Accepted Manuscript

Central mechanisms of airway sensation and cough hypersensitivity

Alexandria K. Driessen, Alice E. McGovern, Monica Narula, Seung-Kwon Yang, Jennifer A. Keller, Michael J. Farrell, Stuart B. Mazzone

PII: S1094-5539(17)30016-0
DOI: 10.1016/j.pupt.2017.01.010
Reference: YPUPT 1584

To appear in: Pulmonary Pharmacology & Therapeutics

Received Date: 12 January 2017
Accepted Date: 25 January 2017


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Central mechanisms of airway sensation and cough hypersensitivity

Alexandria K. Driessen\textsuperscript{1}, Alice E. McGovern\textsuperscript{1}, Monica Narula\textsuperscript{2}, Seung-Kwon Yang\textsuperscript{2}, Jennifer A. Keller\textsuperscript{2}, Michael J. Farrell\textsuperscript{3} and Stuart B. Mazzone\textsuperscript{1*}

\textsuperscript{1}Department of Anatomy and Neuroscience, The University of Melbourne, Australia

\textsuperscript{2}School of Biomedical Sciences, The University of Queensland, Australia

\textsuperscript{3}Biomedicine Discovery Institute and Department of Medical Imaging and Radiation Sciences, Monash University, Australia

*Author for correspondence: Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, Melbourne, VIC 3010, Australia

Email: stuart.mazzone@unimelb.edu.au

Phone: +61 3 8344 6457

Funding: NHMRC of Australia #1078943
Abstract

The airway sensory nervous system is composed of two anatomically distinct processing pathways that allow for the production of respiratory reflexes and voluntary evoked respiratory behaviours in response to sensing an airway irritation. Disordered sensory processing is a hallmark feature of many pulmonary disorders and results in the development of cough hypersensitivity syndrome, characterised by chronic cough and a persistent urge-to-cough in affected individuals. However, the mechanism underpinning how the airway sensory circuits become disordered, especially at the level of the central nervous system, is not well understood. In this mini-review we present well-defined mechanisms that lead to the development of chronic pain as a framework to explore the evidence that cough disorders may manifest due to neuroplasticity and sensitisation of important components of the airway sensory circuitry in the brain. We highlight recent discoveries of how airway sensory processing occurs in the brain in health and disease and additionally suggest areas where gaps exist in our current knowledge on the topic, with the goal of providing a better understanding of how airway circuits become dysfunctional in disease. This may in turn help identify novel therapeutic targets for restoring normal airway sensory processing and alleviating excessive cough.

Keywords: Central sensitization; Vagal afferents; Brainstem; Descending inhibition; Neuroinflammation; fMRI
1.0 Introduction

The airways are innervated by heterogeneous populations of jugular and nodose vagal ganglia sensory neurons that respond to a wide range of chemical and mechanical stimuli. Activation of these sensory neurons results in the transmission of information about the airway environment, firstly to the brainstem and then to higher order brain regions, allowing for the production of both respiratory and autonomic reflexes, as well as more complex behavioural respiratory responses. Collectively, these reflexes and respiratory behaviours serve to protect the airways from endogenous or exogenous stimuli that are potentially damaging and by doing so they contribute to the maintenance of adequate physiological respiratory function. The characterisation and physiology of airway vagal afferent neurons has been reviewed in detail, as has the neuroanatomical organisation of airway vagal afferent pathways in the brain (Widdicombe, 2001, Carr and Undem, 2003, Canning et al., 2006, Driessen et al., 2016, Mazzone and Undem, 2016), and as such the present review will only briefly cover these topics. Rather, we will focus on plasticity in the central neural circuitry involved in airway sensory processing and the current understanding of how this may provide insight into the airway sensory dysfunctions that underpin two prominent and related clinical presentations in pulmonary disease; excessive coughing and the urge-to-cough. In doing so, we will present some original data and draw from the advanced work that has been conducted in a comparable area of research (chronic pain), with the aim of highlighting important gaps in our current understanding of central cough neurophysiology.
2.0 Neuroanatomy of airway sensation

