma: association with chronic cough

The relationship between nasal diseases and chronic cough has suffered from similar issues to those for GORD, with the addition of a controversy about the most appropriate terminology in spite of a poor understanding of the underlying mechanisms. Originally named the ‘post nasal drip syndrome’, it was thought that nasal secretions caused coughing by mechanical stimulation of cough receptors either in the larynx or in the trachea if aspirated, but little evidence is available to support such suggestions. Nasal secretions are normally carried backwards into the pharynx by ciliary action, and many patients with increased nasal secretions do not cough. However, the influence of nasal stimuli on the cough reflex has been confirmed in both animal and human studies. Intra-nasal administration of capsaicin does not cause coughing, but the cough responses to both mechanical (Aδ-fibre) and chemical (C-fibre) airway stimuli in guinea pigs are enhanced.
This sensitization can be reproduced in animal models of allergic rhinitis, and by a nasal capsaicin challenge in human healthy volunteers and nasal histamine in subjects with allergic rhinitis. As distinct neural pathways mediate mechanical- and chemical-induced coughing, it has been suggested that nasal afferent stimulation can modulate the cough reflex via central mechanisms in a similar manner to that already described for oesophageal reflux events. Thus central sensitization in patients with chronic cough could link nasal stimuli and rhino-sinusitis to coughing. Recent data have supported this notion by demonstrating that intra-nasal capsaicin activates neurones not only in the trigeminal nucleus but also those in the nTS, providing evidence for brainstem convergence of trigeminal and vagal afferents.

Cough is an important symptom in asthma, but the mechanisms behind cough in asthma are complex and relatively unexplored. For example, many asthma patients also have coexistent triggers such as nasal disease and/or oesophageal reflux. Airway inflammation could cause peripheral sensitization of airway nerves. Subjective symptom scores and cough reflex sensitivity are poor surrogates for objective cough frequency in asthma. While objective ambulatory cough counts do not correlate with bronchial hyper-responsiveness, pulmonary function or exhaled NO levels, they are related to asthma control. Sputum eosinophil counts and cough frequency independently predict asthma control, implying that coughing is not just a reflection of airway inflammation. Some patients presenting with chronic cough also have features of asthma including bronchial hyper-reactivity, and reversible airflow obstruction termed ‘cough-variant asthma’. In these patients, cough seems to be disproportionate to other measures of asthma control and apart from sputum eosinophilia, airway inflammation is similar to other patients with chronic cough. This suggests that in some patients a different process drives coughing and central sensitization could be important. We need to develop improved phenotyping of asthma patients if treatments for cough are to be targeted and successful.

Two types of cough?

In humans, it seems likely that there are two important mechanisms which can initiate a cough. Aspiration is detected by ‘cough receptors’ which initiate an airway protective motor reflex driving an immediate protective cough, which is not preceded by any period of an urge to cough.

Patients with chronic cough complain of a persistent sense of irritation, usually located in their throat, associated with an urge to cough, suggesting a sensory disturbance rather than an abnormal
reflex. The triggers of these sensations may be trivial (changes in temperature) or even physiological (eating). Coughing in response to such sensations is analogous to scratching an itch. It is largely a voluntary motor response to an unpleasant sensation. This sensory-driven cough is generally not an immediate motor response and is under a degree of voluntary control, depending upon the intensity of the sensations, but the urge to cough can become so intense that it is difficult to resist, just as it is difficult to resist pulling your hand away from a painful stimulus.

How sensory-driven coughing and the protective cough reflex are integrated in the CNS is not clear. It would seem likely that rather than discrete processes, these mechanisms form part of a spectrum from coughing which is entirely voluntary (under complete conscious control and independent of any sensory input) through to sensory-driven coughing (as the intensity of sensory experiences increases, the ability to consciously modulate cough responses will decrease) and finally to very intense sensations where the urge to cough may be impossible to consciously control (indistinguishable from a reflex cough). It is possible that such intense stimuli may exceed a threshold to activate a protective cough reflex. In pathological states the gain may be increased on such a system by sensitization of somatic and visceral afferent pathways. A defect of inhibitory control could also play an important role (see Fig. 1).

**Implications for the development of new treatments**

Unfortunately there are currently no effective cough suppressants. The efficacy of over-the-counter cough medications remains unproven.
Cough syrups for children containing dextromethorphan have been withdrawn in the United States by the FDA because of lack of data on efficacy and the presence of significant side effects. Low-dose morphine over 4 weeks did improve the quality of life for patients with treatment-resistant chronic cough compared with placebo, but the sample size was small, and there were no objective cough counts.64

Successful drug development depends on a greater understanding of the mechanisms responsible for chronic cough in order to identify promising therapeutic targets.

There is no evidence that suppression of the excessive urge to cough is harmful. Anti-tussive drugs could be targeted either against the sense of throat irritation, or the urge to cough, but designed to leave the 'protective' cough unmodified. Potential candidates might range from TRPV1 or TRPA1 antagonists to modify C-fibre function and reduce peripheral sensitization, to gamma-aminobutyric acid agonists or NMDA receptor antagonists which impact on central sensitization. The pharmaceutical industry has finally realized the unmet need and is putting major resources into novel therapeutics.

Conflicts of interest: Dr Smith and Prof Woodcock are co-inventors on a patent application from the University Hospital of South Manchester NHS Trust and Vitalograph for an ambulatory cough monitor. Neither have received any personal financial benefit from this. Dr Smith and Prof Woodcock have both acted as consultants to GSK, Almirall, Schering Plough and Merck on the development of novel tussive agents.

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References

New insights in cough


New insights in cough


1.3 Anti-tussive Effects of Memantine in Guinea-pigs
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ANTI-TUSSIVE EFFECTS OF MEMANTINE IN GUINEA PIGS

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Running head: Memantine for Cough

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Conflict of Interest: Drs. Smith and Canning are named as co-inventors on a provisional method of use patent filed by Johns Hopkins University entitled, “Memantine for the treatment of cough”. Dr. Young and Ms. Saulsberry have no conflicts of interest.
ABSTRACT

Background: The treatment of cough is a significant clinical unmet need, as there is little
evidence that current therapies are effective. Based on evidence supporting a role for N-methyl
D-aspartate receptors (NMDAR) in cough, we hypothesised that memantine, a low affinity,
uncompetitive NMDAR channel blocker in routine use for the treatment of Alzheimer’s disease,
could be an effective, well-tolerated anti-tussive therapy. The aim of this study was to establish
pre-clinical evidence that memantine has anti-tussive effects.

Methods: We studied the influence of memantine on experimentally induced coughing in
response to citric acid and bradykinin inhalation in guinea pigs. We also compared the potency
and efficacy of memantine as an anti-tussive to other NMDAR antagonists, dextromethorphan
and ketamine, and to the GABA<sub>δ</sub> receptor agonist baclofen.

Results: Compared with control, 10 mg/kg memantine significantly reduced the cumulative
number of coughs evoked by both citric acid [median 24.0 coughs (IQR 13.0-25.5) versus 1.5
(IQR 0.3-10.3), p=0.012] and bradykinin aerosols, [median 16.0 coughs (IQR 9.5-18.5) versus
0.0 (IQR 0-0.75), p=0.002]. Memantine 10mg/kg produced a similar reduction in the cumulative
number of coughs to baclofen 3mg/kg, and demonstrated comparatively greater cough
suppression than 30mg/kg dextromethorphan or 30 mg/kg ketamine. This dose of memantine
produced no sedative or respiratory depressive effects.

Conclusions: This study illustrates that memantine has marked anti-tussive effects in guinea pigs,
most likely mediated via NMDAR channel blockade. Memantine therefore has the potential to be
a safe, effective and well-tolerated anti-tussive agent.
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INTRODUCTION

Effective treatments for cough are a significant clinical unmet need. There is little evidence that current therapies are effective and many are associated with significant side effects. The N-methyl D-aspartate receptor (NMDAR) blocker, dextromethorphan has been used as an antitussive agent for decades and is frequently a component of over-the-counter cough remedies. Dextromethorphan is a low affinity, uncompetitive NMDAR channel blocker, but also a sigma-1 receptor agonist\textsuperscript{5,9}\textsuperscript{,} and voltage-gated calcium channel blocker\textsuperscript{6,4} and has anti-tussive effects that translate from animal models to human studies\textsuperscript{5,9}. However, in the only study to objectively quantify the effect of dextromethorphan compared with placebo in subjects with acute cough, the impact on cough frequency was modest, with a reduction in cough frequency of just 12%\textsuperscript{5}. Recent concerns about the safety of dextromethorphan and other over-the-counter cough medications, especially in children, has led to restrictions in their use\textsuperscript{10,11}.

