Brainstem Structures Involved in the Generation of Reflex Cough

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3.1 Introductory Remarks

Cough is a very complex motor act that can be voluntary, behavioral, or simply reflex aimed at defending airway integrity. In awake mammals, especially in humans, the contribution of the higher brain structures is prominent, not only when coughing is voluntarily initiated for different purposes (vocal fold clearing, psychological behaviors, etc.) but also when triggered by nociceptive airway stimulation either under physiological or pathological conditions. As it is usual for nociceptive stimulation leading under certain circumstances to pain sensation (the highest level of body defense), airway stimulation causes in awake subjects specific sensations ("urge-to-cough") that may trigger irrepressible coughing. Thus, it is very difficult to differentiate between reflex cough and cough produced under cognitive and emotional influences. Actually, nociception (especially when associated with pain) displays various components, such as those sensory-discriminative, cognitive, emotional, and reflex [1–3]. Cough and associated sensations are obviously defensive responses to nociceptive stimulation; thus, it is not surprising that cough and pain share similar features at both peripheral and central levels. This notion has been recently incorporated into the current knowledge of physiology and pathophysiology of cough [4]. Here we deal in particular with the cough reflex as produced in decerebrate or anesthetized animals as well as in anesthetized humans. Cough-related afferents terminate in the nucleus tractus solitarii (NTS) and have extensive projections both to the brainstem to generate reflex actions and to the higher brain structures to produce cough associated with the other nociception-related components. Interestingly, like pain sensation, it seems that also the

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"urge-to-cough" and consequent coughing displays placebo/nocebo responses (see, e.g., [1, 3, 5, 6]). Cough-related afferents generate the cough reflex by involving both respiration-related and non-respiration-related brainstem areas in a fairly complex way, although the prominent role is played by the neural structures containing respiration-related neurons and constituting the brainstem respiratory network. The central action of neuromodulators or neurotransmitters at the level of the various brainstem structures could help to understand the neural circuitry underlying this reflex.

3.2 Central Terminations of Cough-Related Afferents

The central projections of airway vagal afferents terminate in the brainstem where they innervate second-order neurons that in turn project to other brainstem nuclei and contribute to both reflex and higher circuits encoding various involuntary and voluntary motor responses as well as perceivable sensations. Neuroanatomical and electrophysiological studies in both cats and rats have demonstrated that the main central termination sites of primary afferents of rapidly adapting stretch receptors (RARs) that mediate cough-related inputs are the medial subnucleus of the NTS and the caudal aspects of the NTS (cNTS), especially the lateral portion of the commissural subnucleus. RAR second-order neurons (RAR cells) are located in these NTS regions. On the other hand, also bronchopulmonary C-fiber afferents convey tussigenic inputs and have their central terminations mainly in the medial portion of the commissural subnucleus, although a certain degree of overlapping exists between termination sites of RARs and C-fiber afferents ([7–11]; see for review [12]). Interestingly, RAR cells are excited by ammonia inhalation (a well-known tussigenic stimulus) and display monosynaptic EPSP in response to vagal stimulation. Studies in the rabbit [13, 14] using excitatory amino acid (EAA) receptor antagonists implicate that cough-related afferents activated by the stimulation of the tracheobronchial tree terminate in the cNTS consistently with previous findings. The results of investigations in guinea pigs both by anatomical tracing studies and by microinjections of EAA receptor antagonists [15, 16] led to the suggestion that afferent fibers activated in response to the stimulation of "cough-receptors" located in the tracheal mucosa terminate mainly in slightly more rostral and lateral NTS sites (lateral to the commissural subnucleus and, perhaps, in the medial subnuclei). The difference with previous findings in the cat, rat, and rabbit was attributed chiefly to the different site of tussigenic stimulation (trachea vs. tracheobronchial tree).