The central representation of jugular and nodose airway afferents has been investigated using a variety of physiological and neuroanatomical techniques (McGovern et al., 2012a, McGovern et al., 2012b, Driessen et al., 2015, McGovern et al., 2015a, McGovern et al., 2015b). Such studies have described in detail a primary termination site for airway vagal afferents in the brainstem nucleus of the solitary tract (nTS) and not surprisingly this nucleus has underpinned a substantial body of work on airway sensory neurotransmission (Katz and Karten, 1983, Kubin et al., 1991, Panneton, 1991, Ranson et al., 1995, Mazzone and Canning, 2002, Alheid et al., 2011, Zoccal et al., 2014). However, our own tracing studies from the airways using a novel Herpes Simplex Virus 1 strain H129 (HSV1 H129) anterograde transynaptic tracer recently described a second primary termination site for airway vagal afferents in the brainstem paratrigeminal nucleus (Pa5) (McGovern et al., 2012a, McGovern et al., 2015a, McGovern et al., 2015b). The Pa5 is a small obscure region of brainstem neurons located within the tract of the medullary spinal trigeminal nucleus, previously described as playing a role in somatic nociception, but not recognised for playing a role in airway sensation (Phelan and Falls, 1989; Lapa and Watanabe, 2005; Koepp et al., 2006).

Using conventional retrograde neuroanatomical tracing from the nTS and Pa5 we have further shown that the nodose and jugular vagal ganglia have distinct and specific projections to these two termination sites (Driessen et al., 2015, McGovern et al., 2015b). Thus, the nodose vagal ganglia display terminations only in the nTS, while the jugular vagal ganglia predominately project to the Pa5 (Driessen et al., 2015, McGovern et al., 2015b). This observation is striking and coincides with the reported distinct embryological origins of nodose and jugular neurons – the former derived from the epibranchial placodes and the latter from the somatic neural crest. This anatomical
segregation of nodose and jugular afferent processing at the level of the brainstem is conserved in the ascending higher brain pathways, inasmuch as a comparison of airway specific projections arising from the nTS and Pa5 showed that nodose-nTS afferents project largely to regions previously identified in autonomic and limbic/paralimbic pathways, perhaps representing a central viscerosensory processing circuit in the brain, while the jugular-Pa5 pathway projects largely to regions involved in somatic nociceptive (somatosensory) processing (McGovern et al., 2015b). This notion of multiple cortical circuits involved in airway sensations has been supported by studies in humans using functional brain imaging (Farrell et al., 2012; Ando et al., 2014; Farrell et al., 2014), however whether this relates to the anatomical segregation of airway afferent processing observed in animal models remains unclear.

These data clearly argue for two anatomically distinct airway sensory processing pathways that may form an anatomical framework for functional distinctions between respiratory reflex production and the conscious perception of airway irritations. For example, reflexive cough is a brainstem-mediated process that requires sensory input into the nTS and subsequent alterations to the activity of respiratory cells located in nuclei of the ventral and pontine respiratory groups (Baekey et al., 2003, Poliacek et al., 2009, Mazzone et al., 2011, Smith et al., 2013, Dutschmann et al., 2014, Koshiya et al., 2014, Zoccal et al., 2014, Ferreira et al., 2015, Wang et al., 2015). On the other hand, the central mechanisms that underpin the conscious perception of airway sensations leading to voluntary respiratory behaviours are less well understood and could conceivably be linked to the understudied somatic representation of airway sensation in the brain that is seemingly carried by the jugular-Pa5 circuitry. Although this speculation awaits empirical evidence, it provides intriguing possibilities for
interventional regulation of airway sensations without disturbing fundamental protective reflexes. It also provides unexplored possibilities by which airway sensory processes may become disordered in disease.

3.0 Can airway afferent circuits in the brain be altered in disease?

*Neuronal sensitization.* Disordered airway sensory neural circuit activity, precipitated (for example) by the inflammatory processes induced by acute viral or allergen exposure or that associated with chronic illness in asthma or other respiratory diseases, contribute to excessive coughing and the increased perception of airway irritation common to many pulmonary disorders (Chung et al., 2013, Ando et al., 2014, Hilton et al., 2015, Ando et al., 2016, Zaccone et al., 2016). As such, it has become increasingly important to understand how airway sensory circuits are altered in these disease states, a topic that has been investigated in some detail with respect to the primary afferent neurons themselves (peripheral sensitisation) but largely unexplored in terms of the central neural circuits in receipt of airway sensory inputs (central sensitisation).

