EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

The Effect of Pain Conditioning on Experimentally Evoked Cough: Evidence of Impaired Endogenous Inhibitory Control Mechanisms in Refractory Chronic Cough

Emma Hilton, Imran Satia, Kimberley Holt, Ashley A. Woodcock, John Belcher, Jaclyn A. Smith

Please cite this article as: Hilton E, Satia I, Holt K, *et al*. The Effect of Pain Conditioning on Experimentally Evoked Cough: Evidence of Impaired Endogenous Inhibitory Control Mechanisms in Refractory Chronic Cough. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.01387-2020).

The Effect of Pain Conditioning on Experimentally Evoked Cough: Evidence of Impaired Endogenous Inhibitory Control Mechanisms in Refractory Chronic Cough

*Emma Hilton MB BS PhD[1], *Imran Satia MB BChir PhD[1,2], Kimberley Holt MPhil[1,3], Ashley A Woodcock M.D[1,3], John Belcher PhD[3], Jaclyn A Smith MB ChB PhD[1,3]

Author affiliations:
1. University of Manchester, Division of Infection, Immunity and Respiratory Medicine, and Manchester Academic Health Science Centre, Manchester, UK
2. McMaster University, Department of Medicine, Division of Respirology
3. Manchester University NHS Foundation Trust, Manchester, UK
*Equal contributors

Corresponding Author: Professor Jacky Smith, Division of Infection, Immunity and Respiratory Medicine, University of Manchester, Manchester University Foundation Trust, Wythenshawe Hospital, Education and Research Centre, Level 2, Manchester, M23 9LT. jacky.smith@manchester.ac.uk, +44 (0)161 291 5863

Author's contributions: Conception and design: EH and JAS; Participants screening and recruitment; EH and KH; Data analysis and interpretation: EH, I.S, J.B and JAS; Drafting manuscript: IS, JA. All authors approved the final manuscript submission.

Disclosure Statement: E.H, K.H, JB have no conflicts of interest to report. I.S reports speaker fees from GSK, Astrazeneca, grants and personal fees from Merck Canada, outside the submitted work; A.A.W reports personal fees from GlaxoSmithKline, Novartis, Chiesi, Axalbion, Reacta Biotech, and from the Medicines Evaluation Unit, outside the submitted work; JAS reports grants and personal fees from Glaxosmithkline, NeRRe Pharmaceuticals, Menlo, Bayer, Boehringer Ingleheim, Axalbion, Afferent, Merck, personal fees from Genentech, Neomed, Bellus, Chiesi, AstraZeneca, Algernon, non-financial support from Vitalograph, outside the submitted work; ; AAW and JAS are named inventors on a patent, owned by Manchester University NHS Foundation Trust and licensed to Vitalograph Ltd, describing the detection of cough from sound recordings.

Funding: EH and this study were funded by an MRC doctoral fellowship (ID:91870). I.S was supported by the European Respiratory Society Respire 3 Fellowship (R3201703-00122). The

study was conducted with the support of the NIHR Manchester Clinical Research Facility. JAS is funded by the NIHR Manchester Biomedical Research Centre, a Wellcome Investigator in Science Award and is an NIHR Senior Investigator.

Clinical Trial Registration: ISRCTN31901405

Word Count: 3299

This article has an online supplement

ABSTRACT

The pathophysiology of refractory chronic cough (RCC) is unclear. We hypothesised that endogenous inhibitory control mechanisms, such as those activated by noxiousstimuli inducing pain (conditioned pain modulation (CPM)), may be capable of inhibiting coughing and urge to cough evoked by inhaled capsaicin. Furthermore, these mechanisms may be impaired in patients with RCC.

The objective was to investigate the effects of pain on cough and urge to cough (UTC) in healthy volunteers (HV) and RCC. HV and RCC patients underwent a randomised, controlled, 4-way cross-over study comparing the effect of 4 interventions on capsaicin evoked coughing and UTC. The interventions comprised immersing a hand in i) noxious cold-water, ii) warm water, iii) warm water but instructed to voluntarily supress coughing and iv) no intervention. The co-primary outcomes were numbers of evoked coughs and UTC scores.

Twenty HV (mean age 50.1(±SD14.2), M:F 10:10) and 20 RCC (age 60.1(±7.9), M:F 9:11) participated. Overall, noxious cold water reduced capsaicin evoked UTC scores and cough numbers compared with warm water (1.6 (95% C.I. 1.3-2.0) vs 2.2 (1.8-2.6) $p<0.001$; 4.8 coughs (3.7-6.2) vs 7.9 coughs (6.7-9.5) $p<0.001$, respectively). HV and RCC demonstrated similar reductions in the UTC during noxious cold-water immersion, but noxious cold water and voluntary suppression interventions were less effective in RCC than HV in reducing capsaicin evoked cough ($p=0.041$).

Endogenous inhibitory control mechanisms, specifically those activated by pain, can reduce both coughing and the UTC. Impairment of endogenous inhibitory control mechanisms may contribute to excessive coughing in RCC.

KEY WORDS: chronic cough, chronic pain, conditioned pain modulation, descending inhibitory control

ABBREVIATIONS USED:

ACCP; American College of Chest Physicians, BTS; British Thoracic Society, CPM; conditioned pain modulation, DNIC; diffuse noxious inhibitory controls, ERS; European Respiratory Society, HNCS; heterotopic noxious counter stimulation, HV; healthy volunteers, NTS; nucleus tractus solitariesPa5; paratrigeminal nucleus, PAG; periaqueductal gray,RCC; refractory chronic cough,RVM; rostro-ventral medulla, UTC; urge to cough

INTRODUCTION

Chronic cough is one of the most common reasons for referral to a pulmonologist and has a global prevalence of approximately 10%[1]. Unfortunately, there are no licensed treatments for this debilitating condition and the underlying mechanisms are poorly understood. Cough is a reflex designed to protect the airway from mechanical and noxious chemical irritants but is also under a degree of voluntary control. Patients with chronic cough complain their cough is driven by sensations of irritation in the throat and an urge to cough.

