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International Symposium on:

PAIN AND COUGH

Florence (Italy), March 19-21, 2015

Organized by

DEPARTMENT OF HEALTH SCIENCES
DEPARTMENT OF EXPERIMENTAL AND CLINICAL MEDICINE
UNIVERSITY OF FLORENCE

DEPARTMENT OF GERIATRICS AND MEDICINE
DEPARTMENT OF CARDIOVASCULAR AND THORACIC MEDICINE
AZIENDA OSPEDALIERO UNIVERSITARIA CAREGGI

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FONDAZIONE
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ABSTRACT BOOK

Convitto della Calza
Piazza della Calza, 6

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CONTENTS

Central and Peripheral Sensitization: from Pain to Cough

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Both chronic cough and chronic pain are critical clinical issues in which a large number of patients remain unsatisfied with available treatments. The basic neurobiological mechanisms and pathologies of these two conditions show substantial homologies. In both pain and cough there is a complex network of primary sensory nerves involved that comprise both unmyelinated C-fibers and small myelinated Aδ fibers. The depolarizing "generator potentials" at the terminals of pain and cough nerves are evoked by similar mediators (bradykinin, eicosanoids, purines, proteases, etc.) interacting with similar nonspecific cation channels (TRPV1, TRPA1, TRPV4, P2X, 5HT3, etc) in both pain and cough pathways (e.g. capsaicin, bradykinin, acid, etc). The generators potentials are enocoded into action potential discharge by the same subtypes of voltage-gated sodium channels, namely NaV 1.7, NaV 1.8 and NaV 1.9. The action potentials are conducted along the axons to the terminals in the central nerves system where the excitatory transmission in pain and cough pathways involved glutamatergic receptors, with modulator influences provided by tachyinins.

The peripheral neuronal pathways in pain and cough can be sensitize by inflammation resulting in terminals that are electrically and chemically more excitable than in healthy tissues. In addition, inflammation can lead to phenotypic changes by altering the gene expression the level of the cell bodies of neurons in the dorsal root ganglia (pain) and vagal sensory ganglia (cough). The sensitization associated with tissue inflammation is not limited to the periphery. Stimulation of nociceptive inputs and phenotypic changes can also lead to a sensitization of synaptic transmission within the CNS at the level of the dorsal horn of the spinal cord (pain) and within the nucleus tractus solitarious (cough).

The peripheral and central changes in excitability along with phenotypic changes results in conditions of hyperalgesia and hypertussivity where the threshold for a pain and cough stimulus, respectively is substantially reduced. These changes can also result in situation where a normally non-painful stimulus such as light touch becomes painful, a phenomenon referred to as allodynia. Likewise these processes can lead to situations where non-tussive stimuli, talking, laughing, breathing cold air, leads to urge to cough sensations; an "allotussivity". Due to the substantial overlap that in the biology of chronic pain and cough, it will be interesting to determine the extent to which the efficacy of therapeutic strategies overlap in these two diseases.

References:

1. O'Neill J1, McMahon SB, Undem BJ. Chronic cough and pain: Janus faces in sensory neurobiology? Pulm Pharmacol Ther. 2013 Oct;26(5):476-85. doi: 10.1016/j.pupt.2013.06.010. Epub 2013 Jul 4.

2. Canning BJ1, Mazzone SB, Meeker SN, Mori N, Reynolds SM, Undem BJ. Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs. J Physiol. 2004 Jun 1;557(Pt 2):543-58. Epub 2004 Mar 5.

3. Cervero F1.Visceral pain: mechanisms of peripheral and central sensitization. Ann Med. 1995 Apr;27(2):235-9.

4. Muroi Y1, Ru F, Kollarik M, Canning BJ, Hughes SA, Walsh S, Sigg M, Carr MJ, Undem BJ. Selective silencing of Na(V)1.7 decreases excitability and conduction in vagal sensory neurons. J Physiol. 2011 Dec 1;589(Pt 23):5663-76. doi: 10.1113/jphysiol.2011.215384. Epub 2011 Oct 17.

5. Mazzone SB, Mori N, Canning BJ. Synergistic interactions between airway afferent nerve subtypes regulating the cough reflex in guinea-pigs. J Physiol. 2005 Dec 1;569(Pt 2):559-73. Epub 2005 Jul 28.

6. Waxman SG1, Zamponi GW. Regulating excitability of peripheral afferents: emerging ion channel targets. Nat Neurosci. 2014 Feb;17(2):153-63. doi: 10.1038/nn.3602. Epub 2014 Jan 28.

Central Control of Cough

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Cough has a key physiological function as a very important airway protective reflex, but can also characterize a debilitating disease under chronic conditions. Tracheobronchial rapidly adapting receptors (RARs) are well known to mediate the cough reflex while the role of bronchopulmonary C-fibers and Aδ-nociceptive afferents is still controversial. Recently, in the larynx and rostral trachea of guinea pigs, "cough receptors" innervated by very slow-conducting Aδ-fibers and activated by punctate mechanical stimulation and acid have been described (1). Previous studies have identified the caudal nucleus tractus solitarii (cNTS; medial subnucleus and, especially, the lateral aspect of the commissural subnucleus) as the predominant site of RAR central projections (2).

Our experiments on the cough reflex evoked by mechanical and chemical (citric acid inhalation) stimulation of the tracheobronchial tree under physiological conditions have been carried out on spontaneously breathing rabbits anaesthetized with pentobarbital sodium mainly making use of microinjection techniques at the level of two medullary regions, i.e. the cNTS and the caudal ventral respiratory group (cVRG) where expiratory premotor neurons are located. We have provided for the first time evidence that ionotropic glutamate receptors, especially non-*N*-methyl-D-aspartate receptors, within the cNTS are primarily involved in the mediation of the cough reflex. Similarly, we have demonstrated that the same receptors are responsible for the excitatory drive to cVRG expiratory neurons and that these neurons are not merely elements of the expiratory output but play an essential role in the genesis of the overall cough motor pattern (3 also for further Refs.).

From the cNTS, cough-related inputs engage other brainstem structures. Recording studies from respiratory neurons as well as c-fos studies have shown that the ponto-medullary network underlying respiratory rhythm generation reconfigures to generate the cough motor pattern, i.e. it is a multifunctional neural network and contains neurons with functional flexibility (1, 4). We have contributed to corroborate this view by means of lesion and recording experiments in the Bötzinger complex that provided evidence that augmenting expiratory neurons there located are involved in the generation of the cough motor pattern and may convey an excitatory drive to the cVRG expiratory neurons.

Previous studies showed that some antitussive drugs act centrally, but the central responsive structures were not determined. We hypothesized that the cNTS and the cVRG could be important, although not exclusive, central sites of action of some antitussive drugs (5, 6). Our studies demonstrated that μ-opioid receptor agonists, GABAA and GABAB receptor agonists and antagonists, NK1 receptor agonists and antagonists exert a potent modulation of the cough reflex at the level of these two central sites.

In agreement with the above mentioned results, peripheral and central mechanisms subserving nociception and cough (that is indeed a defensive response to nociceptive stimuli) appear to share similar features. These latter include for instance the type of afferent fibers (Aδ and C), transient receptor potential vanilloid 1 and transient receptor potential ankyrin 1 channels and central and peripheral sensitization. Thus, the attention of recent studies on cough has been focused on this aspect (7). Accordingly, we have investigated the antitussive effects of agents involved in the central control of pain sensation (8-10). In more detail, our studies showed for the first time that the inhibition of ERK1/2 activation as well as the activation of α2-adrenergic and galanin receptors play an important role in the inhibitory control of the cough reflex induced by both mechanical and chemical stimulations of the tracheobronchial tree at the medullary level.

