
Chapter 2

Electrophysiologically Identified Airway Receptors: Main Characteristics

The most generally accepted classification discriminates mechanosensory “slowly adapting (stretch) receptors (SARs)”, “rapidly adapting stretch receptors (RARs)” and chemosensitive “C-fibre receptors (CFRs)” (Sant’Ambrogio 1982; Widdicombe 2001; Carr and Undem 2003; Canning and Mazzone 2005; Yu 2009).

Mechanoreceptive stretch-sensitive airway afferents have been classified, based on their adaptation properties to sustained inflation, into SARs (slowly adapting) and RARs that discharge action potentials upon lung inflation but then rapidly adapt to the stimulus (Sant’Ambrogio and Widdicombe 2001; Schelegle and Green 2001; Schelegle 2003; Widdicombe 2003a). The mechanoreceptors are categorised as myelinated “A-fibres”, conducting action potentials at a fast rate along their axons (about 10–50 m/s).

Chemosensors in the lungs are commonly referred to as “C-fibres”, conducting action potentials at a slow velocity (about 0.3–2 m/s). CFRs can be further subdivided into those situated deep in the lung (pulmonary C-fibres) and those in the conducting airways (bronchial C-fibres). Regardless of their location, nociceptive C-fibres in the airways can typically be stimulated by capsaicin, a pungent ingredient of red hot chilli peppers (Lee and Pisarri 2001; Lee et al. 2003; Lee 2009).

Electrophysiologically identified RARs, SARs and CFRs have been discriminated based on their physical and chemical sensitivity, adaptation to mechanical stimulation, neurochemistry, origin, myelination, conduction velocity, activity during tidal breathing, reflexes associated with their activity, ganglionic location of the neuronal cell body, possible sites of termination in the airways and lungs, and their involvement in expiratory sensations. Extensive studies have resulted in several classification schemes, none of which is able to take all of these features into account (Sant’Ambrogio 1982; Carr and Undem 2003; Widdicombe 2009). Because of the large variety of electrophysiologically identified airway receptors, especially when species differences are taken into account, several receptor subtypes do not seem to readily fit the classical classification scheme.

RARs and SARs, the two primary classes of mainly mechanosensitive airway receptors are differentiated by their adaptation to sustained lung inflation. However, a considerable number of lung mechanoreceptors represent intermediate

types, based on their responses to lung inflation and adaptation indexes (Bergren and Peterson 1993; Burnet and Hilaire 1999; Carr and Undem 2003; Canning and Mazzone 2005; Lee and Undem 2005; Widdicombe 2006). Sensitivity to capsaicin is considered to be the prototypical feature of chemosensitive C-fibre endings, but some airway CFRs are not activated by capsaicin, even at relatively high doses (Ho et al. 2001; Kollarik et al. 2003). In addition, another type of vagal chemosensory nociceptive nerve fibres, originating in the jugular ganglia and conducting action potentials in the A-fibre range, has been identified. Although these receptors show some resemblance to SARs (albeit with a high mechanical threshold) and CFRs, this group of chemosensitive A-fibres does not correspond to any of the well-defined electrophysiological airway receptors. These sensors have been referred to as “high threshold A-receptors (HTARs)” and have been identified *in vivo* and *in vitro* (Widdicombe 2003a; Yu et al. 2006).

The cough reflex is one of the most effective defence functions against inhaled irritants and is induced exclusively via activation of vagal afferents. “Urge-to-cough” is one of the most common respiratory discomforts and symptoms found in airway disease patients (Lee 2009). However, the questions which airway afferents are responsible for eliciting the cough reflex, and whether a true “cough receptor” that initiates these reflex actions actually exists, have been debated for a long time (Undem et al. 2002; Canning et al. 2004, 2006; Mazzone 2004, 2005; Lee and Undem 2005). Today, substantial evidence supports the hypothesis that at least two separate vagal sensory populations of airway afferents are responsible for initiating cough reflexes (Undem and Carr 2010). One is a capsaicin-insensitive, acid-sensitive, myelinated type of nerve that conducts action potentials in the A-range, is limited to the extrapulmonary airways and large bronchi, and mediates cough evoked by aspiration in both conscious and anaesthetised experimental animals and humans. The other is a C-fibre population that, when selectively stimulated, causes an irritating, itchy urge-to-cough sensation in conscious animals and humans only, mimicking sensations associated with inflammatory airways diseases (Undem and Carr 2010; Canning 2011). All of these imply the existence of two parallel pathways for cough, one essential and homeostatic, the other nonessential and pathophysiological (Canning 2011).