A permissive role of slowly adapting stretch receptors (SARs) in the cough reflex (see also Chap. 1 in this book) has been shown in some animal models, but not in others [16–19]. The importance of volume-related feedback in the regulation of the cough reflex is controversial, although both RARs and SARs may provide a significant contribution [20–24]. It has been shown that lung inflation [25] inhibits RAR cells and that SARs are responsible for inhibitory inputs to RAR cells via NTS second-order neurons, the so-called P cells. Really, two types of SAR second-order neurons exist. The first is represented by P cells, which receive virtually only SAR

(or pulmonary stretch receptors) afferent input, discharge during lung inflation and become silent if lung inflation is prevented (no-inflation test). The second is represented by Iβ (or Rβ) cells, which receive both central inspiratory drive and SAR afferent input and reduce their inspiratory discharge, but do not become silent, during the no-inflation test. SAR afferents project to the NTS subnuclei from the level of the obex to almost 1.5 mm rostral to it (cat). SAR second-order neurons have been found in the ventrolateral NTS, but also in other NTS regions, such as medial, dorsolateral, intermediate, and interstitial subnuclei [10, 26–29]. P cells are GABAergic or may corelease GABA and glycine ([8, 30]; see also [31]). Second-order neurons in the cough afferent pathway have been shown to project to brainstem neural structures involved in breathing pattern formation (see below), such as pontine and medullary respiratory groups [7, 10, 12, 32–36].

A schematic representation of more prominent synaptic inputs to RAR cells, originating especially from airway afferent fibers, is reported in Fig. 3.1 (for Refs. related to this illustration see [4]). Interestingly, several afferent inputs to the NTS imply the release of substance P. These sensory fibers include baro- and chemoreceptor afferents conveyed by vagus and glossopharyngeal nerves, trigeminal

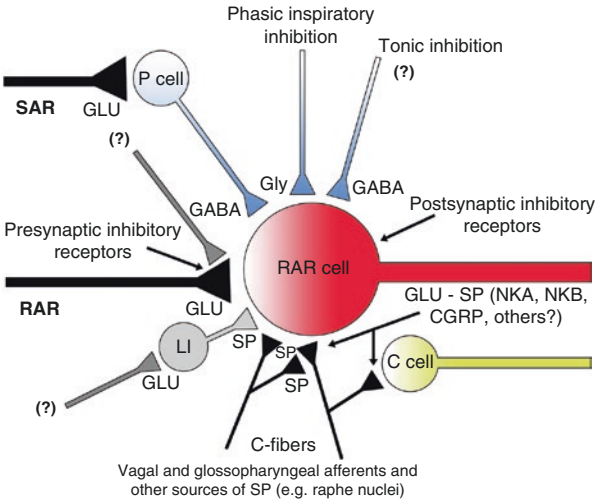

Fig. 3.1 Schematic representation of synaptic inputs to RAR relay neurons (RAR cells, red), a nonhomogenous NTS population of second-order neurons in the RAR afferent pathway which conveys also cough-related inputs. SAR, RAR, and bronchopulmonary C-fiber inputs modulate RAR cell activity through excitatory and inhibitory connections. P cells (largely localized in the medial and ventrolateral NTS regions, blue) are a subset of the entire population of second-order neurons in the SAR afferent pathway. C cells (yellow) are relay neurons of the bronchopulmonary C afferents located primarily in the medial portion of the commissural subnucleus. Convergence of C-fibers onto RAR relay neurons has also been reported. Abbreviations: *CGRP* calcitonin gene-related peptide; *GABA* γ-aminobutyric acid; *GLU* glutamate; *Gly* glycine; *LI* local interneuron; *NKA* neurokinin A; *NKB* neurokinin B; *RAR* rapidly adapting receptor; *SAR* slowly adapting receptor; *SP* substance P; (?) unknown sources that may include also vagal and glossopharyngeal afferents. (Modified from Mutolo [4])

afferents, skeletal muscle afferents, and projections from raphe nuclei (e.g., [37–41]; see also [42]). In addition, bronchopulmonary C-fibers have been suggested to converge onto RAR cells [43–45]. Indeed, the effects of substance P on NTS neurons are fairly complex since it displays not only postsynaptic excitatory effects but also presynaptic depressant effects on glutamatergic transmission between bronchopulmonary afferent fibers and second-order NTS neurons [12, 46, 47]. In addition, local interneurons may release substance P in response to glutamatergic inputs ([48], see also [47] for further Refs.). A comprehensive review on vagal afferent innervation of the airways and related central pathways under healthy or pathological conditions has recently been reported by Mazzone and Undem [49].