Surprisingly, the notion of central sensitisation as a mechanism of airway sensory hypersensitivity has been widely presented in the literature without substantive evidence for central circuit plasticity or even a well-presented framework for what could be expected if airway sensory circuits in the brain undergo disease-induced changes. Such a framework can be laid down by looking at other nociceptive systems where the mechanisms of central neural plasticity have been extensively investigated. Perhaps the best example of this is in the chronic pain field where the inflammatory and neuropathic mechanisms leading to changes in central circuit connectivity, neuronal excitability and gene expression are well described (Latremoliere and Woolf, 2009, Undem et al., 2015, Bettini and Moore, 2016).
The similarities between somatic and airway afferent neurophysiology supports the notion that common mechanisms may underlie the development of pain and cough hypersensitivities (Mazzone et al., 2009, Ji, 2015, Undem et al., 2015, Ando et al., 2016). Indeed, chronic pain and cough are characterised by common phenotypes that relate to underlying inflammatory and/or neuropathic processes (Latremoliere and Woolf, 2009, Chung et al., 2013, Ji, 2015). These include hyperalgesia/hypertussia (increased pain/cough sensitivity to noxious stimuli), allodynia/allotussia (increased pain/cough responsivity to innocuous stimuli) and paraesthesia (abnormal sensations such as numbness or tingling in the affected tissue) (Latremoliere and Woolf, 2009, Chung et al., 2013, Ji, 2015). In chronic pain, these phenotypes occur due to peripheral and/or central sensitisation processes (Latremoliere and Woolf, 2009). Peripheral sensitisation occurs following tissue damage, whereby the release of inflammatory mediators alters sensory neuron excitability and gene expression within the sensory neuron cell bodies and at their peripheral terminals (Latremoliere and Woolf, 2009, Bettini and Moore, 2016). This sensitisation is restricted to a localised area of damage and drives hypersensitivities at the injury site (Latremoliere and Woolf, 2009, Bettini and Moore, 2016). The evidence for peripheral sensitisation in chronic cough has been reviewed elsewhere and won’t be repeated here (Undem et al., 2015; Zaccone and Undem, 2016; Mazzone and Undem, 2016).

*Second order neuron plasticity.* Persistent sensory nerve input to the central nervous system can induce alterations in central neural circuits by changing second order sensory neuron excitability and their projection profiles, although in the case of nerve injury central sensitisation can be induced without prior peripheral sensitisation.
This allows for an uncoupling of responses to any direct noxious stimulation and thus these processes are important for the development of secondary hyperalgesia (hypersensitivity outside of the injury zone) and allodynia (Latremoliere and Woolf, 2009). The induction and maintenance of centrally sensitised pain states depends on both acute and chronic processes, initially at the level of the primary afferent synapse in the spinal dorsal horn (Bettini and Moore, 2016). Alterations that occur in the acute phase are remarkably similar to those seen in peripheral sensitisation, except that they affect second order sensory neurons and their projections throughout the central nervous system (Latremoliere and Woolf, 2009, Cornelison et al., 2016, Liang et al., 2016). That is the release of inflammatory cytokines, neurotrophins and neuropeptides drives hyperexcitability in second order sensory neurons (Tao et al., 2014, Bettini and Moore, 2016). Collectively these processes increase the excitability of the post-synaptic neurons in the spinal dorsal horn and therefore lead to increased activation of neurons elsewhere in key pain processing regions such as the raphe nuclei in the brainstem and the anterior cingulate and somatosensory cortices in the higher brain (Li et al., 2014, Tao et al., 2014, Liang et al., 2016, Potter et al., 2016). This enhanced excitability is largely driven by increased synaptic delivery of ion channels, transporters, neuropeptides and vesicle release machinery that are necessary to facilitate and strengthen glutamatergic transmission (Latremoliere and Woolf, 2009, Tao et al., 2014, Radwani et al., 2016).

With respect to cough, studies have shown that airway afferent second order neurons in the brainstem can similarly become hyperexcitable (Mutoh et al., 2000, Mazzone and Canning, 2002, Mazzone et al., 2005, Kline et al., 2007, Sekizawa et al., 2008, Undem et al., 2015). More specifically, exposure of the airways to environmental pollutants,
such as cigarette smoke, is known to increase excitability of nTS neurons with the neuropeptide substance P playing an essential role in driving increased synaptic neurotransmission (Mutoh et al., 2000, Sekizawa et al., 2008). Consistent with this, the activation of pulmonary C-fibre afferents (many of which express neuropeptides) acutely enhances mechanoreceptor (glutamatergic) reflexes via a brainstem neuropeptide-dependent mechanism (Mazzone and Canning, 2002, Mazzone et al., 2005). While glutamatergic and ionic changes may also be directly altered, it is apparent that dysregulation of neuropeptide expression and transmission is a key component in the development of central sensitisation of airway sensory circuits at the level of the sensory integration nuclei in the brainstem. Whether jugular afferent pathways similarly drive neuropeptide-dependent sensitisation of second order Pa5 neurons has not yet been studied. However, pulmonary infections induced by pneumovirus exposure in mice results in elevated neuronal activity within the Pa5 (Mazzone et al., unpublished data), suggesting that there is persistent sensory input to this region as a consequence of airway inflammation.