A number of mechanistic and clinical translational studies have reported evidence of neuronal hyper-excitability to inhaled irritant stimuli in refractory chronic cough (RCC)[2, 3]. We have shown that RCC patients predominantly exhibit a hyperresponsiveness (rather than hypersensitivity) of cough responses to inhaled capsaicin[3] i.e. they cough approximately twice as frequently in response to a range of doses of inhaled capsaicin compared with healthy controls. These heightened cough responses observed in chronic cough have been compared to the hyperalgesia exhibited by patients with chronic neuropathic pain.

In the study of neuropathic pain, it is thought that heightened neuronal responses occur as a consequence not only of hyperexcitability of afferent sensory pathways but also due to failure of endogenous pain control mechanisms[4]. These mechanisms involve descending pathways that project from specific brain areas to the spinal cord where they may either facilitate or inhibit transmission of noxious information, thus determining the level of pain experienced. Interestingly, spinal neurones have been shown to be inhibited by nociceptive stimuli applied outside of their own receptive fields, a phenomenon previously known as diffuse noxious inhibitory controls (DNIC) and more recently termed conditioned pain

modulation (CPM)[5]. For example, it can be demonstrated experimentally that a localised tonic noxious stimulus applied to the left hand will, via CPM, inhibit the level of pain experienced when a second noxious stimulus is applied to the right hand[6, 7]. The inhibition of pain in the right hand represents a diffuse analgesic effect (all viscera) that persists beyond the stimulation period and is independent of the site of the initial noxious stimulus.

Impaired CPM mechanisms have been found in a number of conditions including irritable bowel syndrome[8], fibromyalgia[9], chronic tension headaches/migraine[10, 11], temporomandibular joint dysfunction as well as chronic neuropathic pain[12]. The study of CPM mechanisms has been useful in quantifying an individual's endogenous inhibition of pain, and has provided insights into disease mechanisms and treatment effects[13]. However, the effectiveness of CPM-like mechanisms in modulating airway sensations and reflexes e.g. "pain-inhibiting-cough", has not been investigated either in healthy volunteers or in patients with RCC. Therefore, the objectives of this study were to assess whether similar inhibitory mechanisms are applicable to experimentally evoked urge to cough and coughing and whether a deficiency in these mechanisms occurs in patients with refractory chronic cough compared with healthy volunteers.

METHODS

Subjects: Patients with RCC were recruited from a tertiary referral specialist cough clinic (Manchester, UK) and were defined by having a cough lasting longer than 8 weeks that was either unexplained or refractory to treatment of identified underlying conditions as per BTS/ERS/ACCP guidelines[14-16]. We excluded current smokers or ex-smokers with >10 pack year history, those with a recent upper respiratory tract infection (<4 weeks),

pregnancy, breastfeeding, diabetes, and use of medication which may have altered cough responses (e.g. ACE Inhibitors, opiates, gabapentin, anti-cholinergics, and theophylline). Healthy subjects were recruited by poster advertisements 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. The protocol was approved by the local research ethics committees (REC10/H1003/104) and registered at www.controlled-trials.com (ISRCTN31901405). All participants provided written informed consent.

Study Protocol and Procedures: All participants attended for two initial screening visits, followed by four randomised visits separated by at least 48 hours (Figure 1). At screening, after performing history and examination, all participants completed several questionnaires *(see online supplement for full list*). The IBS sub-section of the ROME III questionnaire was completed by all participants and healthy volunteers were excluded if they met the criteria of IBS, because CPM mechanisms are known to be impaired in IBS patients [8, 17]. Participants were then fitted with an ambulatory cough monitor (VitaloJAK™; Vitalograph, Buckinghamshire, UK) for the next 24-hours, and returned at least one day later to undergo a full dose capsaicin cough challenge as described previously [3, 18]. The dose of capsaicin that evoked at least half the maximum cough response (ED50) during the full challenge was chosen as the single dose to be administered in the four subsequent visits. Prior to the randomised visits, all subjects practiced placing their non-dominant hand in a water bath (Clifton, stirred water-bath, NE4, Nickel-Electro Ltd, North Somerset, UK) in which the temperature was set to 10^{o}C (accurate to +/-0.5C) for 1 minute and 30 seconds if possible, but the hand could be withdrawn if the pain became unbearable at any stage. This cold-

water immersion method is known as heterotopic noxious counter stimulation (HNCS) and has been used extensively to activate CPM mechanisms when studying pain[19, 20]. Subjects also practiced rating pain intensity using a numerical scale ranging from 0 (no pain) to 10 (worst possible pain).

All subjects then attended four randomised visits in a cross-over design, wearing the ambulatory cough monitor throughout (see Figure 1). At each visit, subjects underwent two cough challenges 1 hour apart; each challenge was performed whilst the subject had their hand immersed in either noxious cold water (10°C), non- noxious warm water (32°C) or no immersion (no intervention). The order of the cold water and voluntary cough suppression intervention blocks were randomised. Subjects were instructed to place their hand in the water bath (or not for no intervention) and then after 20 seconds inhale the pre-determined ED50 dose of capsaicin four times at 15s intervals, whilst the hand remained immersed. During the cold water immersion and no intervention blocks, subjects were instructed to cough freely, however, during one of the warm water immersions subjects were told to "try not to cough". The number of coughs evoked was counted, and the intensity of the urge-to-cough experienced was rated on a modified Borg Scale (0-10)[21, 22] (Figure 1) . Pain intensity was rated on a numerical scale (0-10) immediately prior to the first inhalation and after the last inhalation of capsaicin. This procedure was then repeated an hour later to ensure the stability of ED50 evoked coughs. Blood pressure and pulse rate were measured before and after each intervention block. The number of coughs evoked was later verified from the sound recordings.

Statistical Analysis: Data analysis was performed using SPSS version 25.0 (IBM Inc, NY, USA). Summary data are presented as mean and standard deviation (S.D) or as median and

interquartile range (IQR). Repeatability of both cough and urge to cough evoked by the ED50 capsaicin concentration was assessed using intra-class correlation estimates, calculated using data collected from the warm water hand immersion where blocks 1 and 2 were identical and separated by 60 mins (average measures k=2, absolute agreement, 2-way mixed effects model). The effect of the interventions (warm water, noxious cold water, no intervention), instructions (cough vs. try not to cough), disease group on both UTC and capsaicin evoked coughs were analysed using general estimating equations (GEE, linear and non-linear) with a Bonferroni correction for multiplicity of post-hoc comparisons.