In conclusion, we believe that the study of the basic physiological mechanisms underlying different types of responses to nociceptive stimulation may provide helpful suggestions for novel therapeutic strategies. Admittedly, studies of neuropathic pain or neuropathic cough performed on animal models and humans could be more relevant to develop novel antitussive compounds with proved efficacy and devoid of important side effects.
Finally, since cough has an important role in assuring survival both in normal and pathological conditions, we would like to underline that investigations on cough-potentiating mechanisms would be of great interest.

Part of these studies has been supported by grants from the Ministry of Education, University, and Research of Italy and from the A. Menarini United Pharmaceutical Industries.

References:

1. Widdicombe JG. Reflexes from the lungs and airways: historical perspective. *J Appl Physiol* 101: 628-634, 2006.

2. Kubin L, Alheid GF, Zuperku EJ, McCrimmon DR. Central pathways of pulmonary and lower airway vagal afferents. *J Appl Physiol* 101: 618-627, 2006.

3. Mutolo D, Bongianni F, Cinelli E, Pantaleo T. Role of excitatory amino acids in the mediation of tracheobronchial cough induced by citric acid inhalation in the rabbit. *Brain Res Bull* 80: 22-29, 2009.

4. Pantaleo T, Bongianni F, Mutolo D. Central nervous mechanisms of cough. *Pulm Pharmacol Ther* 15: 227-233, 2002.

5. Mutolo D, Bongianni F, Cinelli E, Fontana GA, Pantaleo T. Modulation of the cough reflex by antitussive agents within the caudal aspect of the nucleus tractus solitarii in the rabbit. *Am J Physiol Regul Integr Comp Physiol* 295: R243-R251, 2008.

6. Mutolo D, Bongianni F, Cinelli E, Pantaleo T. Depression of cough reflex by microinjections of antitussive agents into caudal ventral respiratory group of the rabbit. *J Appl Physiol* 109: 1002-1010, 2010.

7. Dicpinigaitis PV, Morice AH, Birring SS, McGarvey L, Smith JA, Canning BJ, Page CP. Antitussive drugs--past, present, and future. *Pharmacol Rev* 66: 468-512, 2014.

8. Mutolo D, Bongianni F, Cinelli E, Giovannini MG, Pantaleo T. Suppression of the cough reflex by inhibition of ERK1/2 activation in the caudal nucleus tractus solitarii of the rabbit. *Am J Physiol Regul Integr Comp Physiol* 302: R976-R983, 2012.

9. Cinelli E, Bongianni F, Pantaleo T, Mutolo D. Suppression of the cough reflex by alpha 2-adrenergic receptor agonists in the rabbit. *Physiol Rep* 1: e00122, 2013.

10. Mutolo D, Cinelli E, Bongianni F, Pantaleo T. Inhibitory control of the cough reflex by galanin receptors in the caudal nucleus tractus solitarii of the rabbit. *Am J Physiol Regul Integr Comp Physiol* 307: R1358-R1367, 2014.

Peripheral Control of Cough

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Cough preserves lung capacity for gas exchange by promoting clearance of aspirate, accumulated secretions, inhaled particulates and irritants, either inhaled or formed during inflammation. Cough thus serves an important protective role in the airways and lungs. But cough also promotes the spread of life-threatening respiratory tract infections (e.g. influenza, tuberculosis, SARS, *Bordetella pertussis*), and in diseases such as chronic obstructive pulmonary disease (COPD), gastroesophageal reflux disease (GERD), asthma and rhinosinusitis, cough can become excessive, harm the airway mucosa and adversely impact patient quality of life. These contrasting roles of cough indicate the need for balancing therapy targeting cough in a way that preserves the defensive functions of cough but limits the spread of harmful illnesses and diminishes the impact of this symptom on patient quality of life.

Clinical and preclinical studies provide overwhelming evidence for the primary role of bronchopulmonary vagal afferent nerves in the regulation of cough. These vagal afferent nerves can be differentiated into subtypes by their mechanical and chemical responsiveness, their activity during eupnea, as well as their anatomical, embryological and physiological attributes. At least 2 subtypes of vagal afferent nerves can initiate coughing upon activation, and their function and dysfunction likely contributes to the presentation of cough in health and in disease.

The most readily identified vagal afferent nerves in cough are bronchopulmonary C-fibers. C-fibers are unmyelinated and conduct action potentials at <1 m/ second. These vagal afferents are largely unresponsive to lung stretch and are mostly inactive during eupnea. But C-fibers are activated by a variety of pro-inflammatory mediators produced during lung inflammatory responses, including protons and bradykinin, and can also be activated by inhaled irritants including cigarette smoke, pollutants (e.g. toluene diisocyanate (TDI), ozone) and

by chemical irritants that evoke coughing upon inhalation (citric acid, capsaicin, allyl isothiocyanate (AITC). At the molecular level, C-fibers can be identified by their expression of TRP channels often associated with nociceptors innervating somatic tissues, including TRPV1 (which is activated by capsaicin and protons) and TRPA1 (which is activated by AITC, TDI, ozone). All of these C-fiber selective stimuli will initiate coughing upon inhalation in patients and in experimental animals, directly implicating C-fiber activation in cough.

In addition to C-fibers, a second vagal afferent nerve subtype that initiates cough upon activation has been identified in several species and with circumstantial evidence for their presence in human airways. These "cough receptors" are insensitive to many of the chemical stimuli that directly activate C-fibers (e.g. capsaicin, AITC, bradykinin) but are exquisitely sensitive to protons and to mechanical irritation of the airway mucosa. The cough receptors are myelinated and conduct action potentials at ~5 m/ second.

Although C-fibers and cough receptors can initiate coughing independently, their coincident activation may promote the enhanced cough responsiveness, or cough hypersensitivity, associated with conditions such as asthma and GERD. C-fibers may also be sensitized to activation by pro-inflammatory mediators associated with allergic inflammation.

Therapeutic strategies for cough suppression can target multiple mechanisms that may promote cough hypersensitivity. The receptors directly initiating cough upon activation might be targeted. Alternatively, the receptors for pro-inflammatory mediators (e.g. cysteinyl-leukotrienes, thromboxane, prostaglandin E2) that peripherally sensitize vagal afferent nerves to activation may be targeted. Ion channels regulating action potential generation and excitability may also be a target for cough suppression.

References:

1. Mazzone SB, N Mori, Canning BJ (2005). Synergistic interactions between airway afferent nerve subtypes regulating the cough reflex in guinea-pigs. J. Physiol., 569(2):559-573.

2. Canning BJ, N Mori and SB Mazzone (2006). Vagal afferent nerves regulating the cough reflex. Respir Physiol Neurobiol., 152(3):223-242.

3. Muroi Y, Ru F, Kollarik M, Canning BJ, Hughes SA, Walsh S, Sigg M, Carr MG, Undem BJ (2011). Selective silencing of NaV1.7 decreases excitability and conduction in vagal sensory neurons. J Physiol, 589(Pt 23):5663-5676.

4. Dicpinigaitis PV, A Morice, SS Birring, L McGarvey, JA Smith, Canning BJ, CP Page (2014). Anti-tussive drugs – past present and future. Pharmacol Rev., 66(2):468-512.

5. Canning BJ, AB Chang, DC Bolser, JA Smith, SB Mazzone, L McGarvey. Anatomy and neurophysiology of coughing: CHEST guideline and expert panel report. Chest, 146(6):1633-48.