NMDARs are involved in many diverse roles in the central nervous system including synaptic transmission, synaptic plasticity, neuronal protection and survival. NMDARs are glutamate gated ion channels, consisting of four subunits, typically two NR1 subunits and two NR2 subunits, surrounding a central channel pore. The NR1 subunits are obligatory for functionality and can combine with four different NR2 (A-D) and two different NR3 (A and B) subunits. Subunit expression varies during development and with location. In the inactive state, the channel pore is blocked by Mg\textsuperscript{2+}. Partial membrane depolarization is sufficient to relieve this blockade, allowing the influx of Na\textsuperscript{+} and also Ca\textsuperscript{2+}. NMDARs possess multiple extracellular binding sites, allowing a variety of molecules to modulate their function.
Like dextromethorphan, memantine (used clinically to treat moderate to severe Alzheimer’s disease) is a low affinity, uncompetitive NMDAR blocker, binding preferentially to open NMDA receptor channels. Memantine therefore only blocks activated receptors, providing higher levels of blockade in the presence of high concentrations of glutamate and relatively lower levels of blockade during normal physiological transmission. This mode of action may explain why memantine treatment is well tolerated by patients; a recent review suggested adverse effects were reported in <10% of those treated for dementia. In addition to blocking NMDAR channels, memantine may also block 5-HT₃ and nicotinic acetylcholine receptor channels at similar concentrations.

Based on the available evidence supporting a role for NMDARs in cough, we hypothesised that memantine may be a well-tolerated anti-tussive therapy. The aims of this study were to establish pre-clinical evidence that memantine has anti-tussive effects on experimentally induced coughing in guinea pigs. We also compared the potency and efficacy of memantine as an antitussive to that of the NMDA receptor blockers dextromethorphan and ketamine, as well as the GABA₉ receptor agonist baclofen.

METHODS

Animals

Male Hartley guinea pigs (200-700g) were studied and all experiments were first approved by the institutional Animal Care and Use Committee.
Citric Acid Induced Cough

Animals were placed in a transparent chamber (Busco Research Systems, Wilmington, NC) with a continuous flow of air and exposed to increasing concentrations of citric acid (0.01M-0.3M) delivered by an ultrasonic nebuliser generating aerosol particles of 3-6μm diameter. Coughs were counted during a 5 minute nebulisation period and over the subsequent 5 minutes with the assistance of sound and pressure monitoring from the chamber. Respiratory rate and tidal volume were monitored throughout via a calibrated pressure transducer (Emka Technologies, Falls Church VA).

Bradykinin Induced Cough

Using a similar chamber and nebuliser system, animals were treated firstly with aerosolised peptidase inhibitors (captopril 0.1μM and thiorphan 0.1μM, 5 minutes nebulisation) to reduce bradykinin degradation and enhance tussive responses evoked by bradykinin (unpublished observations). Animals were then exposed to increasing concentrations of aerosolised bradykinin (0.1-3μg/ml), again for 5 minute periods. Coughs were counted during this and the subsequent 5 minutes. Pressure changes within the chamber were used to monitor respiratory rate (Biopac Systems Inc, Goleta, CA).

Responses to Intravenous 2-Methyl 5-Hydroxytryptamine and Mecamylamine

Animals were anaesthetised (1.5g/kg, i.p. urethane) and cannulae placed in the carotid artery and jugular vein to monitor arterial pressure and to administer drugs, respectively. A cannula was also placed in the extra-thoracic trachea, through which animals spontaneously breathed warmed, moistened air whilst ventilatory pressures were monitored via a side-port. Arterial and airway pressures were recorded digitally (Biopac Systems). Animals were treated with either i.p. memantine (10mg/kg) or vehicle (0.9% saline), given 30 minutes before the administration of 2-
methyl 5-hydroxytryptamine (2M5HT) and mecamylamine. Bolus doses of 2M5HT (10 and
100μg/kg) followed by mecamylamine (1 and 3mg/kg) were given at 5 minute intervals
intravenously.

NMDAR Subunit Expression

Animals were euthanized with phenobarbital (150mg/kg) and tissue harvested from the nodose
and jugular ganglia, cerebellum, lung parenchyma and by punch biopsy taken from the nucleus
Tractus Solitarius (nTS). Tissues were then frozen in RINaLater (Qiagen Inc, Valencia, CA) prior
to RNA isolation using RNasy Plus Mini Kit (Qiagen Inc) according to the manufacturer’s
instructions. Synthesis of cDNA was performed using Ominscript Reverse Transcriptase
(Qiagen Inc). The resulting cDNA product was then used to carry out PCR reactions. After an
initial activation at 95°C for 15 minutes, cDNAs were amplified with custom-synthesised primers
(Invitrogen Corporation, Carlsbad, CA) (Table 1 online supplement) by 50cycles, denaturation at
94°C for 30s, annealing at 60°C for 30s, extension at 72°C for 1min followed by a final extension
at 70°C for 10min. Products were then visualized in ethidium bromide-stained 1.5% agarose gels.

Compounds and Materials

Memantine, citric acid, bradykinin, mecamylamine, captopril, thiorphan, ondansetron and
baclofen were supplied by Sigma-Aldrich (St. Louis, MO); dexamethormphan by MP
Biomedical (Solon, OH), 2-Methyl 5-Hydroxytryptamine by Tocris Bioscience (Ellisville, MO)
and ketamine by Vedco Inc (St Joseph, MO). All drugs were dissolved in 0.9% saline except
citric acid (dissolved in distilled water) and thiorphan (dissolved in ethanol and then diluted in
0.9% saline). Memantine, ketamine, baclofen, ondansetron and placebo were administered by
intra-peritoneal injection, 30 minutes prior to cough challenges; the experimenters were not
blinded to the agent identity. Dosing, and administration were based on studies in rats where
memantine half-life is 2.5hrs at 10mg/kg\textsuperscript{17} and receptor occupancy approximately one third at
doses of 12.5mg/kg/day\textsuperscript{18}. Any sedation caused by these agents was assessed subjectively, and
classified as severe if animals were unable to stand, moderate if unstable when walking and mild
if just showing reduced spontaneous movement.

Statistical Analysis

Data were analysed using SPSS (version 15, SPSS Inc, Chicago, Ill) and graphs produced using
Prism (version 4, Graphpad Ltd). A 5% level of significance was used throughout. Cumulative
numbers of coughs to citric acid/bradykinin were expressed as median and inter-quartile ranges
as the data was positively skewed. Comparisons of cumulative cough numbers for treatment
groups were therefore compared using non-parametric tests (Kruskal Wallis and Mann-Whitney
U tests).

RESULTS

Citric Acid Induced Cough

Compared with vehicle, 10 mg/kg memantine markedly reduced the number of cumulative
coughs evoked by 0.01-0.3M citric acid (p<0.012) (Figure 1A and Table 1). At 1 and 3mg/kg,
there was no significant effect of memantine over vehicle. No side effects associated with the
10mg/kg memantine treatment were apparent. As expected, tripling the dose of memantine
administered to 30mg/kg similarly reduced cough responses but produced signs of neurologic
effects and slight sedation.

In contrast to 10 or 30 mg/kg memantine, dextromethorphan (30mg/kg; \(n=8\)) and ketamine
(30mg/kg; \(n=8\)) failed to produce a statistically significant reduction in cumulative coughs
evoked by 0.01-0.3M citric acid (\(p=0.328\) and \(p=0.645\), respectively) (Figure 1B and Table 1).
Dextromethorphan but not ketamine significantly reduced the cumulative number of coughs evoked by lower concentrations of citric acid (0.01-0.1M) (p=0.038). Mild to moderate sedation was observed in 62.5% of the dextromethorphan-treated animals and 87.5% of the ketamine-treated animals. When administered at 30mg/kg, the sedation produced by dextromethorphan and memantine was long-lasting (>2 hours) and persisted throughout the citric acid challenge while ketamine-induced sedation rapidly reversed during the cough challenge (40-50 minutes post-injection).

The 50mg/kg doses of ketamine and dextromethorphan caused severe sedation, precluding assessment of cough responsiveness even though breathing frequency declined only slightly and insignificantly [controls mean breathing frequency 117.7±26.3 breaths/min versus ketamine 92.3±18.8 breaths/min (p=0.25) and dextromethorphan 90.0±13.7 breaths/min (p=0.18)] with no significant change in tidal volume (p=0.293) or expiratory time (p=0.14).

Baclofen (3mg/kg) significantly reduced the cumulative number of coughs evoked by 0.01-0.1M and 0.01-0.3M concentrations of citric acid (p=0.035 and p=0.034 vs. controls, respectively) whereas baclofen 0.3mg/kg had no effect (Figure 2). Animals treated with 10mg/kg baclofen (n=3) were overtly sedated with difficulty standing, making cough challenge impossible. Respiratory rates were markedly reduced by 10mg/kg baclofen [controls mean breathing frequency 120.5±18.1 breaths/min versus baclofen 54.7±20.6 breaths/min, p=0.014], while expiratory time and tidal volume were unaffected (p=0.53 and 0.23, respectively). Subjective signs of sedation were also apparent with 3mg/kg baclofen treatments. Memantine 10mg/kg and baclofen 3mg/kg produced a similar reduction in the number of cumulative coughs (p=0.35).
Relative to vehicle treated animals, ondansetron (10mg/kg ip, n=4) had no significant impact on citric acid (0.01-0.3M) evoked coughing (p=0.39; see Figure E1). This dose of ondansetron abolished 5-HT evoked cardiopulmonary responses (unpublished observations).