RESULTS

Subjects:

Twenty healthy controls and 20 RCC patients completed the study between January and October of 2011. There was no significant difference in age, FEV1 % predicted, FVC % predicted, pack years of previous smoking history or body mass index between the groups (Table 1). However, RCC patients demonstrated significantly higher scores on the sino-nasal outcome test (SNOT) [23]and gastro-oesophageal reflux disease (GORD) questionnaires compared to healthy volunteers (HV)[24].

As expected, patients with RCC demonstrated exaggerated and hyper-sensitive cough responses indicated by a significantly higher Emax, and lower ED50 by one double dose (Table 1). This was associated with a higher 24-hour cough frequency in RCC patients, with the greatest difference during waking hours. Patients with RCC also had significantly higher anxiety and depression scores compared to HV, but no significant group differences for perceived stress, body vigilance or pain catastrophizing (Table E1).

Repeatability of Endpoints

There was no missing data for either cough frequency or the UTC. The UTC scores and cough frequency measures exhibited very good repeatability. The mean difference in the UTC sensation with capsaicin inhalations 1h apart during the warm water hand immersion was 0.10 (S.D.±1.07) on the mBorg scale, with an intra-class correlation co-efficient of 0.88, $p<0.001$. The mean difference in the number of capsaicin evoked coughs over 4 inhalations 1hr apart in the same conditions was 2.5 coughs (S.D.±7.6) with an intra-class correlation co-efficient of 0.79, $p<0.001$.

Factors Influencing Evoked Cough and Urge-to-Cough

The factors influencing the co-primary endpoints where explored in the whole data set (healthy controls and RCC patients), using GEE modeling (urge-to-cough linear model, coughs Poisson model). Overall, the urge-to-cough ratings during inhalation of capsaicin ED50 doses were not influenced by age ($p=0.71$) or gender ($p=0.87$). In contrast, the numbers of coughs evoked by the ED50 capsaicin concentration increased slightly with increasing age ($B=0.021$, $p=0.006$) but were not influenced by gender (females 6.6 coughs (4.9-9.0) vs. males 7.3 coughs (5.5-9.3), $p=0.61$). Additionally, the urge to cough ($B=0.26$, $p<0.001$) also significantly predicted the number of coughs evoked.

Effect of Noxious Cold Stimulus and Voluntary Suppression on Evoked Urge-to-Cough

A GEE model (linear) showed a significant effect of the interventions on the urge-to-cough ($p<0.001$) in the combined subject groups, see figure 2A. Noxious cold water immersion significantly reduced the urge-to-cough compared with warm water immersion (1.6 (95%CI 1.3-2.0) vs 2.2 (1.8-2.6), $p<0.001$), cough suppression during warm water immersion (2.2 (1.7-2.7) $p=0.02$) and no intervention (2.4 (1.8-3.0), $p=0.002$) (Figure 2A). There were no

significant differences between the urge to cough with warm water immersion, cough suppression during warm water immersion and no intervention. There were no significant effects of intervention, sequence or period.

Effect of Noxious Cold Stimulus and Voluntary Suppression on Evoked Cough

A GEE model (Poisson) showed a significant effect of the interventions on number of coughs evoked by inhaling the ED50 dose of capsaicin ($p<0.001$), adjusted for the influences of age ($p=0.005$) and the urge to cough ($p<0.001$). In the combined data (all subjects), noxious cold-water immersion significantly reduced the number of evoked coughs compared with warm water immersion control (4.8 coughs (95%CI 3.7-6.2) vs 7.9 coughs (6.7- 9.5), $p<0.001$). Voluntary cough suppression had a similar inhibitory effect on coughing (3.8 coughs (2.3-6.3) $p<0.001$) whereas no intervention was similar to warm water immersion (6.7 (5.3-8.4), $p=0.06$) (Figure 2B). There were no significant effects of intervention sequence or period in the model.

Comparison of Refractory Chronic Cough with Healthy Volunteers

Interestingly, there were no significant differences in the urge to cough reported by RCC patients compared with HV when inhaling the ED50 capsaicin concentration. Both groups experienced a similar reduction in the urge to cough during cold water immersion compared with the warm water, cough suppression with warm water and no intervention, see figure 3A, $p<0.001$. In contrast, despite reporting similar urge to cough, RCC patients coughed much more frequently than HV during all the interventions, see figure 3B [HV 3.4 (2.3-4.9) vs. RCC 9.1 (7.5-11.1), $p<0.001$]. The effect of the interventions also differed between the patient groups (group*intervention interaction ($p=0.042$)). Post-hoc comparisons corrected for multiplicity (Bonferroni) suggested that whilst the different interventions significantly

modulated the number of evoked coughs in the HV group, i.e. cold-water immersion and voluntary suppression both significantly reduced coughing compared with warm water immersion and no intervention, this was not the case for RCC. In the RCC group, although evoked cough was numerically reduced by these interventions, the differences did not achieve statistical significance.

Pain Intensity during interventions

Pain scores were higher in RCC patients compared with HV after the hand was immersed in noxious cold water for 20 seconds before any capsaicin was inhaled (3.7 (2.6) in HV vs. 5.9 (2.5) in RCC patients, $p<0.001$). Pain intensity increased after the cold water bath, but there were no differences in the change reported between the groups; mean difference (SD) in HV was 2.2 (1.7) vs. 2.0 (1.5) in RCC, $p=0.27$). During warm water control interventions none of the subjects reported any pain.

Changes in physiology during interventions

There were no significant differences in the changes in systolic blood pressure (sBP) or pulse rate (PR) between the two interventions on each study day (mean of $<\pm 3$mmHg or $<\pm 3$bpm). There was no significance difference in the change in sBP/PR for CC patients compared to HV during any of the interventions, see online Table E2.