Coughing and breathing

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The regular alternation of diaphragmatic contractions and passive expirations automatically generated by brainstem neural networks is essential to blood oxygen, carbon dioxide and $[H^+]$ homeostasis. The metabolic role of breathing requires afferent feedback from chemoreceptors and slowly adapting pulmonary stretch receptors in order to adjust tidal volume and breathing rate. The respiratory muscles also are involved in behavioural activities, such as phonation, that implies various groups of motoneurons, coordinated at the level of both the brainstem and the somatosensory cortex. Metabolic and behavioural drives may thus be in competition, so that for the time of phonation for instance the ventilatory response to CO_2 is blunted. Cough has a three phase motor act: initiation by a deep inspiration, glottis closure and a forceful expiratory effort. It occurs primarily as a reflex protecting the airways by removing inhaled foreign particles and clearing the lower respiratory tract from accumulated mucus and secretions. Therefore, although cough contributes to maintaining the metabolic role of breathing, breathing and coughing differ significantly not only in their motor patterns but also their regulatory mechanisms (1). The first is imbedded in an automatic regulatory loop where O_2 and CO_2 concentrations are major end-points, while the second is reflex, triggered by activation of chemically and mechanically sensitive vagal afferent fibres. On the other hand, both behaviours are also under volitional control, through which they can be consciously initiated and, to some extent, inhibited.

Ventilation and cough may interact in many ways. Increase in ventilation provoked by the hypoxic stimulation of peripheral chemoreceptors (2) or the increased inspired fraction of CO_2 (3) is associated with suppression of the mechanically or chemically induced

cough. Also, the incidence of the cough response to fog is blunted during exercise in humans (4). Decreased ventilation induced by severing the carotid sinus nerve or blocking the discharge by inhalation of pure oxygen, leads also to suppression of mechanically and chemically induced cough in animal models (5). Ventilation decreases during slow wave sleep and the experimental evidence exists in dogs that for tracheal or laryngeal stimulation to provoke a cough response, arousal is mandatory. A similar observation was made during REM sleep; with the particularity that arousal threshold to bronchial or laryngeal stimulation was considerably higher compared to slow wave sleep (6). The cough response to stimulation of airway irritant receptors that is markedly dependent on waking contrasts with the apnoea induced by stretch receptor stimulation that does not cause arousal from sleep, is readily elicited during slow wave sleep, but profoundly depressed during REM sleep (6). The nociceptive stimulation of the airways in dogs may also induce a brief expiratory effort that does not require arousal and may be triggered during waking, slow wave sleep and REM sleep (7). The expiration reflex is also known to resist general anaesthesia and antitussive medications and to be present at birth in some animal species.

Human development is associated with considerable evolution in control of breathing. The neonatal period is characterized by a low hypoxic ventilatory drive (8), depressed response to laryngeal or tracheobronchial stimulation that induces apnoea rather than cough in babies and experimental animals (9). The cough reflex develops afterwards and the incidence is reported to increase in clinical situations, from the early period after birth where cough seems to be absent, to a few weeks of age, where it has become a major symptom of respiratory tract infection or foreign body inhalation. The first months of life are characterized by the high proportion of REM sleep, during which the experimental evidence exists that arousal to airway stimuli, and the associated cough response are depressed.

Therefore it appears that the drive to breathe competes with - and overcomes - the drive to cough in those situations where more ventilation is demanded by increased chemical drive or metabolism.

On the other hand, cough inhibition also appears to be associated with depression of the chemical ventilatory drive or of arousal systems.

References:

1. Bolser D, Bolser DC, Poliacek I, Jakus J, Fuller DD, Davenport PW. Neurogenesis of cough, other airway defensive behaviors and breathing: A holarchical system? Respir Physiol Neurobiol. 2006 Jul 28;152(3):255-65. Epub 2006 May 24. Review.

2. Tatár M, Korpás J, Polácek H, Záhradný V.: Effects of hypoxia on respiratory defence reflexes. Effects of thirty hours' oxygen deficiency on cough, the expiration reflex and sneezing in awake cats. Bratisl Lek Listy. 1985 Aug;84(2):157-64.

3. Kulik AM,Rtarakanv IA, Tatar M, Korpas J: Cough reflex in hypercapnia. Bull. Eur. Phyiopathol. Respir. 23 (suppl 10),63.

4. Lavorini F, Fontana GA, Chellini E, Magni C, Duranti R, Widdicombe J. Desensitization of the cough reflex by exercise and voluntary isocapnic hyperpnea. J Appl Physiol (1985). 2010 May;108(5):1061-8.

5. Hanacek J, Brozmanova M, Tatar M, Karcolova D, Strapkova A, Szepe P: Effects of long-term high oxygen concentration breathing on defensive respiratory reflexes in guinea-pigs. Pulm Pharmacol. 1996 Oct-Dec;9(5-6):367-8.

6. Sullivan CE, Kozar LF, Murphy E, Phillipson EA. Arousal, ventilatory, and airway responses to bronchopulmonary stimulation in sleeping dogs. J Appl Physiol Respir Environ Exerc Physiol. 1979 Jul;47(1):17-25.

7. Sullivan CE, Murphy E, Kozar LF, Phillipson EA. Waking and ventilatory responses to laryngeal stimulation in sleeping dogs. J Appl Physiol Respir Environ Exerc Physiol. 1978 Nov;45(5):681-9.

8. Carroll JL. Developmental plasticity in respiratory control. J Appl Physiol 2003;94:375e89.

9. Thach, B.T.(2007). Maturation of cough and other reflexes that protect the fetal and neonatal airway. Pulm Pharmacol Ther 20,365–370.

Brainstem Neurones Involved in the Upper Airway Control

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Upper airways (UA) represent an organic structural and functional component of the respiratory tract. UA serve to respiration, respiratory tract protection and defence, phonation, deglutition, etc. Substantial regulation of the airway resistance is provided by motor control of the oral, pharyngeal, and laryngeal structures. UA stiffen and widen during the inspiration mainly due to inspiratory (I) activation of the alae nasi muscles, genioglossus m., pharyngeal dilators, and laryngeal abductor the posterior cricoarytenoid m. (PCA). Tonic activity of some UA muscles (e.g. PCA, laryngeal adductor thyroarytenoid, or cricopharyngeus m.) and/or their expiratory (E) modulation (e.g. thyropharyngeus m.) were reported as well, depending on species, anesthetic, and respiratory drive (e.g. Iscoe 1988).

Primary component of the respiratory tract protection is laryngoconstriction. During defensive airway reflexes laryngeal, pharyngeal, but also lingual muscles may provide vigourous highly coordinated action resulting in appropriate changes in UA calibre and tonus. Laryngeal movement consists of abductions during I and E phase of cough and adductions in the cough compression and subsequent constriction phase (Poliacek et al. 2005). Simpler patterns at least in some motoneurons were found in decerebrate animals (e.g. Baekey et al. 2001). During all cough the cricopharyngeus m., in E phase the superior pharyngeal constrictor (Satoh et al. 1998) are activated and thyropharyngeus m. is suppressed at least in cat. In the sneezing expiration also styloglossus and levator veli palatini m. are activated (Satoh et al. 1998).

Unlike in breathing or coughing, where UA serve to respiratory-protection-defence, the swallow is essentially made by coordinated action of UA muscles. During the oro-pharyngeal phase of swallowing progressively and/or simultaneously majority of pharyngeal, lingual (e.g. styloglossus) muscles, and laryngeal adductors are vigorously activated with an early relaxation followed by activation of the cricopharyngeus m. (upper esophageal sphincter) all performed in an orderly fashion (e.g. Pitts et al. 2013). Motoneurons driving the UA muscles are located primarily in the hypoglossal (HgN) and ambigual (NA) nuclei. Motor pattern of individual motoneuronal pools is determined by activation-inhibition-modulation from pre-motoneurons and neurons upstream of reflex circuits. Many of these neurons are involved in the control of primary behavior e.g. breathing, coughing, vomiting, etc, in order to provide coordination of airway functions with the needs of particular behavior.