**Glia and neuroinflammation.** The transition from acute to chronic central sensitisation includes activation of glia that induce a state of spinal neuroinflammation, which may reflect the persistent or altered primary sensory neuron input to the central nervous system that promotes the development of central neuroinflammation (Gwak et al., 2012, Ikeda et al., 2012, Trang et al., 2012, Chen et al., 2014, Gu et al., 2016, Lei et al., 2016, Liu et al., 2016). Therefore, both peripheral nerve injury and inflammation can upregulate the expression of glia, as marked by increased expression of CD11b (OX-42) or ionized calcium binding adaptor molecule 1 (Iba1) for microglia activation and Glial Fibrillary Acidic Protein (GFAP) for astrocyte activation, in both sensory ganglia
themselves and in the central nervous system (Ikeda et al., 2012, Romero et al., 2013, M’Dahoma et al., 2015, Liu et al., 2016, Lei et al., 2016). More importantly, pharmacological inhibition of glia has been shown to largely abolish enhanced neuronal excitation in models of chronic pain, suggesting that glial cells are the primary drivers of the neuronal hyperexcitability underpinning painful phenotypes (Ikeda et al., 2012). Interestingly, in a model of nerve injury, spinal dorsal horn neurons become hyperexcitable and this coincides with an increase in connexion-43 expression in astrocytes (Chen et al., 2014). Connexin-43 is a gap junction protein that facilitates glia-neuron cross talk, and when inhibited leads to suppression of neuronal excitation, suggesting that more complex interactions between activated glia and neurons after injury may further enhance the neuroinflammatory state (Chen et al., 2014). Since glia are not electrically active they interact with neurons via neurotransmitter release, such as cytokines or other mediators, and in line with this there is a growing body of evidence that suggests adenosine tri-phosphate (ATP), chemokine CXCL12, interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNFα) and/or Brain Derived Neurotrophic Factor (BDNF) may be the primary mediators released to maintain a state of central sensitisation after peripheral injury and inflammation (Henn, 1976, Guthrie et al., 1999, Fields and Burnstock, 2006, Ulmann et al., 2008, Trang et al., 2011, Trang et al., 2014, M’Dahoma et al., 2015, Bai et al., 2016, Gu et al., 2016, Lalo et al., 2016). Furthermore, the neuroinflammatory state in chronic pain is known to persist due to epigenetic changes to second order sensory neurons, including histone modifications on important genes including BDNF (Crow et al., 2013, Tao et al., 2014).

There is a paucity of data to indicate whether vagal ganglia or the brainstem processing sites of airway afferents display glial activation in models of airway inflammation.
However, recent studies may support this notion (Chounlamountry et al., 2015, Spaziano et al., 2015). Allergen sensitisation as well as ozone exposure cause gliosis within the nTS, and it has been speculated that this upregulation plays a role in airway hyper-responsiveness (Chounlamountry et al., 2015, Spaziano et al., 2015). Gliosis at glutamatergic synapses may indicate that the activation of glia could strengthen glutamatergic transmission and in turn drive neuronal hyper-excitability in second order neurons (Chounlamountry et al., 2015). In our own studies, neonatal mice infected with murine pneumovirus demonstrate a modest increase in Iba1 expression (microglial cells) and a robust increase in GFAP expression (astrocytes), along with increased cytokine expression, in the region of the nTS (Figure 1). This gliosis may reflect both migration and proliferation of glia cells given that we also noted an increase in the cell proliferation marker PCNA at this site (Figure 1). Correspondingly, influenza infection alters nTS neuron resting membrane potential and baseline action potential firing, consistent with a centrally sensitised state (Figure 1). Nevertheless, more extensive studies still need to be conducted to better understand the role of activated glia in altering airway sensory neurons but data to date suggests that it is likely an avenue worth investigating to better understand the role of central neural plasticity in respiratory disease.