DISCUSSION

To our knowledge, this is the first study to directly investigate the modulatory effects of pain on coughing. Our main findings were, i) a noxious cold-water stimulus applied to the hand inhibited the urge-to-cough and coughing induced by capsaicin inhalation, ii) asking patients to “try not to cough” also reduced capsaicin-evoked coughing, but not the urge-to-cough, and iii) noxious cold-water and voluntary cough suppression both appear less effective in inhibiting cough in patients with RCC compared with HV. These findings are consistent with our hypothesis that both the urge to cough and cough can be inhibited by activating endogenous inhibitory control mechanisms, unlike voluntary cough suppression which has no influence on the urge to cough. Notably, these distinct mechanisms were both impaired in patients with RCC compared with healthy volunteers, implying failure of cough control mechanisms is more extensive than previously appreciated, and includes endogenous inhibitory controls.

Classical CPM pathways have been well characterised in both pre-clinical models and in translational studies in healthy volunteers and patients with well-defined neurological deficits [25, 26]. Descending inhibitory signals are thought to originate in the hypothalamus and amygdala and are transmitted via the peri-aquaductal grey (PAG) and rostro-ventral medulla (RVM) to the dorsal horn of the spinal cord [27-32]. The consequence of activation of these descending controls is diffuse analgesia that can be demonstrated by a reduction in the pain experienced by a second stimulus at a different site (test stimulus). Our findings provide the first evidence for an analogous endogenous inhibitory pathway activated by a remote noxious cold water stimulus and capable of inhibiting the urge to cough and coughing evoked by an inhaled irritant. Whilst further exploration is needed to understand

the exact location of the action of pain conditioning and descending inhibitory controls on cough pathways, possibilities include the PAG, RVM, and the sites where sensory airway nerves first synapse in the brainstem i.e. in the nucleus tractus solitarius and paratrigeminal nuclei (nTS/Pa5).

Previous studies have investigated other types of cough inhibition such as voluntary cough suppression. Healthy controls can significantly inhibit cough responses to inhaled irritants and in comparison, RCC patients have a reduced ability to voluntarily supress cough[33]; this finding has been recently replicated[34]. As illustrated by the current study, voluntary suppression is very different from endogenous inhibitory controls. The former is a conscious process, cognitively driven, in which patients actively try to resist coughing in response to the urge to cough. The latter is sub-conscious and in contrast to voluntary suppression, reduces both the urge to cough and coughing to capsaicin.

The inability to voluntarily suppress cough is undoubtedly a problem for chronic cough patients, who often complain of a constant urge to cough. However, impaired voluntary cough suppression may well reflect the severe, persistent urge to cough experienced by chronic cough patients and thus represent an epiphenomenon rather than a fundamental component of the condition. In health, voluntary cough suppression is only important on occasions where exposure to environmental irritants occurs. Conversely, endogenous inhibitory control mechanisms (including CPM) may be tonically active, modulating perceived airway sensations and preventing inappropriate triggering of coughing episodes. Impairment of such inhibitory controls could therefore both explain the persistent urge to cough and consequential excessive coughing in RCC and is accordingly plausible as component of the pathophysiology of RCC. Of note, in this study we were only able to

demonstrate a significant difference between RCC and HV in efficiency of pain conditioning to reduce coughing; the absolute reductions in urge to cough were numerically greater in healthy controls than refractory cough patients, however the difference did not reach statistical significance. Urge to cough scores are subjective and inherently more variable than cough numbers and therefore a larger sample size would be required to demonstrate significant differences between these groups for that endpoint. Of interest, RCC patients did report significantly higher pain scores on exposure to noxious cold at baseline and throughout the study compared with HV, an observation consistent with disordered pain conditioning in this group. Whether pre-existing impaired inhibitory controls predispose individuals to developing chronic cough or occur as a consequence of the condition would be difficult to establish. Interestingly, longitudinal studies of post-operative pain support the notion that pre-existing impaired CPM predisposes subjects to develop chronic pain[35, 36]. Furthermore, studies in chronic pain states have implicated opioids in activating descending pathways mediating CPM[37],a potential explanation for the significant benefits of low dose morphine in a substantial subset of patients with RCC.

Inhibition of cough, in the form of placebo responses, has also been observed in RCC patients participating in clinical trials of novel therapies influencing not only subjective reports of cough severity and quality of life, but also reducing objectively measured cough frequency by approximately 30%[38, 39]. Like voluntary cough suppression, placebo responses are a consequence of cognitive processes, whereby the knowledge that the patient may be taking a new therapy and the expectation that this therapy may be beneficial act to reduce cough frequency. In healthy volunteers, placebo effects following placebo conditioning have been shown to reduce capsaicin evoked urge to cough by up to

45% and a follow up fMRI study suggested this was associated with increased activation in the prefrontal and posterior parietal cortices [40]. Unfortunately, fMRI studies designed to evaluate central inhibition of cough (by any mechanism) in RCC patients are lacking. A single study did compare cerebral activations during capsaicin evoked urge to cough in RCC with healthy controls and although voluntary cough suppression was not specifically studied, RCC patients seemed to have reduced activations compared with healthy controls in areas thought to be involved in voluntary cough suppression in healthy controls[41].

There are some limitations to this study worth noting. Firstly, a noxious cold stimulus could be considered a distraction, an alternative mechanism via which the urge to cough and cough could be reduced. However, this question has been addressed in classical CPM studies and distraction found to be a distinct effect from CPM[42]. Secondly, the study could not be fully blinded. By the very nature of the interventions the subjects become immediately aware of the temperature of the water bath on immersion of their hands, however it is highly unlikely that they have particular expectations of how these may influence their cough. Finally, this first study was small in size and so has limited power to understand the effects of gender and other characteristics on the ability of noxious stimuli to modify coughing or the variability of its impairment in RCC. Nonetheless, these positive findings provide the motivation for further larger studies to characterise these endogenous inhibitory mechanisms in both heath and disease and to investigate whether these changes in experimentally evoked cough also have importance in clinical spontaneous cough.

CONCLUSIONS

In conclusion, this study provides the first evidence that conditioning with a noxious stimulus modulates airway sensations and reflex responses such as the urge to cough and

the cough response evoked by inhaled capsaicin. The inefficiency of this mechanism in RCC patients compared with healthy controls implicates impairment of endogenous inhibitory control mechanisms in the pathophysiology of RCC which may be an important therapeutic target.