**Bradykinin Induced Cough**

Compared to vehicle, memantine (10mg/kg) substantially reduced the cumulative number of coughs evoked by 0.1-3mg/mL bradykinin, [memantine median 0.0 coughs (IQR0-0.8) versus controls 16 coughs (IQR9.5-18.5), p=0.002; see figure 4]. A single vehicle treated animal coughed 14 times during pre-treatment with the peptidase inhibitors, but then had a similar response to other controls during bradykinin challenge, coughing a further 15 times during inhalation of 3mg/ml bradykinin.

**Respiratory Parameters**

With the exception of baclofen 10mg/kg, none of the treatments studied altered breathing frequency or expiratory times (see online supplement Figure 2E) during citric acid challenge. Tidal volume was significantly increased with increasing citric acid concentration in all treatment groups. Bradykinin induced a small reduction in breathing frequency in control animals but a small increase in breathing frequency following 10 mg/kg memantine treatment (p=0.012).

**Responses to Intravenous 2-Methyl 5-Hydroxytryptamine and Mecamylamine**

Control animals showed no significant changes in mean arterial pressure (MAP), respiratory rate (RR) or heart rate (HR) to boluses of 2M5HT at 10μg/kg whereas 100μg/kg induced a fall in MAP, a rise in RR but still no change in HR. In memantine treated animals (10mg/kg) responses were similar except that the rise in RR with 2M5HT 100μg/kg was blunted and slower in onset (see online supplement Figure 3E and Figure 4E). There were no significant differences.
whatsoever between control and memantine treated animals in their responses to mecamylamine
at doses of 1mg/kg or 3mg/kg (see online supplement Figure 5E).

**NMDA Subunit Expression**

NR1 subunit gene expression was detected in all tissues evaluated (data not shown). PCR of
neural tissue suggested some differences in expression of NR2 receptor subunit genes; see Figure
4 (also Table 1E online supplement). Nodose ganglia and nTS rarely expressed the NR2A gene,
comparably to 50% of jugular ganglia and most cerebellar samples. The NR2B subunit was,
however, present in most ganglia tissue and in 50% of nTS samples whilst NR2C and 2D
expression was detected in the majority of these tissues. Lung tissue did not express NR2A but
other subunits were present.

**DISCUSSION**

This study provides the first evidence that memantine has anti-tussive activity with significant
inhibition of both citric acid and bradykinin induced cough in guinea pigs. The degree of
inhibition was similar to that seen with baclofen but without the associated sedation, and was
comparably more effective than high doses of other NMDAR antagonists. As there was little
evidence of significant activity at nicotinic acetylcholine or serotonin receptors, it is most likely
that the inhibition of cough by memantine was NMDAR dependent. Receptor subunit expression
suggested the presence of NMDARs in both the central and peripheral tissues involved in the
cough reflex, with some degree of differential expression of NR2 subunits that might permit
targeting specific pathways in cough with NMDAR-selective blockade.

Coughing is known to be initiated by activation of vagal afferent fibres in the larynx and large
airways, via acid and mechanically sensitive Aδ fibres, and by activation of capsaicin-sensitive C
fibres that are also responsive to acid (via the TRPV1 channels) and bradykinin. These fibres
terminate in the nucleus Tractus Solitarius (nTS), where microinjection of NMDAR antagonists
have been shown to significantly inhibit coughing in response to citric acid\textsuperscript{20,21}. The effect of
memantine on both bradykinin and citric acid evoked cough is consistent with inhibition of the
C-fibre pathway but an additional effect on A\textsubscript{6}-fibres cannot be excluded.

NMDAR subunit gene expression was detected in the nTS, the primary site of vagal afferent
termination in the brainstem, but also in vagal ganglia. The latter supports the notion that
NMDARs may be present in the airway nerve terminals and/or may modulate neurotransmitter
release presynaptically in the CNS. Consistent with this hypothesis, we have observed NMDAR
dependent Ca\textsuperscript{2+} influx in dissociated nodose and jugular ganglia neurons projecting to the
airways (unpublished observations). The differential expression of NR2A subunits in the jugular
and nodose ganglia raises the possibility of targeting specific populations of afferent nerves
therapeutically, if these receptors can be shown to be functionally important in modulating
cough. This assertion awaits a more systematic evaluation of NMDAR subunit expression in
identified neurons.

Although NMDARs are reported to be mechanically sensitive\textsuperscript{22} and our PCR studies are
consistent with a peripheral expression of NMDARs by vagal afferents, a central site of action
for memantine seems most likely, especially as the anti-tussive effects of dextromethorphan are
absent when delivered to the airway\textsuperscript{7,23}. Nevertheless, memantine treatment did not obviously
cause sedation or suppress respiration at doses that almost completely inhibited coughing. This
may be a consequence of the use-dependant action of memantine and NMDAR specificity at the
doses used in this study. The selective effects of memantine on cough might also be explained
by its greater affinity for NMDAR subtypes\textsuperscript{24} or effects at extra-synaptic NMDARs\textsuperscript{25}. In
contrast, high doses of dextromethorphan and ketamine did not suppress coughing as effectively
as memantine, despite clear evidence of sedation. Perhaps the relative lack of side effects seen with memantine treatment in this study is attributable to its inability to interact with the additional targets for dextromethorphan and ketamine (e.g. Sigma-1 receptors, HCN1 channels).2,26,27

Baclofen was chosen as an additional positive control as it is known to suppress coughing in animals28, reduces cough reflex sensitivity in humans29 and has been used clinically to treat cough although its utility is hampered by its sedative effects30. Indeed, animals treated with high dose baclofen were overtly sedated, with depressed breathing frequency. Moderate doses of baclofen were better tolerated and produced anti-tussive effects similar to those of memantine without respiratory depression.

Other properties of NMDARs provide circumstantial evidence for a role in modulating the cough reflex. Females have a more sensitive cough reflex than males when challenged with inhaled irritants31 and have higher frequency of coughing when suffering from chronic cough32. Oestrogens have significant influence on NMDARs. In the hippocampus, elevated oestradiol levels are associated with increased synaptic receptor density, increased transmission and an enhanced long term potentiation33,34. Furthermore, cross-organ sensitisation between the uterus and urethra is mediated by phosphorylation of NR2B subunits and modulated significantly by the oestrous cycle35,36. Such NMDAR dependent interactions between organs may be analogous to the coughing associated with extra-pulmonary disorders such as gastro-oesophageal reflux disease37.

In conclusion, this study provides the first evidence that memantine has anti-tussive activity, most likely NMDAR dependent, a mechanism previously shown to translate from animal models to clinical effects on cough in humans. Memantine therefore has the potential to be a safe,
effective and well-tolerated treatment for acute cough but also perhaps for patients with chronic
intractable coughing where there are few effective treatment options.

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interpretation of data, drafting and revisions of the manuscript and approved the final version of
the manuscript.

Dr Young contributed to the study design, acquisition, analysis and interpretation of data,
revisions of the manuscript and approved the final version of the manuscript.

Ms Saulsberry contributed to the study design, acquisition, analysis and interpretation of data,
revisions of the manuscript and approved the final version of the manuscript.

Dr Canning contributed to the original concept of the study, study design, acquisition, analysis
and interpretation of data, revisions of the manuscript, approved the final version and is the
guarantor of the manuscript.

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provisional method of use patent filed by Johns Hopkins University entitled, “Memantine for the
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measurements and Mathew Hewitt for guidance in the cough studies.

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Training Fellowship and NIH [Grant HL083192].
### TABLES

**Table 1**: Cumulative numbers of cough with increasing concentrations of citric acid for treatment groups and contemporaneous controls, *indicates significant difference in cough from controls, data are median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Citric Acid Concentration</th>
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<tbody>
<tr>
<td></td>
<td>0.01M</td>
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<tr>
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<td>Baclofen (n=8)</td>
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<td>3mg/kg</td>
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<td>Controls (n=12)</td>
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<td>Ondansetron (n=4)</td>
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<tr>
<td>10mg/kg</td>
<td>(0.0-0.0)</td>
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FIGURES LEGENDS

Figure 1 (A) Cumulative numbers of coughs in response to citric acid aerosols in control animals compared with increasing doses of memantine (MEM). Compared to controls MEM 10mg/kg significantly reduced the cumulative number of coughs (p=0.012)*. (B) Compared to control animals, 30mg/kg ketamine (KET) and 30mg/kg dextromethorphan (DEX) did not significantly reduce the numbers of coughs evoked by 0.01-0.3M citric acid (p=0.65 and p=0.33, respectively) but memantine 30mg/kg did (p=0.013)*. Dextromethorphan and memantine but not ketamine significantly reduced the coughs evoked by lower concentrations of citric acid (0.01-0.1M) (p=0.038 and p=0.031).