FIGURE 1 HERE
Figure 1. Brainstem neuroinflammation following respiratory tract viral infection in mice. Central sensitisation in chronic pain is typically underpinned by glial cell activation and central inflammation, which contributes to synaptic plasticity and neuronal hyperresponsiveness. (A) 7 day old neonatal mice infected with an intranasal inoculation of 5 plaque forming units of pneumovirus show increased immunostaining density for the glial markers GFAP (astrocytes) and IBA1 (microglia) 3-10 days post-inoculation (DPI) in the nucleus of the solitary tract. Virus induced increase in the number of cell nuclei expressing the proliferation marker PCNA is consistent with glial cell expansion at this site (data represent the mean ± SEM of 5-7 animals per group). (B) Consistent with this, pneumovirus infected mice have elevated transcripts for the inflammatory mediators Tumor Necrosis Factor alpha (TNFα) and Interleukin 1β in brainstem homogenates (quantitative PCR, mean ± SEM fold change expression over β-actin, n=3 per group). (C) Representative patch clamp electrophysiological recording of two neurons in nucleus of the solitary tract in brainstem slices of two mice, one inoculated intranasally 7 days earlier with vehicle (VEH, lower trace) and the other inoculated with 103 plaque forming units of influenza Pr8 strain (FLU, upper trace). Of note is the more depolarised resting membrane potential and induction of spontaneous action potential discharge after viral infection. Mean data show an average 40mV shift in the resting membrane potential of nucleus of the solitary tract neurons after infection (mean ± SEM resting membrane potential of 10 neurons per group recorded from 3-4 separate preparations per treatment). *, P<0.05 significantly different to vehicle inoculated (VEH) controls.

*Higher level plasticity. Plasticity and sensitisation following peripheral injuries and inflammation are not confined to second order neurons in primary afferent processing sites, but rather can occur at all levels of the central sensory processing circuit. In this
regard, it is interesting to note that unlike the data described above, which is almost
exclusively obtained from animal studies, higher brain plasticity in sensory
hypersensitivities has been studied in both animals and humans. Indeed, functional
magnetic resonance imaging (fMRI) has uniquely allowed higher brain physiological
sensory circuits to be studied in humans in both healthy and disease states (Farrell et al.,
2005, Mazzone et al., 2009, Wager et al., 2013, Farrell and Mazzone, 2014, Hodkinson et al.,
2015, Ando et al., 2016, Flodin et al., 2016). The available evidence would
suggest that sensory hypersensitivity can result from both an enhanced activity of the
brain regions encoding sensation as well as dysfunctional responses in the brain circuits
that ordinarily provide descending control over primary afferent processing (the
descending analgesia system).

In patients with chronic pain, fMRI studies have reported that there is an increased
functional connectivity at most levels of the higher order pain circuit (Zambreanu et al.,
2005, Hodkinson et al., 2015, Flodin et al., 2016). A region of particular importance in
this circuit is the somatosensory cortex where the conscious perception of sensory
stimulation for each body part is mapped onto a restricted cortical region (Endo et al.,
2007). fMRI studies in rodents have shown that in models of chronic pain the integrity
of the primary somatosensory cortex organisation is lost and instead activity extends
beyond the boundaries of the affected region resulting in an increased receptive field
and hyperalgesia (Endo et al., 2007, Potter et al., 2016). Although these changes may
result due to increased afferent input, local mechanisms within the primary
somatosensory cortex have also been identified in rodent models of chronic pain. These
include upregulation of excitatory synapses by significantly increased vesicular
glutamate transporter 1 (VGlut1) synaptic density as well as activation of local
astrocytes (Kim et al., 2016, Potter et al., 2016). These profound alterations drive an excitatory-inhibitory imbalance allowing for dysfunctional pain processing to persist. Similarly, imaging studies in humans with painful trigeminal neuropathy show signs of decreased grey matter volume and blood flow to the primary somatosensory cortex as well as the thalamus (Henderson et al., 2013). These changes represent functional cortical plasticity as they are correlated with reported increased ongoing pain (Henderson et al., 2013). In line with human imaging studies, the thalamus and subthalamus have also been implicated in rodent models of chronic pain whereby changes in the gating and processing of nociceptive information have been observed (Whitt et al., 2013, Masri et al., 2009). For example, thalamic sensory regulation is in part dependent on tonic inhibitory signals from the zona incerta (a subthalamic nucleus) and after spinal cord injury, neurons of the zona incerta have a decreased firing rate leading to increased thalamic neuron discharge in response to noxious stimulation (Whitt et al., 2013, Masri et al., 2009). Thus, the balance between excitatory and inhibitory control within the pain circuitry is lost and pain signals are processed without appropriate gating allowing for both hyperalgesia and allodynia to result.