ACKNOWLEDGMENTS

We thank all the patients and healthy volunteers for their participation. We are grateful for the National Institute of Health Research (NIHR) Clinical Research Facility in South Manchester for providing the infrastructure to perform all the study visits.

REFERENCES:

1. Morice AH, Jakes AD, Faruqi S, Birring SS, McGarvey L, Canning B, Smith JA, Parker SM, Chung KF, Lai K, Pavord ID, van den Berg J, Song WJ, Millqvist E, Farrell MJ, Mazzone SB, Dicpinigaitis P, Chronic Cough R. A worldwide survey of chronic cough: a manifestation of enhanced somatosensory response. *Eur Respir J* 2014: 44(5): 1149-1155.

2. Belvisi MG, Birrell MA, Khalid S, Wortley MA, Dockry R, Coote J, Holt K, Dubuis E, Kelsall A, Maher SA, Bonvini S, Woodcock A, Smith JA. Neurophenotypes in Airway Diseases. Insights from Translational Cough Studies. *Am J Respir Crit Care Med* 2016: 193(12): 1364-1372.

3. Hilton EC, Baverel PG, Woodcock A, Van Der Graaf PH, Smith JA. Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point. *J Allergy Clin Immunol* 2013: 132(4): 847-855 e841-845.

4. Popescu A, LeResche L, Truelove EL, Drangsholt MT. Gender differences in pain modulation by diffuse noxious inhibitory controls: a systematic review. *Pain* 2010: 150(2): 309-318.

5. Yarnitsky D. Role of endogenous pain modulation in chronic pain mechanisms and treatment. *Pain* 2015: 156 Suppl 1: S24-31.

6. Willer JC, Roby A, Le Bars D. Psychophysical and electrophysiological approaches to the pain-relieving effects of heterotopic nociceptive stimuli. *Brain* 1984: 107 (Pt 4): 1095-1112.

7. Mitchell LA, MacDonald RA, Brodie EE. Temperature and the cold pressor test. *J Pain* 2004: 5(4): 233-237.

8. Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut* 2004: 53(11): 1595-1601.

9. Julien N, Goffaux P, Arsenault P, Marchand S. Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain* 2005: 114(1-2): 295-302.

10. Galioto R, O'Leary KC, Gunstad J, Thomas JG, Lipton RB, Pavlovic JM, Roth J, Rathier L, Bond DS. The role of migraine headache severity, associated features and interactions with overweight/obesity in inhibitory control. *Int J Neurosci* 2018: 128(1): 63-70.

11. Sandrini G, Rossi P, Milanov I, Serrao M, Cecchini AP, Nappi G. Abnormal modulatory influence of diffuse noxious inhibitory controls in migraine and chronic tension-type headache patients. *Cephalalgia* 2006: 26(7): 782-789.

12. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB, Eccleston C, Kalso E, Bennett DL, Dworkin RH, Raja SN. Neuropathic pain. *Nat Rev Dis Primers* 2017: 3: 17002.

13. Damien J, Colloca L, Bellei-Rodriguez CE, Marchand S. Pain Modulation: From Conditioned Pain Modulation to Placebo and Nocebo Effects in Experimental and Clinical Pain. *Int Rev Neurobiol* 2018: 139: 255-296.

14. Morice AH, McGarvey L, Pavord I, British Thoracic Society Cough Guideline G. Recommendations for the management of cough in adults. *Thorax* 2006: 61 Suppl 1: i1-24.

15. Morice AH, Fontana GA, Belvisi MG, Birring SS, Chung KF, Dicpinigaitis PV, Kastelik JA, McGarvey LP, Smith JA, Tatar M, Widdicombe J, European Respiratory S. ERS guidelines on the assessment of cough. *Eur Respir J* 2007: 29(6): 1256-1276.

16. Gibson P, Wang G, McGarvey L, Vertigan AE, Altman KW, Birring SS, Panel CEC. Treatment of Unexplained Chronic Cough: CHEST Guideline and Expert Panel Report. *Chest* 2016: 149(1): 27-44.

17. Heymen S, Maixner W, Whitehead WE, Klatzkin RR, Mechlin B, Light KC. Central processing of noxious somatic stimuli in patients with irritable bowel syndrome compared with healthy controls. *Clin J Pain* 2010: 26(2): 104-109.

18. Satia I, Tsamandouras N, Holt K, Badri H, Woodhead M, Ogungbenro K, Felton TW, O'Byrne PM, Fowler SJ, Smith JA. Capsaicin-evoked cough responses in asthmatic patients: Evidence for airway neuronal dysfunction. *J Allergy Clin Immunol* 2017: 139(3): 771-779 e710.

19. Fidanza F, Varanini M, Ciaramella A, Carli G, Santarcangelo EL. Pain modulation as a function of hypnotizability: Diffuse noxious inhibitory control induced by cold pressor test vs explicit suggestions of analgesia. *Physiol Behav* 2017: 171: 135-141.

20. Torta DM, Legrain V, Rossetti Y, Mouraux A. Prisms for pain. Can visuo-motor rehabilitation strategies alleviate chronic pain? *Eur J Pain* 2016: 20(1): 64-69.

21. Davenport PW. Urge-to-cough: what can it teach us about cough? *Lung* 2008: 186 Suppl 1: S107-111.

22. Davenport P, Sapienza C, Bolser D. Psychophysical assessment of the urge-to-cough. *European Respiratory Review* 2002: 12(85): 249-253.

23. Baumann I. Subjective outcomes assessment in chronic rhinosinusitis. *The Open Otorhinolaryngology Journal* 2010: 4(1).

24. Shaw MJ, Talley NJ, Beebe TJ, Rockwood T, Carlsson R, Adlis S, Fendrick AM, Jones R, Dent J, Bytzer P. Initial validation of a diagnostic questionnaire for gastroesophageal reflux disease. *The American journal of gastroenterology* 2001: 96(1): 52-57.

25. Bouhassira D, Le Bars D, Bolgert F, Laplane D, Willer JC. Diffuse noxious inhibitory controls in humans: a neurophysiological investigation of a patient with a form of Brown-Sequard syndrome. *Ann Neurol* 1993: 34(4): 536-543.