The classification of vagal airway afferents is further complicated by substantial species differences in the afferent properties of some airway receptors, as well as by phenotypic switches that these sensory receptors may undergo in certain pathophysiological conditions, such as inflammatory airway disease (Barnes 2001; Schelegle and Green 2001; Lee and Undem 2005; Undem and Kollarik 2005; Undem and Nassenstein 2009; Lieu and Undem 2011).

The present review certainly does not have the intention to reinvent or reorganise the categorising system of the physiologically identified airway receptors, and we will, as much as possible, further on stick to the classical well-accepted divisions based on stereotypical characteristics discriminating RARs, SARs and CFRs.

2.1

Slowly Adapting (Stretch) Receptors

The original designation of slowly adapting receptors or SARs was based on the observation that inflation of the lung increases receptor discharge, and that this increase in discharge essentially does not adapt when lung inflation is maintained (Fig. 2.1) (Knowlton and Larrabee 1946; Widdicombe 1954). Nerve fibres that give rise to SARs correspond to myelinated fibres that conduct action potentials in the A-fibre range (mainly A β and A γ) and have their origin in the nodose ganglion (Jordan 2001; Kubin et al. 2006). Based on the indirect functional evidence, it is believed that SARs are anatomically located within the airway smooth muscle layer in several mammalian species, including humans (Larsell 1921; Larsell and Dow 1933; von Düring et al. 1974; Bartlett et al. 1976). Although SARs can be found throughout the entire tracheobronchial tree, considerable variation in the distribution of SARs exists between mammalian species. In cats, guinea-pigs and rats, SARs are more abundant in the intrapulmonary airways, whereas dogs tend to have more SARs in the extrapulmonary airways (Widdicombe 1954; Miserocchi et al. 1973; Bartlett et al. 1976; Schelegle and Green 2001). SARs outside the lungs

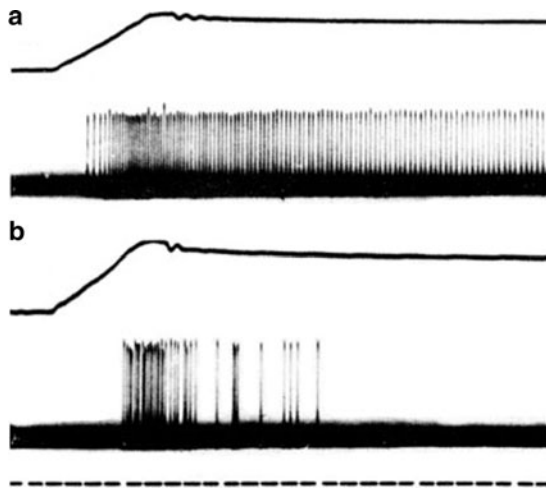


Fig. 2.1 Discharge pattern of two types of afferent nerve fibres in the vagus nerve of the cat, as recorded under maintained lung inflation, discriminating the two predominant types of airway mechanoreceptors. *Upper traces* represent the intratracheal pressure, *lower traces* the recorded action potentials. (a) This first receptor adapts slowly to maintained lung inflation and can be identified as a slowly adapting receptor (SAR). (b) The second receptor adapts much more rapidly to sustained lung inflation compared to the SAR and is therefore designated as a rapidly adapting receptor (RAR). From Knowlton and Larrabee (1946)

may exert different features compared to those in the intrapulmonary bronchi (Schelegle and Green 2001).

Different types of SARs have been described based on adaptation rate, volume threshold and sensitivity to inflation (Paintal 1973; Sant'Ambrogio 1982; Bergren and Peterson 1993; Schelegle and Green 2001; Schelegle 2003). A commonly used classification subdivides SARs into "high and low threshold" receptors. "High threshold" receptors generate action potentials when high tensions are reached during inspiration, these types of SARs are mainly found in the intrapulmonary airways. "Low threshold (type II)" receptors are activated at much lower excitation levels and are also active during expiration. Type II receptors are predominantly present in the extrapulmonary airways. Although the different subtypes of SARs may exhibit diverse patterns of discharge, it is not known whether they give rise to different reflexes and/or sensations (Paintal 1973; Schelegle and Green 2001; Schelegle 2003; Widdicombe 2009).