A well described descending circuit provides top down control over ascending nociceptive processing, and recent studies suggest that this circuit may also undergo changes in patients with chronic pain. The most extensively studied of these descending circuits is that involved in pain inhibition, also known as the descending analgesia system, largely controlled by a neuronal circuit that originates in the frontal cortex and descends upon the spinal dorsal horn via connections in the midbrain periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) (Boadas-Vaello et al., 2016). The RVM contains both ON and OFF cells, which enhance or inhibit pain respectively
(Boadas-Vaello et al., 2016). ON cells receive inhibitory control from the PAG in order to allow for the firing of OFF cells and it is this balance that ultimately provides endogenous anti-nociceptive benefits (Boadas-Vaello et al., 2016). However, in chronic pain conditions alterations in the PAG change the balance of excitatory to inhibitory control, leading to the development of primary and secondary hyperalgesia (Liu et al., 2010, Hahm et al., 2011, Schwedt et al., 2014, Ho et al., 2015, Boadas-Vaello et al., 2016). For example, spinal nerve ligations in the rat resulted in both altered glutamatergic and GABAergic transmission in the PAG and both the PAG and RVM display signs of neuroinflammation following peripheral injuries, which is presumably involved in the maintenance of a centrally sensitised state (Ho et al., 2015, Zhuang et al., 2016). In particular, models of Parkinson’s disease, where pain is a commonly reported symptom, the dorsal lateral PAG has increased activation of pro-inflammatory cytokines, while microglia have been shown to be activated in the RVM under conditions of peripheral inflammatory pain (Roberts et al., 2009, Zhuang et al., 2016).

In human functional brain imaging studies, experimentally induced hyperalgesia following subcutaneous injections of capsaicin results in a rapid induction of PAG neuronal activity, suggestive that alterations in the descending analgesia pathway plays an essential role in the development of hyperalgesia (Zambreanu et al., 2005). Consistent with this, it has also been shown that these descending pain circuits have altered connectivity in chronic pain conditions that present with allodynia (Schwedt et al., 2014).

Patients with chronic cough may similarly display alterations in their higher brain processing of airway irritant stimuli (Ando et al., 2016). We recently reported that patients with chronic cough demonstrated enhanced urge-to-cough sensitivity during
inhaled capsaicin challenges and that this was associated with elevated activity in midbrain regions known to be involved in descending nociceptive control. Thus, in fMRI studies the PAG and neighbouring cuneiform nucleus showed elevated activity in response to inhaled capsaicin, regardless of whether capsaicin concentrations were the same for healthy and cough participants or if they were tailored to produce equivalent behavioural outcomes in the two groups (i.e. to match the urge-to-cough intensities) (Ando et al., 2016). Remarkably, the midbrain areas activated in our study overlap with the areas active during experimental induction of pain hypersensitivity (Figure 2), strongly suggesting that common central mechanisms are indeed involved in the development or maintenance of cough and pain hypersensitivity. The midbrain, however, was not the only higher brain region demonstrating changes in activity. Cough hypersensitivity patients showed diminished activity compared to healthy controls in parts of a brain network previously implicated in the voluntary suppression of coughing (Mazzone et al., 2011, Farrell et al., 2012; Farrell et al., 2014, Ando et al., 2016). Collectively these findings suggest that altered descending control and/or altered voluntary cough suppression may contribute to excessive coughing and the urge-to-cough in airways disease. However, more research is needed in this area as we don’t know if cough hypersensitivity coincides with any changes in sensory representations in the brain, nor do we understand the mechanisms that precipitate these changes seen with fMRI. Furthermore, as our study described above was conducted by investigating brain responses during inhaled irritant challenges, it will be important to know whether there are any changes in brain activity, connectivity and/or brain morphometry in cough patients at baseline in the absence of exogenous stimuli. In addition, whether current or future antitussive therapies can correct altered brain activity is also unknown.
Figure 2. A common mechanism of central sensitisation in pain and cough: functional brain imaging studies in humans. Left panel, Subjects were treated unilaterally on the right lower leg with a combination of heat and capsaicin to induce hyperalgesia. Subsequent mechanical stimulation of the area evoked increased pain sensations which were associated with unilateral increased neural activity in the midbrain nucleus cuneiformis (reproduced with permission from Zambreanu et al., 2005). Right Panel, Comparable activations in the midbrain nucleus cuneiformis extending into the periaqueductal grey (PAG) in subjects with chronic cough hypersensitivity exposed to inhaled capsaicin challenges (data adapted from Ando et al., 2016). Not shown is an absence of midbrain activity in control subjects, consistent with the midbrain playing a specific role in the development or maintenance of hypersensitivity.