Figure 2 Cumulative numbers of coughs in response to citric acid aerosols in control animals and with increasing doses of baclofen. Baclofen 3mg/kg significantly reduced the cumulative number of coughs evoked by 0.01-0.1M and 0.01-0.3M concentrations of citric acid (p=0.035 and p=0.034, respectively)* whereas 0.3mg/kg baclofen had no demonstrable effect.

Figure 3 Cumulative numbers of coughs in response to bradykinin aerosols in control and memantine treated animals. Memantine 10mg/kg significantly reduced coughing compared with controls (p=0.002)*.

Figure 4 Representative PCR gel showing the presence of NR2 subunit genes A-D in nodose and jugular ganglia and nucleus tractus solitaries (nTS) from a single animal. The table shows the proportion of tissues, taken from 3-8 animals, expressing the genes for the NR2 subunits A-D.
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252x217mm (300 x 300 DPI)
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<th>Nodose</th>
<th>Jugular</th>
<th>nTS</th>
<th>Trachea</th>
<th>Parenchyma</th>
<th>Cerebellum</th>
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ONLINE-ONLY MATERIAL

TABLES

Table 1E NMDA receptor subunit primers for PCR

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<th>NMDA Receptor Subtype</th>
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<td>TGAAAAGCCAGCA</td>
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Figure 1E Cumulative cough responses to citric acid aerosols in control animals compared with Ondansetron treated animals (OND).

- Controls (n=12)
- OND 10mg/kg (n=4)
Figure 2E Respiratory Parameters during citric acid cough challenges in controls and animals treated with memantine (MEM) and baclofen (BAC).
Figure 3E: Representative traces of arterial pressures and airway pressures from a control and a memantine treated animal challenged with intravenous 2-methyl 5-hydroxytryptamine (2M5HT) 100μg/kg.
**Figure 4E** Cardio respiratory responses to intravenous 2-methyl 5-hydroxytryptamine (2M5HT) in controls and memantine (MEM) treated animals.
Figure 5E: Cardio respiratory responses to intravenous 2-methyl 5-hydroxytryptamine (2M5HT) in controls and memantine (MEM) treated animals.
ANTI-TUSSIVE EFFECTS OF MEMANTINE IN GUINEA PIGS
Jaclyn A. Smith, Emma C. Young, Loren Saulsberry and Brendan J. Canning
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1.4 The Effect of Mindfulness Meditation on Cough Reflex Sensitivity
The effect of mindfulness meditation on cough reflex sensitivity

E C Young,1 C Brammer,2 E Owen,2 N Brown,2 J Lowe,2 C Johnson,2 R Calam,3 S Jones,3 A Woodcock,1 J A Smith1

ABSTRACT

Background: Chronic cough is common, and medical treatment can be ineffective. Mindfulness is a psychological intervention that aims to teach moment-to-moment non-judgemental awareness of thoughts, feeling, and sensations.

Method: 30 healthy subjects and 30 patients with chronic cough were studied in two sequential trials. For both studies, cough reflex sensitivity to citric acid (CS) was measured on two occasions, with urge to cough rated following each inhalation; between challenges subjects were randomised to (1) no intervention, (2) mindfulness or (3) no intervention but modified cough challenge (subjects suppress coughing). For the healthy volunteers, measures were 1 h apart and mindfulness was practiced for 15 min. For the patients with chronic cough measures were 1 week apart and mindfulness was practiced daily for 30 min.

Results: In healthy volunteers, median change (inter-quartile range) (IQR) in cough reflex sensitivity (logCS) for no intervention, mindfulness and suppression was -1.0 (0.0 to +1.3), -2.0 (+1.0 to +3.0) and +3.0 (+2.8 to +5.0) doubling concentrations (p = 0.003), there were significant reductions for both mindfulness (p = 0.003) and suppression (p = 0.002) over no intervention. In patients with cough, median change (IQR) in logCS for no intervention, mindfulness training and voluntary suppression was -0.0 (-1.0 to +1.0), +1.0 (-0.3 to +1.0) and -1.0 (+1.0 to +2.0) doubling concentrations (p = 0.046). There was a significant reduction for suppression (p = 0.02) but not mindfulness (p = 0.35). Uge to cough did not change after mindfulness compared with control in either healthy subjects (p = 0.33) or those with chronic cough (p = 0.47).

Conclusion: Compared with control, mindfulness decreased cough reflex sensitivity in healthy volunteers, but did not alter cough threshold in patients with chronic cough. Both groups were able to suppress cough responses to citric acid inhalation.

Chronic cough, defined as cough lasting >8 weeks, has a prevalence of around 12% in the general population and is associated with significant morbidity including anxiety and depression. Despite comprehensive investigation, it is increasingly recognised that a proportion of patients fail to respond to treatments targeted at identified potential causes. For these patients, both effective antitussive treatments and supportive interventions are lacking despite the acknowledgement that coughing is associated with significant physical, psychological, and social burdens. The effect of psychological interventions on cough reflex sensitivity and whether such interventions are useful in the management of patients with chronic cough have not been investigated.

An outpatient Mindfulness-Based Stress Reduction (MB-SR) programme developed by Kabat-Zinn, teaches moment-to-moment non-judgemental awareness of thoughts, feelings and sensations, and has proven effective in the management of several chronic disease states including chronic pain, depression, fibromyalgia, and psoriasis. Mindfulness training is classically led by experts and practiced in group sessions over an 8- to 10-week period with regular homework activities to encourage integration of the coping strategies into everyday life. A 10-week MB-SR group-based programme directed at patients with fibromyalgia induced subjective ratings of physical and psychological symptoms. Prolonged psychological benefits were demonstrated in a 4-year study of 225 patients with chronic pain who had participated in an intensive 8-week MB-SR programme although the initial reductions in the Pain Rating Index (PRI) returned to pre-intervention levels at 4 months. However, there is some evidence to suggest that mindfulness training can be effective at reducing disease severity. In moderate to severe psoriasis, customised mindfulness tape recordings, played during individual phototherapy treatment, accelerated rates of skin clearing compared with a “no-tape” control group.

Cough can be experimentally induced by chemical stimulation of pulmonary afferent nerve fibres. The concentration of inhaled citric acid or capsaicin eliciting five or more coughs in the first minute after inhalation, known as the C5, is a reproducible measure of cough reflex sensitivity and is known to be moderately correlated with objective cough frequency in respiratory conditions. Cough reflex sensitivity cannot be used to differentiate reliably between health and disease since there is considerable overlap of C5 in healthy subjects and patients with chronic cough, but on average subjects with chronic cough have a lower C5 (more sensitive reflex), which increases following successful treatment of the cough.

Anecdotally, patients with chronic cough often describe a sensation of irritation located in the throat which provokes spontaneous coughing. An urge to cough also occurs during the inhalation of capsaicin and citric acid, increasing in magnitude dose dependently and preceding the motor cough response. However, the cough reflex is under considerable voluntary control. Healthy volunteers are able to resist the urge to cough and voluntarily suppress coughing during capsaicin challenge.
The urge to cough is an unpleasant sensation that may be associated with negative cognitions, especially in circumstances where coughing would be maladaptive. Psychological interventions aimed at reducing the negative cognitions associated with experiencing the urge to cough may reduce the magnitude of the urge to cough sensation and therefore reduce cough reflex sensitivity. We hypothesised that a mindfulness intervention would reduce perceived urge to cough on inhaling noxious agents and therefore reduce motor cough response and cough reflex sensitivity. Two randomised controlled trials were performed assessing the effect of mindfulness on cough reflex sensitivity; first testing the immediate effect of mindfulness in healthy volunteers and secondly a short duration outpatient intervention programme in patients with chronic cough.

**METHODS**

**Subjects**

Thirty healthy volunteers and 30 patients presenting with chronic cough (>8 weeks duration) to a tertiary referral clinic (University Hospital of South Manchester) were studied. Subjects performed a baseline citric acid cough challenge and were excluded from further participation if they did not have a measurable C5—that is, did not cough five times after inhaling the maximum concentration of citric acid. Other exclusion criteria were a history of recent upper respiratory tract infection (<4 weeks) or current treatment with opiates, antihistamine-converting enzyme inhibitors or any over-the-counter cough medicines. Current smokers were also excluded. The chronic cough patients had received targeted 8-week treatment trials for underlying gastro-oesophageal reflux disease, asthma and/or upper airway cough syndrome (UACS). None of the patients had undergone any behavioural therapy for their cough. Ethics approval was granted by the local research ethics committee and written informed consent was obtained from all subjects.