SARs, predominantly activated by mechanical stimuli, are the vagal airway afferents that are responsible for eliciting reflexes evoked by moderate lung inflation. Activation of SARs leads to reflexes that are known to play an important role in regulating the pattern of breathing (Schelegle and Green 2001; Schelegle 2003). In contrast to the observations in animal models, the role of SARs in controlling the pattern of breathing in humans seems to be minor, at least during quiet breathing (Sant'Ambrogio and Sant'Ambrogio 1997). Nevertheless, it is generally agreed that SAR activity regulates the Hering-Breuer reflex, which terminates inspiration and initiates expiration when the lungs are adequately inflated. Once a certain volume threshold is achieved, the inspiratory off-switch provided by the SARs input results in a termination of inspiration (Hering 1868; Adrian 1933; Sant'Ambrogio 1982). It has been proposed that a certain pattern of breathing corresponds with a certain demand of the respiratory muscles. SARs appear to be responsible for the volume feedback necessary to adjust the breathing pattern to the optimal load: once a threshold volume is reached, SARs control in a reflex manner the tidal volume and respiratory rate (Widdicombe and Nadel 1963b; Widdicombe 2006). SAR activation also initiates reflex bronchodilation by inhibiting tonic parasympathetic cholinergic drive to the airway smooth muscle, resulting in a reduction in baseline cholinergic airway tone (Widdicombe and Nadel 1963a, b), but the role that SARs play in determining normal airway tone is poorly understood. At low inflation pressures, SARs are also believed to be the vagal afferents that are responsible for initiating a cardiovascular reflex that results in tachycardia (Agostoni et al. 1957; Daly 1986; Kaufman and Cassidy 1987; Looga 1997). Although SARs are generally considered to be pure mechanoreceptors and relatively insensitive to chemical stimuli (compared with other lung receptors), their activity during eupneic breathing has been shown to be inhibited by increasing CO₂ partial pressure in the pulmonary arterial blood (Coleridge and Coleridge 1978; Sheldon and Green 1982, 2001).

2.2

Rapidly Adapting (Stretch) Receptors

An important feature to discriminate RARs from the slowly adapting mechanoreceptors in the airways is their rapid adaptation to maintained lung inflation (Fig. 2.1) (Keller and Loeser 1929; Adrian 1933; Knowlton and Larrabee 1946; Widdicombe 1954). RARs have thin myelinated nerve fibres that conduct action potentials in the A-fibre range (mainly A δ) exhibit an irregular discharge and respond typically to the dynamic phase of a mechanical stimulus (Widdicombe 1954; Sant'Ambrogio 1982; Sant'Ambrogio and Widdicombe 2001; Widdicombe 2003a). These receptors are found throughout the respiratory tract from nasopharynx and larynx to bronchi, but are scanty in the smaller bronchi and none have been identified in the bronchioles and alveoli (Widdicombe 1954; Sant'Ambrogio 1982; Lee et al. 1992). RARs are usually not very active during quiet breathing but do react to rapid changes in airway volume, discrete probing of the airway mucosa or gross deformation of the airways, such as those induced by a large distension or collapse of the lung. In addition to these mechanical modalities, RARs can readily be recruited by inhalation of irritants (e.g., ammonia, ethyl ether, sulphur dioxide and cigarette smoke) and the release of inflammatory or immunological mediators (Sant'Ambrogio and Widdicombe 2001; Widdicombe 2001, 2003a). Several investigators, however, have suggested that RARs are relatively insensitive to "direct" chemical stimuli (Coleridge and Coleridge 1984; Joad et al. 1997; Ho et al. 2001; Belvisi 2003). The responses of RARs to inhaled substances would then result from secondary effects initiated by the action of these irritants on the mechanical properties of the airways and lungs. Stimuli-dependent release of secretion products of unmyelinated C-fibre receptors may evoke bronchospasm and mucus-secretion-induced airway obstruction, mimicking the mechanical consequences of lung inflation and deflation, and thus increase RAR activity. Nevertheless, there is also convincing evidence for "true" chemosensitive properties of RARs (Joad et al. 1997; Sant'Ambrogio and Widdicombe 2001; Widdicombe 2001, 2003a; Canning 2006). Cigarette smoke, for example, exerts an immediate stimulatory effect on RARs, before any change of the bronchomotor tone can take place (Kou and Lee 1990, 1991).