3.3 Brainstem Respiratory Network

Since cough is a modified respiratory act, it is appropriate to recall that the respiratory cycle is divided into three phases, i.e., inspiration, postinspiration or E1 phase, and expiration or E2 phase (active expiration) on the basis of the activity of the diaphragm and vocal fold adduction muscles (for details see Chap. 1 in this book). This triphasic organization is mirrored in the functional properties of the brainstem neural network underlying respiratory rhythm generation and pattern formation (see below).

Respiratory rhythm in adult mammals probably results from synaptic interactions between respiratory neurons located in the lower brainstem, particularly in the medulla oblongata (see e.g. [29, 50–54]). Several brainstem structures have been found to have a respiratory function. Respiratory neurons have been reported to be present in the rostral pons at the level of the parabrachial and Kölliker-Fuse nuclei, a region designated as the pontine respiratory group (PRG). Medullary respiratory neurons appear to be concentrated in two main aggregates, the dorsal respiratory group (DRG) and the ventral respiratory column (VRC). The DRG is closely associated with the NTS and contains mainly bulbospinal inspiratory premotoneurons. The VRC is located in the ventrolateral medulla and corresponds to a longitudinal column of neurons extending from the cervical spinal cord to the facial nucleus and comprises several rostro-caudally arranged compartments. The more rostrally located portion has been recently described and includes the retrotrapezoid nucleus (RTN) with the closely related parafacial respiratory group (pFRG), and the "Postinspiratory Complex" (PiCo).

Caudad there is a section of the column, initially named ventral respiratory group (VRG), which includes the Bötzinger complex (BötC), the pre-Bötzinger complex (preBötC), the inspiratory portion of the ventral respiratory group (iVRG), and the caudal expiratory component of the ventral respiratory group (cVRG). In the cervical spinal cord, two respiration-related regions have been described called the upper cervical inspiratory group (intermediolateral substance gray; [55]) and the high cervical respiratory group (close to the ventral surface; [56]). Both these two respiratory groups have not been reported to have any major role in the generation of respiratory activity and reflex cough.

The rostral VRG that comprises the BötC and the preBötC has a pivotal role in respiratory rhythm generation, while the iVRG and the cVRG are chiefly considered output systems. The iVRG compartment contains bulbospinal inspiratory neurons that project to spinal phrenic and intercostal inspiratory motoneurons. These neurons receive an excitatory input from preBötC excitatory neurons and an inhibitory input during expiration from BötC expiratory neurons. Both these inputs (along with other modulatory drives) shape and control the characteristic ramp-like pattern of inspiratory activity [57, 58]. Expiratory neurons are mainly concentrated in the cVRG and the BötC. Most of the cVRG expiratory neurons are bulbospinal neurons that project to spinal thoracic and lumbar expiratory motoneurons and receive their excitatory input from more rostral regions of the medulla ([29, 50, 59]; see also [52]). The BötC, which contains especially augmenting expiratory neurons, exerts extensive inhibition on medullary respiratory neurons. Convergent inputs from the BötC to the cVRG contribute to shape the pattern of discharge of expiratory cVRG neurons that drive expiratory motoneurons during eupneic breathing [29, 50]. The preBötC is located between the BötC and the iVRG and comprises several types of respiratory propriobulbar neurons. It has been proved to be the core circuit responsible for the generation of the inspiratory rhythm ([60]; for review see [52]).

Rostral to the BötC, the region located ventrolateral to the facial nucleus, i.e., the pFRG, contains pre-inspiratory neurons that usually discharge both prior to and after the phrenic nerve activity. This region was found to be activated in the respiratory cycle before any other respiration-related regions of the brainstem [61] and was first considered the source of both inspiratory and expiratory rhythmic activity. Successively, other studies proposed that this region is essential for expiratory rhythm generation in newborn, juvenile, and adult rats [62–66] since it contains many neurons silent under control conditions, but rhythmically active during the expiratory phase in response to pharmacological disinhibition or optogenetic excitation. When activated, the pFRG sends rhythmic excitatory drive inputs to cVRG expiratory neurons and, hence, to abdominal muscles (active expiration). However, at least in adult rodents, active expiration requires an ongoing rhythmic preBötC activity sufficient to drive inspiratory motor output [63, 66]. Expiratory muscle activation is also caused by neurons of this area under hypercapnic conditions [58, 64, 66, 67] or following peripheral chemoreceptor stimulation [68]. Moreover, the activation of serotoninergic or cholinergic muscarinic mechanisms within this region contributes to the appearance of neuronal expiratory activity and promotes the recruitment of expiratory motoneurons and active expiration [69, 70].