Anatomical and functional studies pointed out multiple brainstem areas with various density of neurons connected to or related to UA motor control: solitary tract nucleus (NTS), dorsal reticular formation near the HgN, lateral tegmental field (FTL) represented by the parvocellular and other reticular nuclei, spinal trigeminal ncl., medullary ventromedial tegmentum, area postrema, retroambigual area, caudal raphé, parabrachial region of the pons including Kölliker-Fuse ncl. (KF), the cubcoeruleus region, pontine trigeminal nuclei, the ventral and ventrolateral medulla (including retrofacial and parafacial area), and higher brain levels including periaqueductal grey (e.g. Boers et al. 2005, Gestreau et al. 2005). Activation of neuronal populations in these areas vary depending on the momentary action of UA and the behavior induced, e.g. Fos labeling related to laryngeal adduction was limited to NTS, FTL, area postrema and NA. Vice versa, several neurons involved in UA control including motoneurons of HgN and NA are multifunctional (e.g. Gestreau et al. 2005).

Laryngeal and hypoglossal activity is during breathing and coughing under command of respiratory/cough central pattern generator (CPG) located in ventrolateral medulla. Excitatory drive to I laryngeal

(PCA) motoneurons, expressing various firing patterns, may come primarily from augmenting (Iaug), less from decrementing (Idec) I neurons. All these units increased they firing rate during coughing corresponding to augmented pattern of laryngeal abduction in cough. E laryngeal (adductor) motoneurons receive excitatory drive putatively from a subpopulation of Edec neurons. Inhibitory connections may arise from Eaug, and Edec to I motoneurons, from Eaug, Edec, I aug, and Idec to E motoneurons (Baekey et al. 2001, O'Connor et al. 2012). Respiratory control of hypoglossal motoneurons (mainly I activity) is provided *via* I premotoneurons scattered throughout FTL and interneurons within the HgN (e.g. Gestreau et al. 2005). Significant premotoneurons and/or higher order units shaping respiratory laryngeal activity and essential for the development of post-I phase of respiratory cycle are located in KF area of pons (Bautista et al. 2010).

In addition to aformentioned connections from CPG to laryngeal motoneurons, working during coughing and sneezing (Shiba et al. 1999), modulation of primary cough neuronal network induced variable effects in laryngeal motor output. Elimination of cough motor pattern by lesions in medullary raphé, FTL, and pontine parabrachial region mostly preserved the elementary laryngeal adductor responses and respiratory pattern of UA (Poliacek et al. 2005). Significant modulation of ventilatory motor output from the retroambigual region, caudal medial reticular formation, or by baroreceptor stimulation induced only limited changes in UA motor pattern during coughing, likely employing additional pathways that those from CPG (also Shiba et al. 1999).

Majority of respiratory neurons including Eaug units are inhibited during swallowing reducing inhibition of E laryngeal motoneurons and allowing vigorous adductor swallow activation (Shiba et al. 2007). Swallowing related neurons are located in NTS, medullary FTL, the gigantocellular and paragigantocellular reticular formations, the ventrolateral medulla, raphé, KF and intertrigeminal pontine region. Also premotor control of tongue muscle suggests participation of reconfigured respiratory neuronal network in non-respiratory behaviors (Gestreau et al. 2005).

Abundant connectivity of the neuronal network that controls UA patency employ NMDA, AMPA/kainate, opioid, GABA receptors as well as glycinergic, cholinergic, serotonergic, tachykinine, catecholamine and possibly other types of modulation. Irregularities in UA control may result in dysphagia, dystussia etc. Primarily cholinergic, norepinephrine, and serotonergic tonic drives are implicated in disorders related to reduced UA tone, such as obstructive sleep apnea. Pharmacological and frequently simple surgical interventions may improve these conditions in patients.

References:

1. Baekey DM, Morris KF, Gestreau C, Li Z, Lindsey BG, Shannon R: Medullary respiratory neurones and control of laryngeal motoneurones during fictive eupnoea and cough in the cat. J Physiol Lond 2001; 534:565-581.

2. Bautista TG, Burke PGR, Sun QJ, Berkowitz RG, Pilowsky PM. The Generation of Post-Inspiratory Activity in Laryngeal Motoneurons: A Review. Homma I et al. (eds.). New Frontiers in Respiratory Control, Advances in Experimental Medicine and Biology 669, Springer, 2010, pp. 143-149.

3. Boers J, Hulshoff AC, DeWeerd H, Mouton LJ, Kuipers R, Holstege G. Afferent Projections to Pharynx and Soft Palate Motoneurons: A Light and Electron Microscopical Tracing Study in the Cat. J Comp Neurol 2005; 486:18-38.

4. Gestreau C, Dutschmann M, Obled S, Bianchi AL. Activation of XII motoneurons and premotor neurons during various oropharyngeal behaviors. Respir Physiol Neurobiol 2005; 147:159-176.

5. Iscoe SD. Central control of the upper airway. In: Respiratory Function of the Upper Airway, Lung Biology in Health and Disease. Mathew OP, Sant'Ambrogio G (eds), Marcel Dekker, New York, 1988, pp 125-192.

6. O'Connor R, Segers LS, Morris KF, Nuding SC, Pitts TE, Bolser DC, Davenport PW, Lindsey BG. A joint computational respiratory neural network-biomechanical model for breathing and airway defensive behaviors. Front Physiol 2012; 3(264):1-28.

7. Pitts T, Rose MJ, Mortensen AN, Poliacek I, Sapienza CM, Lindsey BG, Morris KF, Davenport PW, Bolser DC. Coordination of cough and swallow: A meta-behavioral response to aspiration. Respir Physiol Neurobiol 2013; 189(3):543-551.

8. Poliaček I, Stránsky A, Szereda-Przestaszewska M, Jakuš J, Baráni H, Tomori Z, Halašová E. Cough and laryngeal muscle discharges in brainstem lesioned anesthetized cats. Physiol Res 2005; 54(6):645-654.

9. Satoh I, Shiba K, Kobayashi N, Nakajima Y, Konno A. Upper airway motoroutputs during sneezing and coughing in decerebrate cats. Neurosci Res 1998; 32(2):131-135.

10. Shiba K, Satoh I, Kobayashi N, Hayashi F. Multifunctional Laryngeal Motoneurons: an Intracellular Study in the Cat. J Neurosci 1999; 19(7):2717-2727.

11. Shiba K, Nakazawa K, Ono K, Umezaki T. Multifunctional Laryngeal Premotor Neurons: Their Activities during Breathing, Coughing, Sneezing, and Swallowing. J Neurosci 2007; 27(19):5156-5162.

Sex differences in pain control

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Pain researchers have now come to some consensus regarding the existence of small quantitative sex differences in the sensitivity to and tolerance of pain in humans.
Differences in the effectiveness of analgesics in men and women are also appreciated. However, broad conclusions regarding the existence and direction of such sex differences are complicated by emerging evidence from laboratory animals that sex differences interact with genetic background. That is, male and female mice of only certain genetic backgrounds display sex differences.
Even the direction of sex differences (male>female vs. female>male) may depend on genetic factors. In addition to these quantitative sex differences, evidence is rapidly emerging that the sexes may differ *qualitatively* in their neural mediation of pain and analgesia. That is, different neural circuits, transmitters, receptors and genes may be relevant to pain processing in males and females.
I will present new data from our laboratory demonstrating that the specific genetic, cellular and neurochemical mediation of chronic pain processing in the spinal cord in male and female mice are radically different.