**Study design**

Two sequential studies were performed. First, the immediate effects of a mindfulness intervention on cough reflex sensitivity were tested in healthy volunteers to assess the ability of a relaxation technique to modulate the cough reflex. Secondly, we examined the effect of mindfulness on cough reflex sensitivity in subjects with chronic cough. This study was of similar design, but mindfulness was taught and practised over a period of 1 week to investigate the longer term effects of mindfulness in a patient group and therefore its potential as a treatment.

**Study 1: immediate effect of mindfulness in healthy volunteers**

Cough reflex sensitivity was measured at baseline using a citric acid challenge, following which subjects were randomised to one of three possible groups: (1) no intervention (control), (2) mindfulness training or (3) voluntary suppression. Randomisation by minimisation was performed and subjects were stratified according to cough reflex sensitivity and gender. 10

**Control**

Repeat citric acid challenge was according to the standard protocol described below after 1 h.

**Mindfulness intervention**

A 15 min mindfulness training exercise was performed just prior to their second cough challenge at 1 h.

**Voluntary suppression**

No intervention was performed between baseline and repeat citric acid challenges at 1 h, but at the beginning of the repeat challenge...
subjects were instructed to try not to cough and reminded of this following each subsequent inhalation of citric acid. Patients were not provided with any particular strategies to achieve this.

**Study 2: effect of mindfulness course in subjects with chronic cough**

As for study 1, patients with chronic cough had cough reflex sensitivity testing at baseline, but also the Spielberger State–Trait Anxiety Inventory (STAI), and then were randomised/ stratified in the same manner to (1) no intervention, (2) mindfulness training or (3) voluntary suppression. However, repeat cough challenges for this study were performed after a longer interval, 7–10 days later, to allow a more prolonged mindfulness intervention. For this mindfulness intervention patients with cough took part in an educational interview delivered by the researcher during which meditative techniques were explained. The interview was recorded on an audio-cassette for home practice, at least 30 min per day until their return visit. Patients did not keep a record of their compliance with home practice.

**Procedures**

**Citric acid challenge**

Citric acid cough challenge was performed using the single-breath, doubling dose method, delivered by a dosimeter (Koko dosimeter, Ferraris Ltd, Herrford, UK) with inspiratory flow rate limitation. In brief, serial doubling concentrations of citric acid ranging from 0.08 to 4 M were administered 1 min apart with three interspersed placebo inhalations (normal saline) to which both researcher and subject were blinded. Subjects were instructed to cough freely and not try to suppress coughing (except in voluntary suppression challenge). The number of coughs, defined as explosive sounds, that occurred in the minute after each inhalation, was recorded and the test stopped when the subject coughed at least five times (CS). To ensure consistency the same nebuliser pot was used throughout the experiment for each individual, and re-calibrated at regular intervals. Spirometry was performed before and after each challenge and if forced expiratory volume in 1 s (FEV1) fell by >20% following citric acid inhalation subjects were excluded from further study.

**Urge to cough**

Following each inhalation of citric acid, healthy subjects were asked to rate their urge to cough intensity on a visual analogue scale (VAS) from 0 to 100 mm anchored by “no urge” to “severe urge”. Subjects with chronic cough rated their urge to cough on a modified Borg scale ranging from 0 (no need to cough) to 10 (maximum urge to cough) as described by Davenport.

**Mindfulness intervention**

The mindfulness intervention aimed to train subjects to experience sensations without evaluation or judgment. We predicted that mindfulness would enable subjects to experience the urge to cough without focusing either on the possibility of coughing or on any associated distress. The intervention was adapted for this study by psychologists and intended to be delivered by non-experts after brief training. For this study a breathing exercise was used to aid mindfulness. Subjects were given written instructions consisting of an explanation of the principles of mindfulness and then specific directions as to how to perform the breathing and mindfulness exercises (see online supplement). Subjects are asked to notice their breathing and the sensations associated with it but not consciously try to change their breath or relax. They were seated in a quiet room, and told to close their eyes and focus on their breathing: “being with each inhalation” for its full duration and “with each exhalation” for its full duration. Subjects were instructed to return their attention to their breathing each time they noticed their mind had wandered. This exercise was conducted with support from the researcher. Healthy volunteers spent 15 min

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**Figure 2** Changes in urge to cough at the citric acid cough threshold (CS) in healthy volunteers for the control, mindfulness intervention and voluntary cough suppression groups. Horizontal lines represent median values, and error bars represent the interquartile range. VAS, visual analogue scale.

**Figure 3** Changes in cough reflex sensitivity to citric acid in patients with chronic cough for the control, mindfulness intervention and voluntary cough suppression groups. Horizontal lines represent median values, and error bars represent the interquartile range.
Chronic cough

Figure 4. Changes in urge to cough at the citric acid cough threshold (C5) in patients with chronic cough for the control, mindfulness intervention and voluntary cough suppression groups. Horizontal lines represent median values, and error bars represent the interquartile range.

practising the mindfulness exercise prior to the cough challenge. Subjects with chronic cough were given the same instructions and asked to practise the exercise at home for at least 30 min per day until the next visit.

State/trait anxiety

The Spielberger STAI is a self-report scale for measuring state (present) and trait (general) anxiety levels that has been used extensively in research. State anxiety is sensitive to change following intervention. Patients with cough completed the scale at baseline and at repeat cough challenge.

Statistical analyses

Cough reflex sensitivity was log transformed (logC5) prior to analysis. The Kruskal–Wallis test was used to compare change in cough reflex sensitivity, urge to cough rating at C5 and anxiety scores across the three groups. Where the Kruskal–Wallis test suggested a significant difference, post hoc pairwise comparisons were made with the Mann–Whitney test.

RESULTS

Thirty healthy volunteers (24 female, 6 male) and 30 patients with cough (20 female, 10 male) meeting eligibility criteria took part in the studies. Mean age was 34 years (SD 10.5) in healthy subjects compared with 58 years (SD 9.46) in the patients with cough. None of the participants was a current smoker, but 11 (37%) of the patients with cough were ex-smokers >6 months, with a median smoking history of 0.0 (0–45) pack-years. Pre-challenge mean percentage predicted FEV1 was 104.98% (SD 13.45%) in healthy subjects compared with 97.34% (SD 20.45%) in the patients with cough. Age, gender and baseline C5 are compared between randomisation groups in Table 1. Significantly more females were randomised to mindful intervention in the healthy volunteer study, but groups were otherwise well matched.

Study 1: healthy subjects

Baseline median (IQR) logC5 for all healthy volunteers was –0.3M (–0.5 to 0.0). Ten subjects were randomised to each group and median change (IQR) in logC5 for the no intervention, mindfulness intervention and voluntary suppression groups was +1.0 (0.0 to +1.3), +2.0 (+1.0 to +3.0) and +3.0 (+2.0 to +3.0) doubling concentrations, respectively, p = 0.003. The mindfulness intervention significantly reduced cough reflex sensitivity compared with the no intervention group (p = 0.045). Voluntary suppression of coughing also significantly improved cough reflex sensitivity (p = 0.002) but was not significantly better than the mindfulness intervention (p = 0.053) (see fig 1).

Urge to cough data were available in 18 (66%) healthy subjects and showed a log-linear relationship with concentration of citric acid (online supplement fig E). Median (IQR) baseline urge to cough at C5 (rated on a VAS from 0 to 100 mm) was 92 (69 to 99) mm. Median change (IQR) in urge to cough at C5 for the no intervention, mindfulness and voluntary suppression groups was +8 (–4 to +52) mm, +1 (–2 to +5) mm and +13 (+5 to +55) mm, respectively, with a trend towards a difference between the groups (p = 0.069). There was no statistically significant difference in change in urge to cough at C5 between the no intervention and mindfulness groups (p = 0.51) or between the no intervention and voluntary suppression groups (p = 0.39), but there was the suggestion of a lower urge to cough for the mindfulness group compared with voluntary suppression (p = 0.015) (see fig 2).

Study 2: patients with cough

Baseline median (IQR) logC5 for all patients was –0.9M (–1.2 to –0.5), significantly lower than in the healthy volunteer study (p = 0.001). Eleven patients were randomised to no intervention, 10 to the mindfulness programme and 9 to voluntary cough suppression. Median change (IQR) in logC5 for no intervention, mindfulness and voluntary suppression groups was 0.6 (–1 to +1), +1.6 (–0.5 to +1) and +1.0 (+1.0 to +2.0) doubling concentrations, respectively (p = 0.046). There was no statistically significant difference in change in logC5 between the no intervention and mindfulness groups (p = 0.55). Comparison of the control and voluntary suppression groups revealed a significant difference (p = 0.02) with an increase in cough threshold (see fig 3).

Urge to cough data were collected for all patients. Median (IQR) baseline urge to cough at C5 as rated by a modified Borg scale was 4 (3–4). Median change (IQR) in urge to cough at C5 was not significantly different between the no intervention, mindfulness and voluntary suppression groups (0 (0 to +1), 0 (0 to +2) and 0 (0 to +1), respectively (p = 0.70); fig 4).