The high sensitivity of RARs to inhaled irritants and mechanical probing suggests a role in the airway defence mechanism. An intraepithelial location of at least part of the terminals of RARs has been proposed in agreement with this function (Riccio et al. 1996; Widdicombe 2001, 2003a). However, little is known about the membrane receptor properties of RARs that allow them to be activated by a variety of mechanical and chemical stimuli (Widdicombe 2001, 2009).

RARs have different reflex actions on breathing and bronchomotor tone, and discharge during lung inflation, or sometimes during the deflation phase of a respiratory cycle, and become more active as the rate and volume of lung inflation are elevated. Activation of RARs generally leads to increased inspiratory

effort, augmented breaths, a rise in parasympathetic outflow (i.e., bronchoconstriction and mucus secretion). While SARs prolong expiration, RARs have the opposite effect and shorten expiration (Sant'Ambrogio and Widdicombe 2001; Widdicombe 2003a; Lee and Undem 2005).

RARs appear to form a heterogeneous collection of sensory receptors, and their properties, both in relation to their sensitivity to various stimuli and the reflexes they induce, largely depend on the characteristics of the individual receptor and on its location in the airways (Sant'Ambrogio and Widdicombe 2001). RARs in the larynx, trachea and large bronchi are highly mechanosensitive and often referred to as "irritant receptors" (Knowlton and Larrabee 1946; Widdicombe 1954). At least subpopulations of laryngeal and tracheal RARs cause cough (Undem and Carr 2010; Canning 2011), the expiration reflex, and other laryngeal reflexes such as mucus secretion and broncho- and laryngoconstriction, but also stimulate hyperventilation and augmented breaths (Karlsson et al. 1988; Widdicombe 1998, 2003a; Sant'Ambrogio and Widdicombe 2001). RARs occurring in the lung are more sensitive to chemical irritant stimuli, and less to mechanical stimuli. A large body of evidence suggests that the latter receptors stimulate breathing, including augmented breaths, and cause reflex tracheo- and bronchoconstriction, airway mucus secretion and laryngeal closure (Sant'Ambrogio and Widdicombe 2001; Widdicombe 2003a).

As mentioned above, RAR activation may evoke cough, and these receptors indeed fulfil many of the accepted criteria for mediating the defensive cough reflex (Sant'Ambrogio et al. 1984; Widdicombe 1998; Canning 2011). However, for a considerable part of the RARs, it is unlikely that they would initiate the cough reflex, because many of the stimuli that robustly activate RARs (e.g., prostaglandins, histamine, neurokinins, metacholine) are often ineffective or only modestly effective in evoking cough (Barnes et al. 1984; Joos et al. 1987; Fujimura et al. 1992; Sant'Ambrogio and Widdicombe 2001; Widdicombe 2001). Although it is clear that the general population of RARs may not be the primary afferent nerve fibres to evoke cough, RARs throughout the airways are believed to act synergistically with other afferent nerve types to induce coughing (Mazzone 2004, 2005; Canning et al. 2006). Recently, selective subtypes of RAR-like A-fibres have been identified as real cough receptors (Undem and Carr 2010; Canning 2011). These receptors are highly sensitive to punctuate mechanical stimulation, acids and low chloride solutions but are, unlike typical RARs and SARs, not activated by airway distension or collapse. They are likely quiescent during tidal breathing, only becoming active in the presence of stimuli evoking cough.

2.3

C-Fibre Receptors

In addition to the myelinated RARs and SARs, the respiratory tract is also abundantly innervated by a large population of thin unmyelinated afferent

nerve fibres (Agostoni et al. 1957), which conduct action potentials in the C-fibre range and are therefore termed C-fibre receptors (CFRs). Vagal afferent C-fibres comprise a majority of the vagal airway afferents, innervate multiple targets over the entire respiratory tract and project to the nucleus of the solitary tract. Their activity is now believed to play essential roles in the physiological regulation of cardiopulmonary function (Lee 2009). Vagal CFRs are generally quiescent in the healthy, non-inflamed airways but are readily activated by noxious chemicals, including cigarette smoke and inflammatory mediators, and may therefore play a critical role in regulating and/or initiating airway defence reflexes (Paintal 1973; Coleridge and Coleridge 1984; Riccio et al. 1996; Ho et al. 2001; Lee and Pisarri 2001; Undem et al. 2004; Lee and Undem 2005; Kollarik et al. 2010; Lee et al. 2010). CFRs are polymodal, responding to both mechanical and chemical stimuli but are relatively unresponsive to lung inflation, collapse or stretch (Coleridge and Coleridge 1984; Ho et al. 2001). Because CFRs are much more readily activated by chemical stimulants than vagal mechanoreceptors, i.e., RARs and SARs, C-fibres may represent the primary type of chemosensitive or nociceptive afferents in the lung (Lee et al. 2003; Lee 2009).