Transient Receptor Potential (TRP) channels in cough

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TRP channels

Transient receptor potential channels are cation-selective ion channels with 28 such channels having been described. Their ligand interactions are subject to different mechanisms including channel ankyrin repeats. They play various physiological roles mainly in cellular sensing and signalling pathways. Up to 6 TRP channels have been shown to be expressed in some primary sensory neurones including TRP vanniloid 1 (TRPV1), TRPV2 to TRPV4, TRPM8 and TRPA1. They are all temperature sensing from hot (TRPV1 and V2) to cold (TRPA1 and M8). Capsaicin, which is used for testing cough sensitivity gates TRPV1, and stimulates capsaicin-sensitive cough receptors. TRPV1 can be activated by heat and also endogenous stimuli such as low pH, anandamide and some liquids. TRPA1 is on the other hand gated by products of oxidative and nitrative stress and may also co-localise TRPV1. TRPA1 agents include many components of spices including cinnamaldehyde, allyl isothiocyanate and allicin.

Chronic cough

Cough has an important protective function in the airways and lungs and is mediated through afferent sensory neuronal pathways, starting from vagal afferent news innervating the larynx, trachea, carina and large intrapulmonary bronchi with terminations confined to the airway mucosa. In the clinic, chronic cough, which is a cough defined as lasting for more than eight weeks, represents a nuisance, serving no protective purpose and can be associated with an impairment of quality of life. Currently-available antitussives are not usually effective in controlling cough. Increasingly now, chronic cough is being considered as having a "neuropathic" component, and has been often referred to as a vagal neuropathy.

Potential for inflammatory and innate immune mechanisms at the level of the vagal afferent nerves to enhance the cough reflex has been proposed. Cough responses to capsaicin or citric acid are increased in chronic cough, supporting the concept of sensitisation which may involve both peripheral and central mechanisms. Central mechanisms may involve increased neuropeptide in brain stem cough nuclei. Subcortical nuclei in various parts of the upper brain are also activated in the urge-to-cough and could represent sensitised sites.

TRPV1 and TRPA1 in cough

There is immunohistochemical evidence of increased TRPV1 expression in epithelial nerves in the airways of chronic cough subjects. A number of endogenous inflammatory mediators such as prostaglandins E_2 and bradykinin also enhances capsaicin cough sensitivity. Activation of TRPV1-expressing sensory neurons can elicit reflex bronchoconstriction and mucus secretion. A TRPV1 agonist, SB-705498, has been shown to produce a significant improvement in cough reflex sensitivity to capsaicin in subjects with chronic refractory cough without altering objective or subjective cough severity. Other studies are being carried out in patients with chronic obstructive airways disease. However, there is evidence of hyperthermia occurring in subjects on TRPV1 blockers.

Inhalation of TRPA1 agonists such as cinnamaldehyde by normal volunteers induced cough. In guinea-pigs, complete suppression of cough induced by prostaglandin and bradykinin required the use of simultaneous blockade of TRPA1 and TRPV1. This indicates that blockade of these 2 TRP channels may be necessary to control cough. Current trials of TRPA1 blockers are underway.

References:

1. Nilius B, Szallasi A. Transient receptor potential channels as drug targets: from the science of basic research to the art of Medicine. Pharmacol Rwev 2014; 66: 676-814.

2. Kaneko Y, Szallasi A. Transient receptor potential (TRP) channels: a clinical perspective. Br J Pharmacol. 2014;171(10):2474-507.

3. Canning BJ, Chang AB, Bolser DC, Smith JA, Mazzone SB, McGarvey L. Anatomy and Neurophysiology of Cough: CHEST Guideline and Expert Panel Report. Chest. 2014;146(6):1633-48.

4. Farrell MJ, Mazzone SB. Sensations and regional brain responses evoked by tussive stimulation of the airways. Respir Physiol Neurobiol. 2014;204:58-63.

5. Ando A, Farrell MJ, Mazzone SB. Cough-related neural processing in the brain: A roadmap for cough dysfunction? Neuroscience and biobehavioral reviews. 2014;47c:457-68.

6. Chung KF, McGarvey L, Mazzone SB. Chronic cough as a neuropathic disorder. The Lancet Respiratory Medicine. 2013;1(5):414-22.

7. Chung KF. Chronic 'cough hypersensitivity syndrome': a more precise label for chronic cough. Pulmonary pharmacology & therapeutics. 2011;24(3):267-71.

8. Birrell MA, Belvisi MG, Grace M, Sadofsky L, Faruqi S, Hele DJ, et al. TRPA1 agonists evoke coughing in guinea pig and human volunteers. Am J Respir Crit Care Med. 2009;180(11):1042-7.

9. Grace M, Birrell MA, Dubuis E, Maher SA, Belvisi MG. Transient receptor potential channels mediate the tussive response to prostaglandin E2 and bradykinin. Thorax. 2012;67(10):891-900.

10. Groneberg DA, Niimi A, Dinh QT, Cosio B, Hew M, Fischer A, et al. Increased expression of transient receptor potential vanilloid-1 in airway nerves of chronic cough. Am J Respir Crit Care Med. 2004;170(12):1276-80.

11. Khalid S, Murdoch R, Newlands A, Smart K, Kelsall A, Holt K, et al. Transient receptor potential vanilloid 1 (TRPV1) antagonism in patients with refractory chronic cough: A double-blind randomized controlled trial. J Allergy Clin Immunol. 2014.

New Options in Cough Therapy: Anticholinergic Agents

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Cough is induced by activation of vagal afferent nerves terminating in the larynx, trachea and bronchi. Multiple vagal afferent nerve subtypes innervate the airways, including parasympathetic cholinergic nerves.[1] Thus it is logical to investigate cholinergic, or muscarinic, receptor antagonists as potential antitussive agents.

A few small studies performed decades ago suggested that the inhaled antimuscarinic agents ipratropium and oxitropium had antitussive effect against experimentally induced cough as well as acute cough during viral upper respiratory tract infection (URI).[2] More recently, the long-acting antimuscarinic agent (LAMA) tiotropium was demonstrated to acutely inhibit capsaicin-induced cough, in the absence of bronchodilation, in healthy nonsmokers with URI, thus raising the question of the mechanism by which cough reflex sensitivity was inhibited.[2,3] A recent study in guinea pigs has suggested that tiotropium exerts its antitussive effect through the inhibition of TRPV1 receptors.[4]

Significant anecdotal experience supports the use of first-generation antihistamines for acute cough as well as chronic cough due to upper airway cough syndrome (formerly, postnasal drip syndrome; rhinitis). It has been proposed that these agents suppress cough through their action as centrally penetrating anticholinergics, though animal studies do not fully support this hypothesis.[5] Recently, the first-generation antihistamine, diphenhydramine, was shown to inhibit capsaicin-induced cough in otherwise healthy subjects with URI.[6]

The tricyclic antidepressant amitriptyline has been suggested to have efficacy in chronic, refractory cough in two small studies, but prospective, randomized, controlled trials are necessary to further elucidate the potential clinical role of this agent.

A potential mechanism by which amitriptyline exerts an antitussive effect may be antimuscarinic, though the drug also displays adrenergic, serotonergic and histaminergic pharmacological actions as well.[1]

A growing body of evidence suggests a potential therapeutic role for inhaled and oral antimuscarinic agents in acute and chronic cough. Properly executed, prospective, randomized, controlled studies employing appropriate objective and subjective endpoints are required and eagerly awaited.