26. De Broucker T, Cesaro P, Willer JC, Le Bars D. Diffuse noxious inhibitory controls in man. Involvement of the spinoreticular tract. *Brain* 1990: 113 (Pt 4): 1223-1234.

27. Villanueva L, Le Bars D. Indirect effects of intrathecal morphine upon diffuse noxious inhibitory controls (DNICs) in the rat. *Pain* 1986: 26(2): 233-243.

28. Bannister K, Dickenson A. The plasticity of descending controls in pain: translational probing. *The Journal of physiology* 2017: 595(13): 4159-4166.

29. Lockwood S, Dickenson AH. What goes up must come down: insights from studies on descending controls acting on spinal pain processing. *Journal of Neural Transmission* 2019: 1-9.

30. Chebbi R, Boyer N, Monconduit L, Artola A, Luccarini P, Dallel R. The nucleus raphe magnus OFF-cells are involved in diffuse noxious inhibitory controls. *Experimental neurology* 2014: 256: 39-45.

31. Lorenz J, Minoshima S, Casey K. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. *Brain* 2003: 126(5): 1079-1091.

32. Starr CJ, Sawaki L, Wittenberg GF, Burdette JH, Oshiro Y, Quevedo AS, Coghill RC. Roles of the insular cortex in the modulation of pain: insights from brain lesions. *Journal of Neuroscience* 2009: 29(9): 2684-2694.

33. Young EC, Brammer C, Owen E, Brown N, Lowe J, Johnson C, Calam R, Jones S, Woodcock A, Smith JA. The effect of mindfulness meditation on cough reflex sensitivity. *Thorax* 2009: 64(11): 993-998.

34. Cho PSP, Fletcher HV, Turner RD, Jolley CJ, Birring SS. Impaired cough suppression in chronic refractory cough. *Eur Respir J* 2019: 53(5).

35. Yarnitsky D, Crispel Y, Eisenberg E, Granovsky Y, Ben-Nun A, Sprecher E, Best LA, Granot M. Prediction of chronic post-operative pain: pre-operative DNIC testing identifies patients at risk. *Pain* 2008: 138(1): 22-28.

36. Wilder-Smith OH, Schreyer T, Scheffer GJ, Arendt-Nielsen L. Patients with chronic pain after abdominal surgery show less preoperative endogenous pain inhibition and more postoperative hyperalgesia: a pilot study. *J Pain Palliat Care Pharmacother* 2010: 24(2): 119-128.

37. Willer JC, Le Bars D, De Broucker T. Diffuse noxious inhibitory controls in man: involvement of an opioidergic link. *Eur J Pharmacol* 1990: 182(2): 347-355.

38. Smith JA, Kitt MM, Morice AH, Birring SS, McGarvey LP, Sher MR, Li YP, Wo WC, Xu ZJ, Muccino DR, AP F. Gefapixant, a P2X3 receptor antagonist, for the treatment of refractory or unexplained chronic cough: a randomised, double-blind, controlled, parallel-group, phase 2b trial [In press]. *Lancet Resp Med* 2020.

39. Smith J BE, Kerr M, Mcgarvey L, Morice A, Sher M, Trower M, Pawsey S. The neurokinin-1 receptor antagonist orvepitant improves chronic cough symptoms: results from a Phase 2b trial. *Eur Respir J* 2019: 54(Suppl 63).

40. Leech J, Mazzone SB, Farrell MJ. Brain activity associated with placebo suppression of the urge-to-cough in humans. *Am J Respir Crit Care Med* 2013: 188(9): 1069-1075.

41. Ando A, Smallwood D, McMahon M, Irving L, Mazzone SB, Farrell MJ. Neural correlates of cough hypersensitivity in humans: evidence for central sensitisation and dysfunctional inhibitory control. *Thorax* 2016: 71(4): 323-329.

42. Moont R, Pud D, Sprecher E, Sharvit G, Yarnitsky D. 'Pain inhibits pain' mechanisms: Is pain modulation simply due to distraction? *Pain* 2010: 150(1): 113-120.

TABLES

Table 1: Subject demographics and baseline characteristics. Mean and S.D showed unless stated otherwise.*Median (IQR). FEV1; forced expiratory volume in 1 second, FVC; forced vital capacity, SNOT; sino-nasal outcome test, GORD; gastro-oesophageal reflux disease, CQLQ; cough quality of life questionnaire, Emax; maximum evoked coughs at any dose of capsaicin, ED50; dose evoking at least half the Emax, c/h; coughs/hour.