With few exceptions – i.e., in humans it still remains a matter of debate (Lee 2009) – CFRs in mammalian lungs are robustly activated by capsaicin. Capsaicin has been widely used to stereotypically identify C-fibre afferent endings and for assessing their physiological effects (Ho et al. 2001; Lee and Pisarri 2001; Lee et al. 2003; Undem et al. 2004). The vanilloid receptor subtype 1 (VR1 or transient receptor potential vanilloid, TRPV1), a ligand-gated, non-selective cation channel, has been recognised as the “capsaicin receptor” on CFRs (Caterina et al. 1997; Helliwell et al. 1998). However, the suggestion of a strict relation of TRPV1 and CFRs is not entirely accurate because it has also been demonstrated that capsaicin can have a stimulatory effect on some thin myelinated A δ afferents, found in various visceral organ systems, including the lung (Riccio et al. 1996; Kajekar et al. 1999; Takemura et al. 2008).

The majority of CFRs, particularly in rats and guinea-pigs, synthesise neuropeptides such as tachykinins and calcitonin gene-related peptide (CGRP), which are located in synaptic vesicles in their central and peripheral terminals (Lundberg et al. 1984; Springall et al. 1987; Baluk et al. 1992; Kummer et al. 1992b; Riccio et al. 1996; Myers et al. 2002; Undem et al. 2004). This neurochemical property has been exploited to describe the distribution of the peripheral terminals of the unmyelinated C-fibre afferents in the airway epithelium. In large airways, CFRs form a dense nerve plexus just beneath the epithelium (Lundberg et al. 1984; Dey et al. 1990; Baluk et al. 1992; Hunter and Undem 1999; Canning et al. 2002). In the gas exchange area, C-fibres have been reported in close apposition of the capillaries, resulting in the term “juxtacapillary receptors” or “J-receptors” (Paintal 1995). Based on these variable anatomical locations, C-fibres have been subclassified into bronchial and pulmonary C-fibres (Coleridge and Coleridge 1984).

Bronchial C-fibres are experimentally identified as those most accessible for manipulation via the bronchial circulation, whereas pulmonary C-fibres are more readily accessed from the pulmonary circulation (Coleridge and Coleridge 1984). The subclassification of bronchopulmonary C-fibres has been extended, at least for mice and guinea-pigs, by considering the ganglionic location, coinciding with their embryonic origin, of the cell somata (Undem et al. 2004; Nassenstein et al. 2010). The jugular (neural crest) C-fibres innervate the larynx, trachea, main bronchi, and intrapulmonary airways (Riccio et al. 1996; Undem et al. 2004; Nassenstein et al. 2010). The nodose (placodal) C-fibres innervate intrapulmonary tissues but are rarely found in extrapulmonary airways. It would seem that the nodose fibres at least loosely correspond to pulmonary C-fibres and that jugular fibres correspond to bronchial C-fibres (Nassenstein et al. 2010). In addition to the different location and neuronal origin, subtypes of C-fibres appear to have different sensitivities to chemical and mechanical stimuli (Coleridge and Coleridge 1994; Ho et al. 2001; Lee and Pisarri 2001; Undem et al. 2004; Chuaychoo et al. 2005; Lee 2009). However, the distinction based on circulatory accessibility is less clear in smaller animals, e.g., rodents, as compared to cats and dogs (Coleridge and Coleridge 1994).