References:

1. Dicpinigaitis PV, Morice AH, Birring SS, et al. Antitussive drugs-past, present, and future. Pharmacol Rev 2014;66:468-512.

2. Dicpinigaitis PV, Spinner L, Santhyadka G, et al. Effect of tiotropium on cough reflex sensitivity in acute viral cough. Lung 2008;186:369-374.

3. Bateman ED, Rennard S, Barnes PJ, et al. Alternative mechanisms for tiotropium. Pulm Pharmacol Ther 2009;22:533-542.

4. Birrell MA, Bonvini SJ, Dubuis E, et al. Tiotropium modulates transient receptor potential V1 (TRPV1) in airway sensory nerves: a beneficial off-target effect? J Allergy Clin Immunol 2014;133:679-687.

5. Bolser DC. Older-generation antihistamines and cough due to upper airway cough syndrome (UACS): efficacy and mechanism. Lung 2008;186(Suppl.1):S74-S77.

6. Dicpinigaitis PV, Dhar S, Johnson A, et al. Inhibition of cough reflex sensitivity by diphenhydramine during acute viral respiratory tract infection. Int J Clin Pharm 2015, in press.

Gabapentin for refractory chronic cough

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Up to 40% of patients with chronic cough remain unexplained following detailed investigations and treatment trials. Patients with chronic cough were twice as likely to be female and the onset of cough is usually around middle age. Chronic cough is associated with significant impairment of quality of life. A key feature of chronic cough is a heightened cough reflex at a peripheral or central level. There are few effective pharmacological therapies available for chronic cough.

There are many similarities between chronic cough and neuropathic pain. Patients with chronic cough commonly describe laryngeal paraesthesia, an abnormal sensation in the throat (such as a tickle, itch, irritation or an urge to clear the throat). They may also have features of hyper-tussia (cough triggered by lower levels of known tussive stimuli, such as strong odours e.g. perfumes or smoke). Some patients have features of allotussia; cough caused by non-tussive stimuli, e.g. speech, laughter, cold air or eating. The neurological pathways that sense and conduct signals centrally from external stimuli also have much in common. For example, transient receptor potential (TRP) receptors, C-fibres and the vagus nerve are important in both neuropathic pain and cough.

Preliminary studies in patients with chronic cough have suggested neuro-modulator drug therapies, such as Amitriptyline, and Gabapentin may be effective. Gabapentin is thought to bind neuronal calcium channels and inhibit the release of neurotransmitters, thereby reducing neuronal hypo-excitability. A recent double-blind, randomised controlled trial of Gabapentin in refractory chronic cough was reported by *Ryan et al*. (1) Gabapentin was titrated to a maximum dose of 1800mgs daily and cough outcome measures were assessed at two months.

Gabapentin led to a clinically significant reduction in cough severity (Visual Analogue Scale), a reduction in objective cough frequency (Leicester Cough Monitor)(2) and improvement in quality of life (Leicester Cough Questionnaire).(3) Following discontinuation of Gabapentin, the cough severity was worse than that assessed at baseline. Gabapentin was well tolerated. Common side-effects such as fatigue, drowsiness and dizziness were manageable with dose reduction. A responder analysis suggested that the number needed to treat (NNT) was 3.6. There was no improvement in cough reflex sensitivity assessed with capsaicin. The best clinical response was in patients reporting symptoms of laryngeal paraesthesia. Further studies are needed to investigate the long-term effects of Gabapentin therapy in chronic cough, and also to investigate the efficacy of other neuro-modulators, such as Amitriptyline and Pregabalin.

References:

1. Ryan NM, Birring SS, Gibson PG. Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial. Lancet 2012 November 3;380(9853):1583-9.

2. Birring SS, Fleming T, Matos S, Raj AA, Evans DH, Pavord ID. The Leicester Cough Monitor: preliminary validation of an automated cough detection system in chronic cough. Eur Respir J 2008 May;31(5):1013-8.

3. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MD, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). Thorax 2003 April;58(4):339-43.

Relevance of aromatic volatile substances (TRPM8 agonists) in the management of cough

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Cough is a troublesome symptom the most frequently experienced in upper airway diseases. In acute respiratory diseases cough plays important role of protective reflex. From the point of airway physiology it should not be desirable to suppress it. Anyhow, patients complaining about dry cough, chest pain while coughing, nocturnal cough, or simply coughing to the extend disturbing their everyday activities frequently seek a consultation.

Many over-the-counter medications are available to relieve upper airway symptoms and/or cough. Many of them contain thyme extract, menthol or cineole. Menthol, as a part of "natural" medicine was accepted by western culture in the beginning of 19th century, but for more than hundreds years had been using in traditional Chinese medicine for its cooling, counter irritating and local anesthetic effects *(Belvisi & Gepetti, 2004)*. Herbal products are frequently used empirically however their efficacy and mechanism of action are not always supported by objective evidence. It is also difficult to predict molecule specific actions, as these products (herbal extracts) are frequently mixtures of more components.

At the molecular level, action of menthol has been ascribed to activation of TRPM8 - the member of the transient receptor potential superfamily of ion channels. TRPM8 is the ion channel activated by cold, l-menthol and other molecules with cooling effect. It is expressed in a subset of small diameter sensory neurons which constitute a functionally distinct population specifically dedicated to innocuous cold and menthol sensing *(Mc Kemy et al., 2002)*.

Menthol, cineole and thyme extract are believed to relieve upper airway symptoms and cough, but the literature shared conflicting evidence about the antitussive effect.

Some authors were reporting antitussive effect of menthol *(Morice et al., 1999)*, while the others documented no effect of inhaled (-) menthol on the cough reflex in children *(Kenia et al., 2008)* or patients pre-treated by (-) menthol prior bronchoscopy *(Haidl et al., 2001)*.

Later, experiments on guinea pigs showed that menthol has significant antitussive effect and further studies on anaesthetized animals with separated upper and lower airways showed, that menthol effect on cough is mediated via trigeminal afferents expressing TRPM8 ion channel. Menthol vapors delivered selectively to the lower airways had no effect on cough induced in experimental set up. These results are in agreement with the data describing expression pattern of TRPM8 channel along the respiratory tract mucosa *(Plevkova et al., 2013)*.

Intranasal administration of (-) menthol, (+) menthol, 1,3 – cineole and thymol reduced the cough threshold, the urge-to-cough and cumulative count of coughs obtained in standardized capsaicin tests in volunteers, supporting this hypothesis *(Buday et al., 2012; Gavliakova et al., 2013)*. Study of other group also showed that menthol decreases cough threshold in humans *(Wise et al., 2012)*. Very important data were obtained in a study which involved also subjects with chronic cough where inhalation of 0.5% and 1% menthol, but no placebo increased the cough threshold and inspiratory flows in these subjects *(Millquist et al., 2013)*.

Herbal products appear to be effective in suppression of cough in subjects with acute cough due to upper respiratory tract infections, also in subjects with chronic cough and subjects with different types of airway irritation. TRPM8 positive sensory neurons innervating the upper airways play important role in this down-regulation of cough and this information is broadly used in a way of administration of nasal drops, sprays, chest rubs and aromatherapy.

Adverse effects of mentholated products were reported mainly in children younger than two years, but they were likely a consequence of misuse and not respecting contraindications of the product rather than menthol “toxicity”.

Mentholated products may interfere with warfarin medication. The mechanism may be related to the potential for menthol to affect the cytochrome P450 system as an inducer and inhibitor of certain isoenzymes that would potentially influence the metabolism of warfarin.