Compared with a working population sample mean, 12 both male and female patients with cough had high trait anxiety levels (mean (SD) in males, 56.60 (16.21) vs 34.89 (9.19); in females, 40.50 (11.00) vs 34.79 (9.22)) but below average state anxiety scores (mean (SD) in males, 50.90 (10.30) vs 35.72 (10.40); in females, 54.15 (8.85) vs 35.20 (10.61)). There were no significant changes in either state (p = 0.23) or trait (p = 0.76) anxiety for any of the patient groups.

DISCUSSION

In this study we have shown that healthy volunteers undergoing a mindfulness intervention had a prompt reduction in cough reflex sensitivity compared with a no intervention control group. Patients with chronic cough performing a brief
outpatient mindfulness programme, however, had no change in cough reflex sensitivity compared with controls. Mindfulness did not significantly alter the perceived urge to cough at the C5 cough threshold in either subject group and did not appear to reduce state/task anxiety levels in the patients with chronic cough compared with the controls. Voluntary suppression of coughing after citric acid inhalation was able to decrease cough reflex sensitivity significantly in both trials, but appeared to be more effective in the healthy volunteers than in the patients with chronic cough.

There are a number of possible explanations for the failure of mindfulness to alter cough reflex sensitivity in the chronic cough study compared with the healthy volunteer study. First, the healthy volunteer study was designed to assess any immediate effect of mindfulness training. In contrast, the chronic cough study assessed whether more prolonged mindfulness training might have a clinical role in this distressing condition. It is possible that any improvements in cough reflex sensitivity following a mindfulness exercise are only very short-lived; however, the optimal duration of mindfulness training is not known. A longer more intensive supervised course may be required to have a prolonged effect in patients compared with healthy volunteers, although this has never been investigated. Secondly, cough reflex sensitivity in patients with chronic cough may be more resistant to change. Voluntary suppression reduced cough reflex sensitivity in patients with chronic cough but appeared to be less effective than in healthy controls. This is in keeping with the experience of patients with chronic cough who complain of an inability to control coughing, leading to social embarrassment. During cough challenges, as the intensity of the urge to cough increases, the ability to suppress coughing decreases until coughing becomes an irresistible response. We speculate that in patients with chronic cough, coughing may not only be an increased sensitivity to irritants but also from poorer conscious control over coughing which may be mediated by impairment of descending cortical pathways. This could also explain why psychological interventions such as mindfulness may be less effective than in healthy volunteer studies. It may be important that this mindfulness training encouraged subjects to focus on their breathing. Attending to the sensations associated with inhalation and exhalation rather than the urge to cough may have motivated the individual to prioritise continued breathing over coughing. This could explain the effect of mindfulness in the healthy volunteer group. It would be interesting to explore whether mindfulness of another non-expectorated bodily sensation or external influence (eg, music) would have a similar inhibitory effect on coughing.

We found that compared with a no intervention control, there was no statistically significant change in the urge to cough experienced following mindfulness intervention or voluntary suppression in either study. However, as a secondary endpoint, the study may well have been inadequately powered to demonstrate such effects. We hypothesised that mindfulness would reduce cough reflex sensitivity by reducing the urge to cough and that voluntary suppression would also reduce cough reflex sensitivity but at the expense of a more intense urge to cough. In the healthy volunteer study, despite some missing data, there was a suggestion that the urge to cough at C5 was lower in the mindfulness group compared with voluntary suppression.

Some studies investigating the effect of a mindfulness intervention in chronic disease have lacked control groups, thereby not excluding a significant placebo effect from such treatment. We compared this mindfulness intervention with a no intervention control group and additionally included a modified cough challenge to act as a positive control, during which volunteers were instructed to “try not to cough”. In the healthy volunteer study, cough reflex sensitivity did improve by one doubling dose in the control group; however, it is interesting to note that in the chronic cough study there was no such improvement with no intervention (decreased C5 in 26 of 50 healthy volunteers vs 11 of 30 patients with chronic cough).

The main limitation of this study is that the duration and intensity of the mindfulness programme may have been inadequate and we cannot be certain that the patients with chronic cough complied with home practice. However, our aim was to assess whether a brief intervention delivered by non-experts could be effective. Other studies with positive outcomes with symptoms other than cough have used an intensive 8- to 10-week programme and taught formal mindful meditative techniques such as yoga, body scan and sitting meditation. Introduction of these formal techniques and modification of the programme by psychologists to be more in line with the specific concerns of a group of patients with chronic cough could improve future results. Also different proportions of females were randomised to the mindfulness intervention in the healthy volunteer study. Subjects were, however, reasonably well matched for baseline cough reflex sensitivity, which is likely to be more important.

In summary, the findings of this study suggest that a short-duration mindfulness outpatient programme cannot be recommended as a psychological intervention for chronic cough, although a potentially beneficial effect following a more prolonged intervention led by experts is not excluded. Since voluntary suppression reduced cough reflex sensitivity in both subject groups, a future randomised controlled study of voluntary cough suppression as a therapeutic strategy for chronic cough would be interesting, particularly to investigate whether prolonged practice improves the ability to suppress coughing successfully. Indeed speech and language therapy is the only supportive intervention shown to be effective in patients with chronic cough, a component of which is strategies to suppress or replace the cough.

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Competing interests: None.

Ethics approval: Ethics approval was obtained from Tamworth and Evesham Local Research Ethics Committee.

Provenance and peer review: Not commissioned; externally peer reviewed.

REFERENCES
Lung alert

Pharmacological manipulation of antituberculous therapies to improve treatment efficacy and compliance with ethionamide

The global multidrug-resistant tuberculosis epidemic has prompted efforts to develop new antituberculous compounds and strategies to improve efficacy and compliance of drugs already in use. Several antituberculous compounds require in vivo metabolic activation to become inhibitory to the mycobacterium. Ethionamide, a thiosemicarbazone-containing drug, is activated by the mycobacterial mono-oxygenase EthA. The production of EthA is inhibited by the transcriptional repressor EthR.

This study investigated the use of inhibitors of EthR to boost activity of EthA, thus increasing the efficacy of ethionamide. The ligands RDM91881 and RDA51545 were identified as compounds that inhibit EthR in vivo, resulting in increased activity of EthA. The minimum concentrations of ethionamide needed in the presence and absence of both ligands showed that RDM91881 and RDA51545 increased the antibacterial potency of ethionamide towards Mycobacterium tuberculosis by factors of 10 and 20 respectively. RDM91881 also improved the potency of thiacetazone, another antituberculous compound, by a factor of 4. In vivo studies in mice infected with M tuberculosis showed that RDA51545 with ethionamide only had a minor effect on bacterial load compared with ethionamide alone, while RDM91881 with ethionamide reduced bacterial load as efficiently as a three times higher dose of ethionamide alone.

This study supports the hypothesis that the sensitivity of M tuberculosis to a produg can be increased by interfering pharmacologically with the regulatory mechanism of drug activation. This could result in lower drug dose requirements, improving side effect profiles and increasing compliance—a potential step forward in the fight against multidrug-resistant tuberculosis.


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Provenance and peer review: Not commissioned; not externally peer reviewed.

### 2.1 Hospital Anxiety and Depression Scale (HADS)

This questionnaire will help you let us know how you are. Read each item and tick the response which comes closest to how you have felt in the last few days. Don't take too long over your replies, your immediate reaction will probably be more accurate than a long thought out response. Tick only one box in each section.

<table>
<thead>
<tr>
<th>I feel tense or ‘wound up’:</th>
<th>I feel as if I am slowed down:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most of the time</td>
<td>Nearly all the time</td>
</tr>
<tr>
<td>A lot of the time</td>
<td>Very often</td>
</tr>
<tr>
<td>Time to time, occasionally</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Not at all</td>
<td>Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I still enjoy the things I used to enjoy:</th>
<th>I get a sort of frightened feeling like ‘butterflies’ in the stomach:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitely as much</td>
<td>Not at all</td>
</tr>
<tr>
<td>Not quite so much</td>
<td>Occasionally</td>
</tr>
<tr>
<td>Only a little</td>
<td>Quite often</td>
</tr>
<tr>
<td>Hardly at all</td>
<td>Very often</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I get a sort of frightened feeling as if Something awful is about to happen:</th>
<th>I have lost interest in my appearance:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very definitely and quite badly</td>
<td>Definitely</td>
</tr>
<tr>
<td>Yes, but not too badly</td>
<td>I don’t take so much care as I should</td>
</tr>
<tr>
<td>A little, but it doesn’t worry me</td>
<td>I may not take quite as much care</td>
</tr>
<tr>
<td>Not at all</td>
<td>I take just as much care as ever</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I can laugh and see the funny side of things:</th>
<th>I feel restless as if I have to be on the move:</th>
</tr>
</thead>
<tbody>
<tr>
<td>As much as I always could</td>
<td>Very much indeed</td>
</tr>
<tr>
<td>Not quite so much now</td>
<td>Quite a lot</td>
</tr>
<tr>
<td>Definitely not so much now</td>
<td>Not very much</td>
</tr>
<tr>
<td>Not at all</td>
<td>Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Worrying thoughts go through my mind:</th>
<th>I look forward with enjoyment to things:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A great deal of the time</td>
<td>As much as I ever did</td>
</tr>
<tr>
<td>A lot of the time</td>
<td>Rather less than I used to</td>
</tr>
<tr>
<td>From time to time but not too often</td>
<td>Definitely less than I used to</td>
</tr>
<tr>
<td>Only occasionally</td>
<td>Hardly at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I feel cheerful:</th>
<th>I get sudden feelings of panic:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>Very often indeed</td>
</tr>
<tr>
<td>Not often</td>
<td>Quite often</td>
</tr>
<tr>
<td>Sometimes</td>
<td>Not very often</td>
</tr>
<tr>
<td>Most of the time</td>
<td>Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I can sit at ease and feel relaxed:</th>
<th>I can enjoy a good book or radio or TV programme:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitely</td>
<td>Often</td>
</tr>
<tr>
<td>Usually</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Not often</td>
<td>Not often</td>
</tr>
<tr>
<td>Not at all</td>
<td>Very seldom</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I can sit at ease and feel relaxed:</th>
<th>I can enjoy a good book or radio or TV programme:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitely</td>
<td>Often</td>
</tr>
<tr>
<td>Usually</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Not often</td>
<td>Not often</td>
</tr>
<tr>
<td>Not at all</td>
<td>Very seldom</td>
</tr>
</tbody>
</table>
2.2 Sino-nasal outcome test (SNOT)