The pFRG partially overlaps with the adjacent chemosensitive RTN region located in a more ventromedial position (for review see [52, 71–74]). Since the pFRG and the RTN share some common features, they are often reported as RTN/pFRG. Neurons located within the RTN detect signals related to CO_2 and/or pH levels and transmit them to the preBötC and other brainstem sites. During the perinatal period its neurons also spontaneously generate late-expiratory bursts that raise preBötC excitability and may entrain preBötC rhythm activity. Then, the RTN loses its rhythmogenic role and functions only as a

chemosensitive center that detects environmental CO_2 and expresses a paired-like homeobox 2b gene (Phox2b). Interestingly, Phox2b mutations have been described in most human cases of congenital central hypoventilation syndrome ([75]; for review see [76]).

Recently, the brainstem region named PiCo, located rostral to the preBötC, dorsal to the BötC, and caudal to the facial nucleus, has been found to be characterized by rhythm-generating properties and has been considered necessary and sufficient for generating postinspiratory activity in both neonatal and adult mice [52, 77, see also 78]. On the basis of these results, a "triple oscillator model" has been proposed in which inspiration, postinspiration, and active expiration are generated by three distinct excitatory rhythmogenic microcircuits, i.e., preBötC, PiCo, and pFRG, respectively. The localization of the structures involved in the generation of the breathing pattern is illustrated in Fig. 3.2.

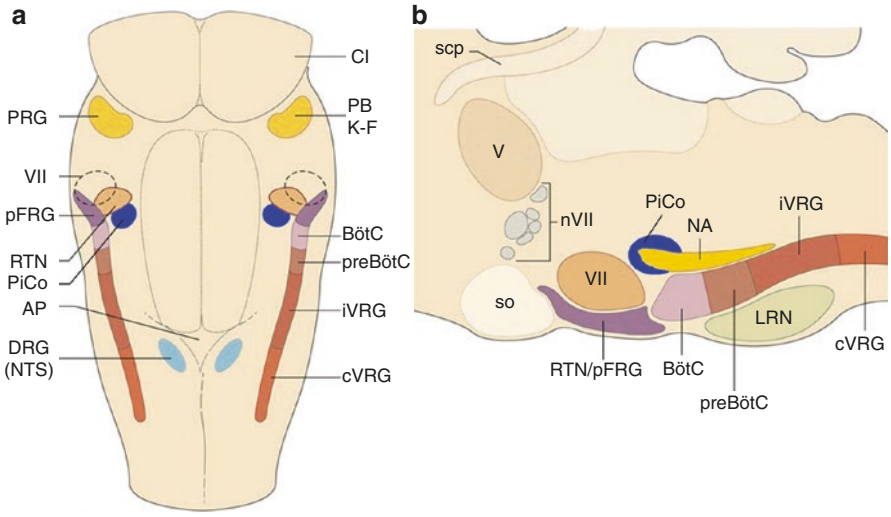

Fig. 3.2 Schematic illustration of the main brainstem neural structures involved in the breathing control. (**a**) Respiration-related regions have been projected on a dorsal view of the mammalian brainstem. *VII* facial motor nucleus; *AP* area postrema; *BötC* Bötzinger complex; *CI* mesencephalic colliculus inferior; *cVRG* caudal expiratory component of the ventral respiratory group; *DRG* dorsal respiratory group; *K-F* Kölliker-Fuse nucleus; *iVRG* intermediate or inspiratory portion of the ventral respiratory group; *NTS* nucleus tractus solitarii; *PB* parabrachial nucleus; *pFRG* parafacial respiratory group; *PiCo* Postinspiratory Complex; *preBötC* preBötzinger complex; *PRG* pontine respiratory group; *RTN* retrotrapezoid nucleus. (**b**) Parasagittal view of the brainstem containing the mammalian respiratory network. *V* trigeminal motor nucleus; *nVII* VII facial nerve and its nucleus; *BötC* Bötzinger complex; *cVRG* caudal expiratory component of the ventral respiratory group; *iVRG* intermediate or inspiratory portion of the ventral respiratory group; *LRN* lateral reticular nucleus; *NA* nucleus ambiguus; *pFRG* parafacial respiratory group; *PiCo* Postinspiratory Complex; *preBötC* preBötzinger complex; *RTN* retrotrapezoid nucleus; *scp* superior cerebellar peduncle; *SO* superior olive