References:

1. Belvisi MG & Geppetti P: Current and future drugs for the treatment of chronic cough. Thorax 2004.

2. Morice et al. Effect of inhaled menthol on citric acid induced cough in normal subjects. Thorax 1999.

3. McKemy DD: How cold is it? TRPM8 and TRPA1 in molecular logic of cold sensation. Mol Pain 2005.

4. Kenia P et al. Does inhaling menthol affect nasal patency or cough? Pediatric Pulmonology 2008.

5. Haidl P et al. Does the inhalation of 1% l- menthol solution in the premedication of fibreoptic bronchoscopy affect coughing and the sensation of dyspnoe? Pneumologie 2001.

6. Millquist et al. Inhalation of menthol reduces cough sensitivity and influences inspiratory flows in chronic cough. Respir Med. 2013.

7. Plevkova et al. The role of trigeminal nasal TRPM8-expressing afferent neurons in the antitussive effects of menthol. J Appl Physiol 2013.

8. Buday et al. Modulation of cough response by sensory inputs from the nose - role of trigeminal TRPA1 versus TRPM8 channels. Cough. 2012.

9. Wise et al. Sweet taste and menthol increase cough reflex thresholds. Pulm Pharmacol Ther. 2012.

10. Gavliakova et al. Antitussive effects of nasal thymol challenges in healthy volunteers. Respir Physiol Neurobiol. 2013.

Central and peripheral sensitization in migraine pain

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Peripheral sensitization

Brief exposure of the dura to inflammatory agents is capable of activating and sensitizing meningeal nociceptors (first-order trigeminovascular neurons) over a long period of time, rendering them responsive to mechanical stimuli to which they showed only minimal or no response prior to their sensitization. During migraine, such *peripheral* sensitization is likely to mediate the throbbing pain and its aggravation during routine physical activities such as coughing, sneezing, bending over, rapid head shake, holding one's breath, climbing up the stairs, or walking.

Central sensitization

Medullary dorsal horn. Brief stimulation of the dura with inflammatory agents also activates and sensitizes second-order trigeminovascular neurons located in the medullary dorsal horn that receive convergent input from the dura and the skin. In this paradigm, the central trigeminovascular neurons develop hypersensitivity in the periorbital skin, manifested as increased responsiveness to mild stimuli (brush, heat, cold) to which they showed only minimal or no response prior to their sensitization. The induction of central sensitization by intracranial stimulation of the dura, and the ensuing extracranial hypersensitivity was taken to suggest that a similar process occurs in patients during migraine.

Extracranial hypersensitivity during migraine was first noted in 1873 and later documented in the 1950. At that time, extracranial hypersensitivity was ascribed to "hematomas that develop hours after onset of headache as a result of damage to vascular walls of blood vessels such as the temporal artery" (Wolff et al., 1953), or "widespread

distension of extracranial blood vessels or spasm of suboccipital scalp muscles". The current view, however, is that extracranial hypersensitivity is a manifestation of central neuronal sensitization rather than extracranial vascular pathophysiology. Recent quantitative stimulation applied to the surface of the skin showed that pain thresholds to mechanical, heat, and cold skin stimuli decrease significantly during migraine. This skin hypersensitivity, termed cutaneous allodynia, is typically found in the periorbital area on the side of the migraine headache. Patients commonly notice cutaneous allodynia during migraine when they become irritated by innocuous activities such as combing, shaving, taking a shower, wearing eyeglasses or earrings, or resting their head on the pillow on the headache side.

Thalamus. In the course of studying cephalic allodynia during migraine, we unexpectedly found clear evidence for allodynia in remote skin areas outside the innervation territory of the trigeminal nerve. In the discussion of that study, we proposed that ipsilateral cephalic allodynia is mediated by sensitization of dura-sensitive neurons in the medullary dorsal horn because their cutaneous receptive field is confined to innervation territory of the ipsilateral trigeminal nerve (Burstein et al., 1998; Craig and Dostrovsky, 1991; Davis and Dostrovsky, 1988; Ebersberger et al., 1997; Strassman et al., 1994; Yamamura et al., 1999) and that extracephalic allodynia must be mediated by neurons that process sensory information they receive from all levels of the spinal and medullary dorsal horn. Our search of such neurons focused on the thalamus since an extensive axonal mapping of sensitized trigeminovascular neurons in the spinal trigeminal nucleus revealed distinguish projections to the posterior (PO), the ventral posteromedial (VPM) and the sub-parafascicular (PF) nuclei.

In 2010 we reported that topical administration of inflammatory molecules to the dura sensitized thalamic trigeminovascular neurons that process sensory information from the cranial meninges *and* cephalic and extracephalic skin. Sensitized thalamic neurons developed ongoing firing and exhibited hyper-responsiveness and hypersensitivity to mechanical and thermal stimulation of extracephalic skin areas.

Relevant to migraine pathophysiology was the finding that in such neurons, innocuous extracephalic skin stimuli that did not induce neuronal firing before sensitization became as effective as noxious stimuli in triggering large bouts of activity after sensitization was established.

To understand better the transformation of migraine headache into widespread, cephalic and extracephalic allodynia, we also studied the effects of extracephalic brush and heat stimuli on thalamic activation registered by fMRI during migraine in patients with whole-body allodynia. Functional assessment of blood oxygenation level-dependent (BOLD) signals showed that brush and heat stimulation at the skin of the dorsum of the hand produced larger BOLD responses in the posterior thalamus of subjects undergoing a migraine attack with extracephalic allodynia than the corresponding responses registered when the same patients were free of migraine and allodynia.

Temporal aspects of sensitization and their implications to triptan therapy. Central sensitization can be either activity-dependent or activity-independent. The induction of sensitization in second-order trigeminovascular neurons, using chemical stimulation of the rat dura, is activity dependent, as evidenced by lidocaine blockade of afferent inputs from the dura. Once established, however, sensitization of the second-order trigeminovascular neurons becomes activity-independent, as it can no longer be interrupted by lidocaine on the dura. Translating theses findings in the context of migraine with allodynia, it appears that central sensitization depends on incoming impulses from the meninges in the early phase of the attack, and maintains itself in the absence of such sensory input later on. This view is strongly supported by the effects of the anti-migraine 5-$HT_{1B/1D}$ agonists, known as triptans, on the induction and maintenance of central sensitization in the rat, and the corresponding effects of early and late triptan therapy on allodynia during migraine. In the rat, triptan administration concomitant with chemical irritation of the dura effectively prevents the development of central sensitization. Similarly, treating patients with triptans early, within 60 min of the onset of migraine, effectively blocks the development of cutaneous allodynia.

However, neither central neuronal sensitization in the rat, nor cutaneous allodynia in patients, can be reversed by late triptan treatment (2 hours after the application of sensitizing agent to the dura in the animal model, and 4 hours after the onset of migraine in allodynic patients). Most importantly, central sensitization appears to play a critical role in the management of migraine headache of allodynic patients. While non-allodynic patients can be rendered pain-free with triptans at any time during an attack, allodynic patients can be rendered pain-free only if treated with triptans early in the attack, namely, before the establishment of cutaneous allodynia (Burstein et al., 2004).