Below you will find a list of symptoms and social/emotional consequences of your nasal disorder. We would like to know more about these problems and would appreciate your answering the following questions to the best of your ability. There are no right or wrong answers, and only you can provide us with this information. Please rate your problems as they have been over the past 2 weeks. Thank you for your participation.

A. Considering how severe the problem is when you experience it and how frequently it happens, please rate each item below on how “bad” it is by circling the number that corresponds with how you feel using this scale.

<table>
<thead>
<tr>
<th>Item</th>
<th>No problem</th>
<th>Very mild Problem</th>
<th>Mild or slight problem</th>
<th>Moderate problem</th>
<th>Severe problem</th>
<th>Problem as bad as it can be</th>
<th>Most important items (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Need to blow nose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sneezing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Runny Nose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal obstruction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of smell or taste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Post-nasal discharge</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Thick nasal discharge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear fullness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dizziness</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Ear pain</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Facial pain/pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficultly falling asleep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wake up at night</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of a good night’s sleep</td>
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<td></td>
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<tr>
<td>Wake up tired</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced productivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frustrated/restless/irritable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sad</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embarrassed</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

B. Please tick the most important item affecting your health (maximum of 5 items)
2.3 Pain catastrophising scale

Instructions: We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

<table>
<thead>
<tr>
<th>RATING</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEANING</td>
<td>Not at all</td>
<td>To a slight degree</td>
<td>To a moderate degree</td>
<td>To a great degree</td>
<td>All the time</td>
</tr>
</tbody>
</table>

When I was in pain...

<table>
<thead>
<tr>
<th>Number</th>
<th>Statement</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I worry all the time about whether the pain will end</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I feel I can’t go on</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>It’s terrible and I think it’s never going to get any better</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>It’s awful and I feel that it overwhelms me</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>I feel I can’t stand it anymore</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>I become afraid that the pain will get worse</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>I keep thinking of other painful events</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>I anxiously want the pain to go away</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I can’t seem to keep it out of my mind</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>I keep thinking about how much it hurts</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>I keep thinking about how badly I want the pain to stop</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>There’s nothing I can do to reduce the intensity of the pain</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>I wonder whether something serious may happen</td>
<td></td>
</tr>
</tbody>
</table>
2.4 Body vigilance scale

Instructions: This measure is designed to index how sensitive you are to internal bodily sensations such as heart palpitations or dizziness. Fill it out according to how you have felt for the past week.

I am the kind of person who pays close attention to internal bodily sensations.

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all like me</td>
<td>Moderately like me</td>
<td>Extremely like me</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I am very sensitive to changes in my internal bodily sensations.

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all like me</td>
<td>Moderately like me</td>
<td>Extremely like me</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

On average, how much time do you spend each day “scanning” your body for sensations (e.g. sweating, heart palpitations, dizziness)?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No time</td>
<td>Half of the time</td>
<td>All of the time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Rate how much attention you pay to each of the following sensations using this scale:

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Slight</th>
<th>Moderate</th>
<th>Substantial</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart palpitations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest discomfort/pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numbness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tingling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short of breath/smothering</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faintness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vision changes</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Feelings of unreality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeling detached from self</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot flash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweating/clammy hands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach upset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choking/throat closing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urge-to-cough</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoarse voice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lump in throat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 2.5 Cough-specific Cough Quality of Life Questionnaire (CQLQ)

Circle the response option that best describes the degree of your agreement concerning each of the following 28 statements.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Family and or close friends can’t tolerate it anymore</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>2</td>
<td>I have experienced prolonged absences from important activities such as work, school, or volunteer services</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>3</td>
<td>I have been completely prevented from engaging in important activities such as work, school, or volunteer services.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>4</td>
<td>I have lost my appetite.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>5</td>
<td>I am sick to my stomach and vomit.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>6</td>
<td>I cough and it makes me retch (dry heaves)</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>7</td>
<td>I have a fear that I might have AIDS or tuberculosis.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>8</td>
<td>I have headaches.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>9</td>
<td>I am concerned that I have cancer.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>10</td>
<td>I am dizzy.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>11</td>
<td>I wet my pants (I am incontinent of urine).</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>12</td>
<td>I soil my pants (I am incontinent of faeces).</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>13</td>
<td>I sweat.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>14</td>
<td>I am hoarse.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>15</td>
<td>It hurts when I breathe.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>16</td>
<td>I broke a rib.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>17</td>
<td>I cannot sleep at night.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>18</td>
<td>I have difficulty speaking on the phone.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>19</td>
<td>I can no longer sing, for instance, in church.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>20</td>
<td>I have stopped going to social activities such as movies, plays, and town meetings.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>21</td>
<td>I have had to change my lifestyle.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>22</td>
<td>I ache all over.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>23</td>
<td>I am exhausted.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>24</td>
<td>I am embarrassed.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>25</td>
<td>I am upset by people thinking that I have something wrong with me.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>26</td>
<td>I want to be reassured that I do not have anything seriously the matter with me.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>27</td>
<td>I am self-conscious.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td>28</td>
<td>I am concerned that I have something seriously the matter with me.</td>
<td>strongly disagree</td>
<td>disagree</td>
<td>agree</td>
<td>strongly agree</td>
</tr>
<tr>
<td></td>
<td><strong>TOTALS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>x1</td>
<td>x2</td>
<td>x3</td>
<td>x4</td>
</tr>
</tbody>
</table>
2.6 State-trait anxiety index (STAI)
SELF-EVALUATION QUESTIONNAIRE STAI Form Y-1

Please provide the following information:

Name __________________________ Date __________ S

Age ____________ Gender (Circle) M F T __________

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

1. I feel calm .......................................................... 1 2 3 4
2. I feel secure .......................................................... 1 2 3 4
3. I am tense ............................................................ 1 2 3 4
4. I feel strained ........................................................ 1 2 3 4
5. I feel at ease ......................................................... 1 2 3 4
6. I feel upset .......................................................... 1 2 3 4
7. I am presently worrying over possible misfortunes .... 1 2 3 4
8. I feel satisfied ....................................................... 1 2 3 4
9. I feel frightened .................................................... 1 2 3 4
10. I feel comfortable ............................................... 1 2 3 4
11. I feel self-confident ............................................. 1 2 3 4
12. I feel nervous ..................................................... 1 2 3 4
13. I am jittery ........................................................ 1 2 3 4
14. I feel indecisive ................................................. 1 2 3 4
15. I am relaxed ...................................................... 1 2 3 4
16. I feel content ................................................... 1 2 3 4
17. I am worried .................................................... 1 2 3 4
18. I feel confused .................................................. 1 2 3 4
19. I feel steady .................................................... 1 2 3 4
20. I feel pleasant .................................................. 1 2 3 4

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SELF-EVALUATION QUESTIONNAIRE
STAI Form Y-2

Name __________________________ Date ____________

DIRECTIONS
A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you generally feel.