C-fibres are activated by a wide range and variety of exogenous and endogenous substances. The stimulatory effect of a number of inhaled chemicals (e.g., capsaicin, bradykinin, citric acid, ozone, ammonia) and volatile anaesthetics on bronchopulmonary CFRs has been extensively described (Paintal 1973; Coleridge and Coleridge 1984, 1994; Lee and Pisarri 2001; Lee et al. 2003; Lee and Undem 2005; Lee 2009). One important example is cigarette smoke, which has a pronounced stimulatory effect on CFRs and may have special significance in the pathogenic effects of smoke on normal airway function (Bonham et al. 2001; Lee et al. 2007, 2010). Endogenous inflammatory and immunological mediators, such as histamine, bradykinin and prostaglandins, are also known to activate C-fibre reflexes (Paintal 1973; Coleridge and Coleridge 1984; Undem and Nassenstein 2009; Lee 2009). Activation of airway C-fibres may cause local neurogenic inflammation. CFRs could be regarded as biosensors that monitor the inflammatory status of the lung by directly interacting with inflammatory mediators (Lee et al. 2007; Yu 2009).

When stimulated, pulmonary and bronchial CFRs can trigger profound airway/respiratory and cardiovascular responses, mediated via both peripheral/local axon reflexes and/or central/CNS reflex pathways. Central respiratory reflexes include airway constriction, mucus secretion, cough, tachypnea and rapid shallow breathing (Coleridge and Coleridge 1984; Lee 2009), while cardiovascular effects of pulmonary C-fibre stimulation include bradycardia, and systemic and pulmonary hypertension (Paintal 1973; Coleridge and Coleridge 1984; Ho et al. 2001; Lee and Pisarri 2001; Lee et al. 2003; Lee and Undem 2005; Lee 2009). Reflex bronchoconstriction and reduced tidal volume, which limits the inhalation of irritants into the lungs, together with the increase in bronchial blood flow to remove the irritants, are consistent with the role of CFRs in the pulmonary defence reflex (Lee and Pisarri 2001). Local actions are mediated by the release

of neuropeptides from C-fibre endings, which probably results from propagation of sensory impulses via the axonal ramifications to other branches of the sensory terminals (Lundberg and Saria 1987). The release of tachykinins and CGRP, which can act on a number of effector cells, may produce potent local effects, such as bronchoconstriction, chemotaxis of inflammatory cells, extravasation of macromolecules and oedema of the airway mucosa (Paintal 1973; Coleridge and Coleridge 1984; Ho et al. 2001; Lee and Pesarri 2001; Lee et al. 2003; Lee and Undem 2005). The great variability may reflect differences in stimuli and/or the possibility that several subtypes of CFRs are involved (Lee 2009). Recently, it has been suggested that the considerable neurochemical and molecular variety of CFRs in rodents may hold the key for the observed variability in sensations evoked by peripheral stimulation (Nassenstein et al. 2010; Adriaensen and Timmermans 2011).

The sensitivity of CFRs to a potential stimulus can be increased by several substances. This process is called sensitisation and can be mediated by certain autocooids (e.g., histamine, prostaglandins) and agents such as ozone, cigarette smoke, adrenaline and adenosine (Coleridge and Coleridge 1984; Ho and Lee 1998; Ho et al. 2000; Lee and Pesarri 2001; Kwong and Lee 2002; Lee et al. 2002; Lee and Undem 2005). The inappropriate and exaggerated activation of the C-fibres may contribute to the airway hyper-responsiveness seen, for example, in asthma patients.

Evidence also supports a role for CFRs in the cough reflex. Capsaicin, bradykinin and citric acid, selective stimulants of airway C-fibres are also potent mediators of cough (Coleridge and Coleridge 1984; Karlsson 1996; Mazzone et al. 2002; Canning et al. 2004). However, these substances only evoke cough in conscious animals and humans, but not in anaesthetised subjects, leading to the suggestion that cough evoked by airway irritation and mediated by CFRs may induce a conscious/voluntary urge-to-cough (Hutchings et al. 1984; Tatar et al. 1988, 1994; Canning et al. 2004; Undem and Carr 2010; Canning 2011), making cough not necessarily completely reflexive in nature. In view of the recent identification of RAR-like “true” cough receptors (see higher Undem and Carr 2010; Canning 2011), C-fibres are believed to be activated by chemical irritation and may be responsible for the recruitment of secondary pathways that evoke or modify cough responses via interactions with the primary cough pathway (Mazzone 2004, 2005; Canning et al. 2006; Canning 2011).

Novel Insights in the Neurochemistry and Function of
Pulmonary Sensory Receptors

Brouns, I.; Pintelon, I.; Timmermans, J.-P.; Adriaensen,
D.

2012, XII, 118 p. 23 illus., 21 illus. in color., Softcover

ISBN: 978-3-642-22771-4