3.0 Concluding remarks
Cough and the urge-to-cough contribute significantly to morbidity in pulmonary diseases and as such understanding the neuronal processes that underpin the development and maintenance of cough hypersensitivity is essential. We now have a reasonably advanced knowledge of the central processing circuits involved in airway sensation that will allow for hypothesis driven investigations of how the brain may contribute to chronic coughing in disease. Although this area of research is still in its infancy, the information that we have presented in this review highlights the likely importance of central plasticity in the development of cough in respiratory disease. Indeed, there is evidence that sensory circuits are subject to disease induced acute
changes in neuronal excitability through alterations in neurotransmission and ion channel activity in the brainstem, and that this enhanced excitability may be maintained and transformed to a chronic neuroinflammatory state by glial cell activation. The net outcome is altered activity of cough regulatory circuits in the brains of patients with chronic cough. However, there are clearly still many gaps in our understanding of the processes and it will be important to define the mechanisms underpinning central sensitisation and plasticity in the airway sensory circuitry as this may help to identify novel therapeutic targets to better treat patients with sensory hypersensitivities associated with respiratory diseases.
References


Figure Legends

Figure 1. Brainstem neuroinflammation following respiratory tract viral infection in mice.

Central sensitisation in chronic pain is typically underpinned by glial cell activation and central inflammation, which contributes to synaptic plasticity and neuronal hyperresponsiveness. (A) 7 day old neonatal mice infected with an intranasal inoculation of 5 plaque forming units of pneumovirus show increased immunostaining density for the glial markers GFAP (astrocytes) and IBA1 (microglia) 3-10 days post-inoculation (DPI) in the nucleus of the solitary tract. Virus induced increase in the number of cell nuclei expressing the proliferation marker PCNA is consistent with glial cell expansion at this site (data represent the mean ± SEM of 5-7 animals per group). (B) Consistent with this, pneumovirus infected mice have elevated transcripts for the inflammatory mediators Tumor Necrosis Factor alpha (TNFα) and Interleukin 1β in brainstem homogenates (quantitative PCR, mean ± SEM fold change expression over β-actin, n=3 per group). (C) Representative patch clamp electrophysiological recording of two neurons in nucleus of the solitary tract in brainstem slices of two mice, one inoculated intranasally 7 days earlier with vehicle (VEH, lower trace) and the other inoculated with 103 plaque forming units of influenza Pr8 strain (FLU, upper trace). Of note is the more depolarised resting membrane potential and induction of spontaneous action potential discharge after viral infection. Mean data show an average 40mV shift in the resting membrane potential of nucleus of the solitary tract neurons after infection (mean ± SEM resting membrane potential of 10 neurons per group recorded from 3-4 separate preparations per treatment). *, P<0.05 significantly different to vehicle inoculated (VEH) controls.
**Figure 2.** A common mechanism of central sensitisation in pain and cough: functional brain imaging studies in humans. Left panel, Subjects were treated unilaterally on the right lower leg with a combination of heat and capsaicin to induce hyperalgesia. Subsequent mechanical stimulation of the area evoked increased pain sensations which were associated with unilateral increased neural activity in the midbrain nucleus cuneiformis (reproduced with permission from Zambreanu et al., 2005). Right Panel, Comparable activations in the midbrain nucleus cuneiformis extending into the periaqueductal grey (PAG) in subjects with chronic cough hypersensitivity exposed to inhaled capsaicin challenges (data adapted from Ando et al., 2016). Not shown is an absence of midbrain activity in control subjects, consistent with the midbrain playing a specific role in the development or maintenance of hypersensitivity.