21. I feel pleasant ................................................................. 1 2 3 4
22. I feel nervous and restless ............................................... 1 2 3 4
23. I feel satisfied with myself ............................................... 1 2 3 4
24. I wish I could be as happy as others seem to be .............. 1 2 3 4
25. I feel like a failure ............................................................. 1 2 3 4
26. I feel rested ................................................................. 1 2 3 4
27. I am “calm, cool, and collected” ........................................ 1 2 3 4
28. I feel that difficulties are piling up so that I cannot overcome them .................................................. 1 2 3 4
29. I worry too much over something that really doesn’t matter .................................................. 1 2 3 4
30. I am happy ................................................................. 1 2 3 4
31. I have disturbing thoughts ............................................... 1 2 3 4
32. I lack self-confidence .......................................................... 1 2 3 4
33. I feel secure ................................................................. 1 2 3 4
34. I make decisions easily ..................................................... 1 2 3 4
35. I feel inadequate ............................................................... 1 2 3 4
36. I am content ................................................................. 1 2 3 4
37. Some unimportant thought runs through my mind and bothers me .................................................. 1 2 3 4
38. I take disappointments so keenly that I can’t put them out of my mind .................................................. 1 2 3 4
39. I am a steady person .......................................................... 1 2 3 4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests ....... 1 2 3 4

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2.7 ROME III Questionnaire
<table>
<thead>
<tr>
<th>IBS Module</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen?</td>
</tr>
<tr>
<td>2. For women: Did this discomfort or pain occur only during your menstrual bleeding and not at other times?</td>
</tr>
<tr>
<td>3. Have you had this discomfort or pain 6 months or longer?</td>
</tr>
<tr>
<td>4. How often did this discomfort or pain get better or stop after you had a bowel movement?</td>
</tr>
<tr>
<td>5. When this discomfort or pain started, did you have more frequent bowel movements?</td>
</tr>
<tr>
<td>6. When this discomfort or pain started, did you have less frequent bowel movements?</td>
</tr>
<tr>
<td>7. When this discomfort or pain started, were your stools (bowel movements) looser?</td>
</tr>
<tr>
<td>8. When this discomfort or pain started, how often did you have harder stools?</td>
</tr>
<tr>
<td>9. In the last 3 months, how often did you have hard or lumpy stools?</td>
</tr>
<tr>
<td>Alternative scale:</td>
</tr>
<tr>
<td>10. In the last 3 months, how often did you have loose, mushy or watery stools?</td>
</tr>
<tr>
<td>Alternative scale:</td>
</tr>
</tbody>
</table>
C1. Irritable Bowel Syndrome

Diagnostic Criteria*

Recurrent abdominal pain or discomfort** at least 3 days/month in last 3 months associated with two or more of criteria #1 - #3 below:

1. Pain or discomfort at least 2-3 days/month (question 1>2)
2. For women, does pain occur only during menstrual bleeding? (question 2=0 or 2)
3. Improvement with defecation
4. Pain or discomfort gets better after BM at least sometimes (question 4>0)
5. Onset associated with a change in frequency of stool
   Onset of pain or discomfort associated with more stools at least sometimes (question 5>0), OR
   Onset of pain or discomfort associated with fewer stools at least sometimes (question 6>0)
6. Onset associated with a change in form (appearance) of stool
   Onset of pain or discomfort associated with looser stools at least sometimes (question 7>0), OR
   Onset of pain or discomfort associated with harder stools at least sometimes (question 8>0)

* Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis

** "Discomfort" means an uncomfortable sensation not described as pain.

In pathophysiology research and clinical trials, a pain/discomfort frequency of at least two days a week is recommended for subject eligibility.

Pain or discomfort more than one day per week (question 1>1)

Criteria for IBS-C

(question 9>0) and (question 10=0)

Criteria for IBS-D

(question 9=0) and (question 10>0)

Criteria for IBS-M

(question 9>0) and (question 10=0)

Criteria for IBS-U

(question 9=0) and (question 10=0)
2.8 Perceived stress scale (PSS)
PERCEIVED STRESS SCALE

Sheldon Cohen

The Perceived Stress Scale (PSS) is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of direct queries about current levels of experienced stress. The PSS was designed for use in community samples with at least a junior high school education. The items are easy to understand, and the response alternatives are simple to grasp. Moreover, the questions are of a general nature and hence are relatively free of content specific to any subpopulation group. The questions in the PSS ask about feelings and thoughts during the last month. In each case, respondents are asked how often they felt a certain way.

Evidence for Validity: Higher PSS scores were associated with (for example):
- failure to quit smoking
- failure among diabetics to control blood sugar levels
- greater vulnerability to stressful life-event-elicted depressive symptoms
- more colds


Temporal Nature: Because levels of appraised stress should be influenced by daily hassles, major events, and changes in coping resources, predictive validity of the PSS is expected to fall off rapidly after four to eight weeks.

Scoring: PSS scores are obtained by reversing responses (e.g., 0 = 4, 1 = 3, 2 = 2, 3 = 1 & 4 = 0) to the four positively stated items (items 4, 5, 7, & 8) and then summing across all scale items. A short 4 item scale can be made from questions 2, 4, 5 and 10 of the PSS 10 item scale.

Norm Groups: L. Harris Poll gathered information on 2,387 respondents in the U.S.

<table>
<thead>
<tr>
<th>Norm Table for the PSS 10 item inventory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>18-29</td>
</tr>
<tr>
<td>30-44</td>
</tr>
<tr>
<td>45-54</td>
</tr>
<tr>
<td>55-64</td>
</tr>
<tr>
<td>65 &amp; older</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>Other minority</td>
</tr>
</tbody>
</table>

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Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

Name ___________________________ Date ___________

Age ______ Gender (Circle): M F Other __________________________

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly? .......................................... 0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life? ...................................... 0 1 2 3 4

3. In the last month, how often have you felt nervous and “stressed”? .......... 0 1 2 3 4

4. In the last month, how often have you felt confident about your ability to handle your personal problems? ........................................ 0 1 2 3 4

5. In the last month, how often have you felt that things were going your way? ........................................................................... 0 1 2 3 4

6. In the last month, how often have you found that you could not cope with all the things that you had to do? ...................................... 0 1 2 3 4

7. In the last month, how often have you been able to control irritations in your life? ................................................................. 0 1 2 3 4

8. In the last month, how often have you felt that you were on top of things?.. 0 1 2 3 4

9. In the last month, how often have you been angered because of things that were outside of your control? ................................. 0 1 2 3 4

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them? ................ 0 1 2 3 4

Please feel free to use the Perceived Stress Scale for your research.

Mind Garden, Inc.
info@mindgarden.com
www.mindgarden.com

References
### Anxiety Sensitivity Index

Please rate each item by selecting one of the five answers for each question. Please answer each statement by circling the number that best applies to you.

<table>
<thead>
<tr>
<th>Item</th>
<th>very little</th>
<th>a little</th>
<th>some</th>
<th>much</th>
<th>very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. It is important not to appear nervous.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. When I cannot keep my mind on a task, I worry that I might be going crazy.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3. It scares me when I feel shaky.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4. It scares me when I feel faint.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5. It is important to me to stay in control of my emotions.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6. It scares me when my heart beat rapidly.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7. It embarrasses me when my stomach growls.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8. It scares me when I am nauseous (sick stomach).</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9. When I notice my heart beating rapidly, I worry that I might be having a heart attack.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10. It scares me when I become short of breath.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11. When my stomach is upset, I worry that I might be seriously ill.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>12. It scares me when I am unable to keep my mind on a task.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>13. Other people notice when I feel shaky.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>14. Unusual body sensations scare me.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15. When I am nervous, I worry that I might be mentally ill.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>16. It scares me when I am nervous.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
## 2.10 Reflux score

**Reflux Disease Questionnaire (RDQ)**

Please answer each question by circling one number per row. Please write the number that corresponds with the answer that best describes your symptoms in the shaded column.

1. Thinking about your symptoms over the last 4 weeks, how often did you have the following?

<table>
<thead>
<tr>
<th></th>
<th>Did not have</th>
<th>Less than one day a week</th>
<th>One day a week</th>
<th>2-3 days a week</th>
<th>4-6 days a week</th>
<th>Daily</th>
<th>Enter Number Here</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. A burning feeling behind your breastbone</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>1a:</td>
</tr>
<tr>
<td>b. Pain behind your breastbone</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>1b:</td>
</tr>
<tr>
<td>c. An acid taste in your mouth</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>1c:</td>
</tr>
<tr>
<td>d. Unpleasant movement of material upwards from the stomach</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>1d:</td>
</tr>
</tbody>
</table>

2. Thinking about your symptoms over the last 4 weeks, how would you rate the following?

<table>
<thead>
<tr>
<th></th>
<th>Did not have</th>
<th>Very Mild</th>
<th>Mild</th>
<th>Moderate</th>
<th>Moderately Severe</th>
<th>Severe</th>
<th>Enter Number Here</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. A burning feeling behind your breastbone</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>2a:</td>
</tr>
<tr>
<td>b. Pain behind your breastbone</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>2b:</td>
</tr>
<tr>
<td>c. An acid taste in your mouth</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>2c:</td>
</tr>
<tr>
<td>d. Unpleasant movement of material upwards from the stomach</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>2d:</td>
</tr>
</tbody>
</table>

Office Use Only

Total Score of Numbers in Shaded Column: __________

---

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