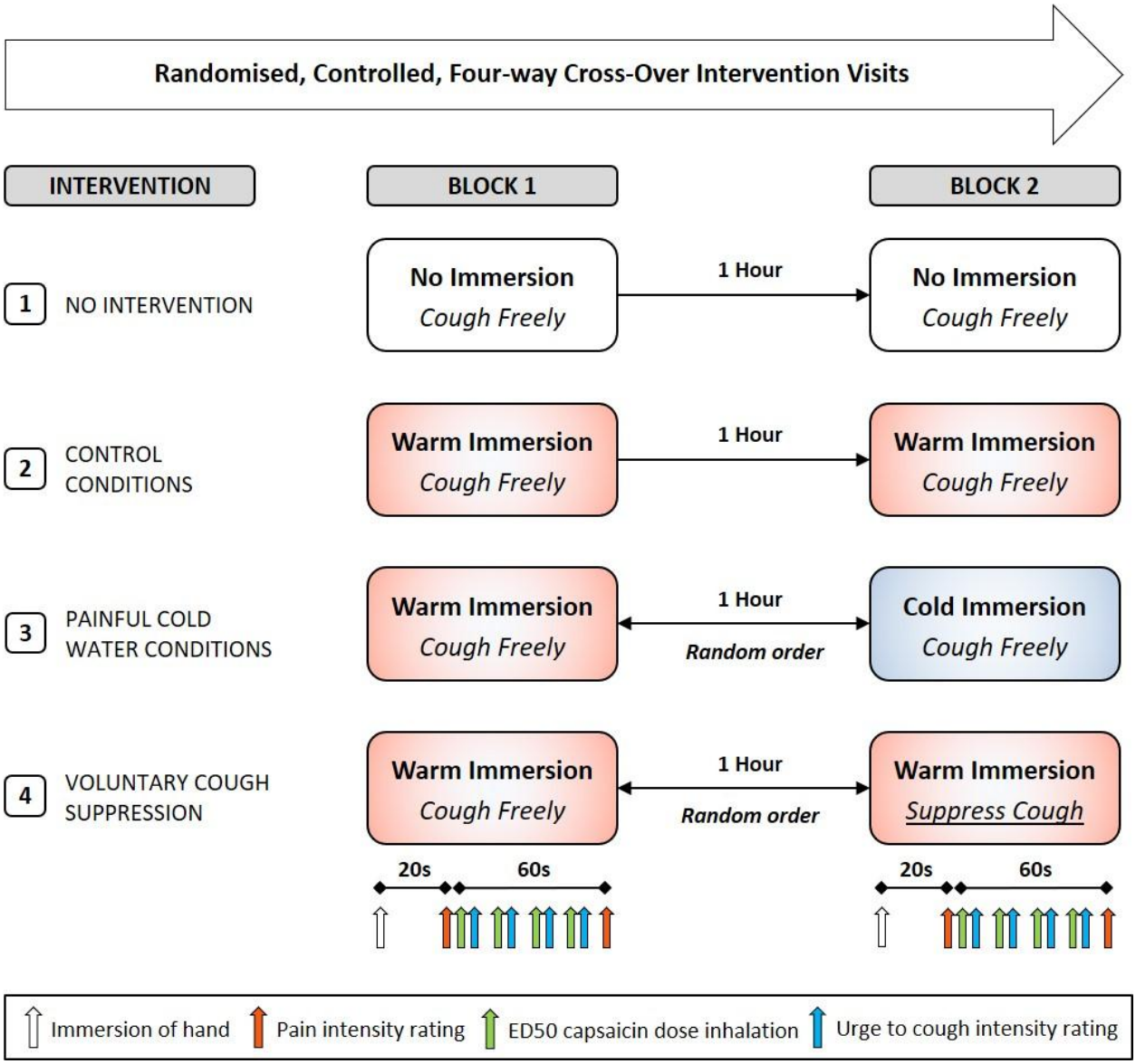
	Healthy Volunteers (HV)	**RCC (RCC)**	**p-value**
Number (n)	20	20	
Age (years)	50.1 (14.2)	60.1 (13.2)	0.22
Gender (M:F)	10:10	9:11	
Duration of Cough (years)		13.0 (9.8)	
Body Mass Index (BMI, kg/m2)	26.6	28.1	0.25
Pack Years*	0.0 (0.0-0.0)	0.0 (0.0-1.8)	0.44
FEV1 % pred	105.7 (13.8)	98.8 (16.5)	0.16
FVC % pred	107.8 (16.0)	102.7 (15.5)	0.31
SNOT Score	0.08 (0.5)	1.3 (1.4)	p<0.001
GORD Score	0.0 (3.5)	7.0 (15.5)	p<0.001
CQLQ		52.5 (12.8)	
Capsaicin Emax*	14.0 (10.5-18.8)	27.5 (21.3-37.0)	p<0.001
Capsaicin ED50 (microM/L)*	15.6 (7.8-31.3)	7.8(3.9-15.6)	P<0.001
24-hr cough frequency (c/h)*	0.8 (0.2-1.3)	10.9 (8.3-23.7)	p<0.001
Awake cough frequency (c/h)*	1.1 (0.3-1.8)	15.4 (12.2-26.7)	p<0.001
Sleep cough frequency (c/h)*	0.1 (0.0-0.6)	1.8 (0.5-5.5)	p<0.001

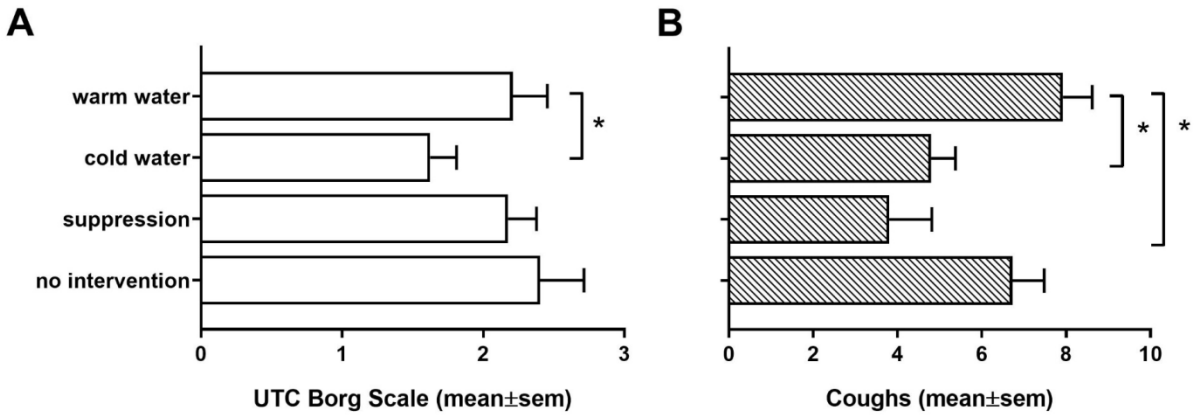
FIGURES

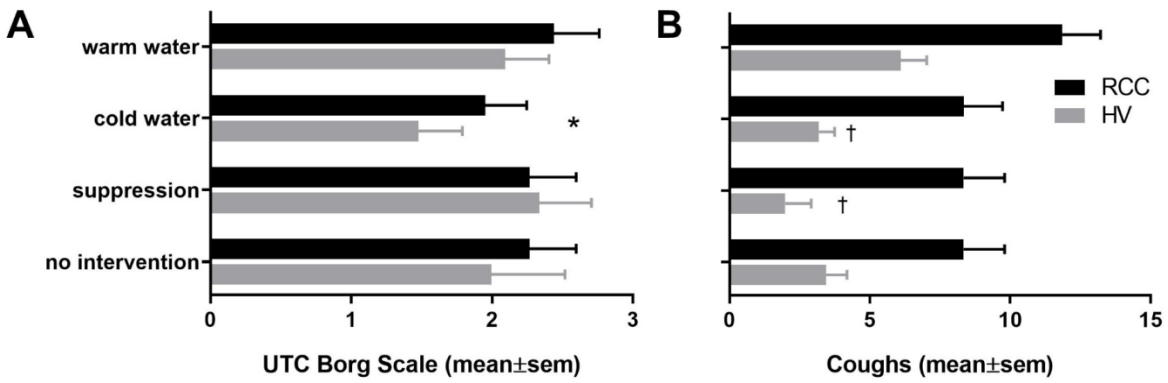
FIGURE 1: Study Design

FIGURE 2: Overall effects of all interventions on urge to cough (A) and capsaicin evoked cough (B) in all participants (n=40). *indicates significant differences for comparison with warm water control, $p<0.001$