Modulation of central sensitization. A growing body of evidence suggests that migraine patients are mostly non-allodynic during the first years of their migraine experience, and are eventually destined to develop allodynia during their migraine attacks. It is therefore possible that repeated migraine attacks over the years have cumulative adverse consequences on the function of the trigeminovascular pathway, one of which is susceptibility to develop central sensitization. The threshold for a central trigeminovascular neuron to enter a state of sensitization depends on the balance between incoming nociceptive signals and their modulation by spinal and supraspinal pathways. Many of the modulatory supraspinal pathways converge on the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM). Recent imaging studies have shown that the PAG is activated during migraine and that it is deposited with abnormally high levels of iron in patients with a long history of migraine. Abnormal PAG functioning can either enhance activity of RVM neurons that *facilitate* pain transmission in the dorsal horn, or suppress activity of RVM neurons that *inhibit* pain transmission in the dorsal horn. This may enhance excitability and, therefore, promote responses of second-order trigeminovascular neurons to incoming nociceptive signals from the meninges, resulting in a reduced threshold for entering a state of central sensitization.

References:

1. Strassman AM, Raymond SA, Burstein R. 1996. Sensitization of meningeal sensory neurons and the origin of headaches. Nature 384(6609):560-564.

2. Burstein R, Yarnitsky D, Goor-Aryeh I, Ransil BJ, Bajwa ZH. 2000. An association between migraine and cutaneous allodynia. Annals Neurol 47:614-624.

3. Burstein R, Jakubowski M. 2004. Analgesic triptan action in an animal model of intracranial pain: A race against the development of central sensitization. Ann Neurol 55(1):27-36.

4. Burstein R, Jakubowski M, Collins B. 2004. Defeating migraine pain with triptans: A race against the development of cutaneous allodynia. Ann Neurol 55(1):19-26.

5. Levy D, Jakubowski M, Burstein R. 2004. Disruption of communication between peripheral and central trigeminovascular neurons mediates the antimigraine action of 5HT 1B/1D receptor agonists. Proc Natl Acad Sci101(12):4274-4279.

6. Burstein R, Jakubowski M, Garcia-Nicas E, Kainz V, Bajwa Z, Hargreaves R, Becerra L, Borsook D. 2010. Thalamic sensitization transforms localized pain into widespread allodynia. Ann Neurol 68(1):81-91.

Cough and vomiting: commonalities and differences

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Cough and emesis are primary mechanisms for the protection of the airways and gastrointestinal (GI) tract, respectively. Both processes serve the removal of material that may impede the flow of air or cause toxic damage of the alimentary canal or whole organism. Cough and vomiting are accomplished by neuronal reflex that share remarkable commonalities, given that they are initiated by vagal afferent neurons and coordinated by a cough pattern generator and emetic centre, respectively, in the brainstem (1,2). There are also close similarities in the efferent reflex arc which governs the activity of the striated muscles of the diaphragm, thorax and abdomen. These physiological commonalities and the fact that heavy cough attacks my trigger vomiting, whereas gastro-oesophageal reflux may give rise to cough (3), advocate a systematic comparison of the mechanisms of cough and emesis. The common physiological attributes of the two reflexes do not extend, however, to the pharmacological management of cough and vomiting, although emerging evidence suggests that both reflex circuits express common molecular targets to be used in novel therapeutic approaches (1,2,4,5).

Vomiting is typically preceded by a prodromal phase that involves nausea, a very special form of visceral pain that is particularly unpleasant if it persists for a longer time (6). Nausea is associated with sympathetic vasoconstriction and tachycardia and parasympathetic salivation and hypersecretion, which cause pallor and cold sweat. In addition, the gastric musculature is relaxed while the duodenum undergoes retrograde peristalsis to collect the vomit in a flaccid stomach. Nausea is accompanied by an urge to vomit and, if emesis is triggered, the stomach stays relaxed and the lower oesophageal sphincter opens, enabling the forceful contraction of abdominal and intercostal muscles and the

diaphragm to expel the gastric contents. A shortening of the oesophagus and retrograde oesophageal contractions add to the clearance of material remaining in the oesophagus.

The vagal afferents mediating the emetic reflex comprise both chemo- and mechanosensitive nerve fibres (1). 5-Hydroxytryptamine (5-HT) released from enterochromaffin cells takes a special place as it excites abdominal vagal afferents by binding to 5-HT_3 receptors on these nerve fibres (4). The emetic centre in the brainstem composed of the nucleus tractus solitarii, Bötzinger complex and vagal motor nuclei also receives inputs from other areas, notably the chemoreceptor trigger zone in the area postrema, vestibular apparatus, limbic system and cortex. Substance P acting via NK_1 receptors in the emetic centre within the blood-brain barrier appears to be a major transmitter of the emetic reflex, given that in animal models NK_1 receptor antagonists suppress virtually any kind of emesis (1).

Although cough is a primary mechanism of protection for the airways, non-productive, inappropriate and excessive coughing is a symptom of many airway diseases (2). The coughing reflex can also be preceded by an urge to cough and, like the emetic reflex, is mediated by mechano- and chemosensitive vagal afferents (2,5). The process of coughing is composed of 3 phases: (i) inspiration, (ii) compression caused by contraction of abdominal and intercostal muscles and the diaphragm while the glottis is closed, and (iii) expiration following the sudden opening of the glottis. The cough pattern generator in the brainstem and pons, involving the nucleus tractus solitarii, Bötzinger complex and ventral respiratory group, receives inputs not only from vagal afferent neurons in the airways, but also from auricular vagal afferents, glossopharyngeal and trigeminal afferents as well as higher cerebral areas. Unlike the emetic reflex, the cough reflex can be voluntarily triggered. Oesophageal vagal afferents may also feed into the cough generator, given that reflux into the lower oesophagus can elicit cough without any sign of microaspiration (3).

While cough reflex sensitization occurs under conditions of inflammation both at the level of the afferent cough receptors and the central cough circuit (2), there is less information on how chronic states of nausea and emesis (e.g., in pregnancy or postoperatively) are brought about. While the mainstay of antiemetic treatment rests on 5-HT_3, NK_1, dopamine D_2, histamine H_1 and muscarinic acetylcholine receptor antagonists and dexamethasone, these drugs have currently limited relevance for cough treatment. In the experimental setting, however, both NK_1 and NK_2 receptor antagonists as well as TRPV1 and TRPA1 channel blockers have antitussive properties. Agonists at μ-opioid receptors which are important antitussive drugs cannot be used as antiemetics because they may themselves cause vomiting. In contrast, cannabinoid receptor agonists inhibit both the cough and emetic reflex.

These pharmacological disparities indicate that the reflexes underlying vomiting and cough are distinct in their neuronal organization and molecular phenotype. On the other hand, they both serve a protective role at the vital entries for food and oxygen and accomplish this task by a remarkably similar clearance process.

Unfortunately, nausea, vomiting and cough occur in many conditions in which they have lost their protective role, and the unmet challenge to control these pathologically triggered reflexes may benefit from a comparative analysis of cough and emesis.

References:

1. Andrews PLR, Rudd JA. The role of tachykinins and the tachykinin NK1 receptor in nausea and emesis. Handb Exp Pharmacol 2004;164:359-440.

2. Nasra J, Belvisi MG. Modulation of sensory nerve function and the cough reflex: understanding disease pathogenesis. Pharmacol Ther 2009;124:354-375.

3. Smith JA, Abdulqawi R, Houghton LA. GERD-related cough: pathophysiology and diagnostic approach. *Curr Gastroenterol Rep* 2011;13:247-256.

4. Johnston KD, Lu Z, Rudd JA. Looking beyond 5-HT_3 receptors: a review of the wider role of serotonin in the pharmacology of nausea and vomiting. Eur J Pharmacol 2014;722:13-25.

5. Canning BJ. Afferent nerves regulating the cough reflex: mechanisms and mediators of cough in disease. Otolaryngol Clin North Am 2010;43:15-25.

6. Sanger GJ, Andrews PLR. Treatment of nausea and vomiting: gaps in our knowledge. Auton Neurosci 2006;129:3-16.

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