FIGURE 3: The effects of all conditions on capsaicin evoked urge to cough and coughing in healthy volunteers and refractory chronic cough patients. For urge to cough (A), there was no difference between healthy volunteers and refractory cough patients, both groups experienced a similar reduction in urge to cough with cold compared with warm water immersion and other conditions, *$p<0.001$. Evoked coughs (B), were significantly greater in refractory chronic cough patients than healthy volunteers for all conditions $p<0.001$, but reductions in coughing were significant for cold water immersion and voluntary suppression in healthy controls (compared with warm water) but not in refractory chronic cough patients †$p\leq0.001$.


Randomised, Controlled, Four-way Cross-Over Intervention Visits
INTERVENTION
BLOCK 1
BLOCK 2
1
NO INTERVENTION
No Immersion
Cough Freely
1 Hour
No Immersion
Cough Freely
2
CONTROL CONDITIONS
Warm Immersion
Cough Freely
1 Hour
Warm Immersion
Cough Freely
3
PAINFUL COLD WATER CONDITIONS
Warm Immersion
Cough Freely
1 Hour
Random order
Cold Immersion
Cough Freely
4
VOLUNTARY COUGH SUPPRESSION
Warm Immersion
Cough Freely
1 Hour
Random order
Warm Immersion
Suppress Cough
20s
60s
20s
60s
Immersion of hand
Pain intensity rating
ED50 capsaicin dose inhalation
Urge to cough intensity rating


A
warm water
cold water
suppression
no intervention
*
0
1
2
3
UTC Borg Scale (mean±sem)
B
*
*
0
2
4
6
8
10
Coughs (mean±sem)


A
warm water
cold water
suppression
no intervention
*
0
1
2
3
UTC Borg Scale (mean±sem)
B
RCC
HV
†
†
0
5
10
15
Coughs (mean±sem)

ONLINE SUPPLEMENT

The Effect of Pain Conditioning on Experimentally Evoked Cough: Evidence of Impaired Endogenous Inhibitory Control Mechanisms in Refractory Chronic Cough

*Emma Hilton MB BS PhD[1], *Imran Satia MA MB BChir PhD[2,3], Kimberley Holt MPhil[2,4], Ashley A Woodcock M.D[2,4], John Belcher[4], Jaclyn A Smith MB ChB PhD[2,4]

Author affiliations:
1. University of Manchester, Division of Infection, Immunity and Respiratory Medicine, and Manchester Academic Health Science Centre, Manchester, UK
2. McMaster University, Department of Medicine, Division of Respirology
3. Manchester University NHS Foundation Trust, Manchester, UK
*Equal contributors

METHODS:

Study 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)

(ii) Hospital Anxiety and Depression Scale (HADS)

(iii) Perceived Stress Scale (PSS): validated to measure the degree to which situations are appraised as stressful and assesses recent (<1 month) stress levels.

(iv) Pain Catastrophising Scale (PCS): a validated self-report scale assessing the individual's tendency towards pain catastrophizing. 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"[1]. Negative correlations between DNIC effect and pain catastrophising score have been shown in healthy subjects[2].

(v) Anxiety Sensitivity Index (ASI): measures the individual's fear of bodily sensations that are interpreted as having potentially harmful physical or psychological consequences.

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

(vii) IBS sub-section of the ROME III questionnaire). Healthy subjects were excluded if they met the criteria for a diagnosis of IBS because DNIC are known to be impaired in IBS patients[3, 4].

(viii) Sino-nasal Outcome Test

(ix) Reflux Symptom questionnaire to determine whether they were symptomatic of reflux disease or post-nasal drip syndrome.

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

RESULTS:

Psychological Questionnaires:

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 E1).

TABLE E1: Comparison of questionnaire scores between healthy controls and patients with chronic cough. HADS-a = Hospital Anxiety and Depression scale for anxiety, HADS-d for depression. Mean (SD)* or median (IQR)** shown.

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

Table E2: Changes in blood pressure and pulse rate before and after each intervention block. Mean (SD) shown.

Intervention	Healthy Controls (HC)	Chronic cough (CC)	p-value
Change in systolic blood pressure (mmHg)			
No intervention	-3.35 (5.41)	-1.65 (5.62)	0.336
Warm water	-0.85 (3.88)	1.40 (9.27)	0.326
Cold water	2.30 (9.95)	1.20 (6.41)	0.680
Suppression	-0.85 (9.37)	-2.10 (11.37)	0.706
Change in pulse rate (beats per minute, bpm)			
No intervention	0.40 (4.12)	3.20 (8.02)	0.176
Warm water	0.40 (5.80)	1.10 (5.89)	0.707
Cold water	-0.60 (6.63)	-0.15 (6.00)	0.823
Suppression	-0.25 (5.50)	0.70 (9.34)	0.697

REFERENCES:

1. Quartana PJ, Campbell CM, Edwards RR. Pain catastrophizing: a critical review. *Expert Rev Neurother* 2009: 9(5): 745-758.
2. Weissman-Fogel I, Sprecher E, Pud D. Effects of catastrophizing on pain perception and pain modulation. *Exp Brain Res* 2008: 186(1): 79-85.
3. Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut* 2004: 53(11): 1595-1601.
4. Heymen S, Maixner W, Whitehead WE, Klatzkin RR, Mechlin B, Light KC. Central processing of noxious somatic stimuli in patients with irritable bowel syndrome compared with healthy controls. *Clin J Pain* 2010: 26(2): 104-109.