3.4 Role of Brainstem Structures in the Generation of the Cough Reflex

Transection and lesion experiments in the cat have shown that the rostral pons and the cerebellum have modulatory effects on the motor pattern of the cough reflex, but the fundamental structures responsible for this reflex appear to reside in the medulla oblongata [79, 80]. For some previous reviews on the central neural mechanisms involved in the generation and/or regulation of the cough reflex, see, e.g., Fontana et al. [81], Pantaleo et al. [34], and Mutolo [4]. Former attempts to localize a medullary "cough center" within the NTS by using electrical microstimulation or lesion experiments have been reported (see, e.g., [34, 82]). However, these techniques do not allow to differentiate the role of neuronal structures and cough-related afferent fibers. In the light of the results of subsequent investigations on brainstem structures involved in the cough reflex, we can infer that the NTS is primarily the relay station of cough-related afferent inputs that are transmitted to the central neural network responsible for the generation of the cough motor pattern.

An interesting approach aimed at understanding the central mechanisms underlying the generation of the cough reflex has been that of investigating the behavior of medullary respiratory neurons during coughing in animal models, mainly cats. Earlier studies investigated the behavior of cVRG expiratory neurons in response to mechanical stimulation of the tracheobronchial tree or electrical stimulation of the superior laryngeal nerve [83, 84]. Expiratory neurons display excitatory responses during the expiratory phase of coughing. Furthermore, "latent" or almost quiescent neurons under normal breathing conditions can be recruited during coughing. Later studies reported that iVRG inspiratory and cVRG expiratory neurons are activated during the inspiratory and expiratory phases of coughing, respectively [85–87]. The same caudal expiratory neurons are activated during different types of expiratory efforts such as cough, sneeze, and expiration reflex.

All these studies mainly deal with the output system of the medullary respiratory network. More recent studies have investigated the behavior of different types of neurons located in more rostral rhythmogenic VRG regions during fictive coughing induced by superior laryngeal nerve stimulation in decerebrate, paralyzed, artificially ventilated cats. They have demonstrated that different types of inspiratory and expiratory neurons are activated during the appropriate phases of coughing [88]. Other studies carried out in similar preparations [89] revealed that a few inspiratory bulbospinal and propriobulbar neurons of the DRG are involved in cough response, thus suggesting that inspiratory premotoneurons responsible for the activity of phrenic motoneurons during the inspiratory phase of coughing are located elsewhere, possibly in the iVRG, in agreement with previous results [85–88]. Further studies have investigated the central mechanisms involved in the cough response evoked by mechanical stimulation of the tracheobronchial tree in anesthetized, spontaneously breathing cats focusing the attention on BötC expiratory neurons [90]. The majority of neurons encountered within this region display excitatory responses during the expulsive phase of coughing, in parallel with the main components of the abdominal electromyographic bursts and the corresponding increases in

tracheal pressure. The important role of the BötC neurons not only in providing the synaptic drive to cVRG expiratory neurons but also in determining the overall characteristics of the cough motor pattern has been corroborated by the suppression of both the inspiratory and expiratory components of the cough motor pattern observed during lignocaine blockades or following kainic acid lesions within this region in anesthetized, spontaneously breathing rabbits [91]. An important advance has been obtained by Shannon and collaborators that have extensively studied the behavior of rostral VRG respiratory neurons, including those located in the BötC and the pre-BötC, during fictive cough induced by mechanical stimulation of the intrathoracic trachea in decerebrate, paralyzed, artificially ventilated cats [36, 92–94]. Multi-site recordings employing microelectrode arrays and cross-correlational methods were used to functionally characterize respiratory neurons. They presented a model of the cough network and related synaptic interactions based on the behavior of respiratory neurons. The general conclusion of all these studies is that the rostral VRG neurons involved in the generation of the eupneic pattern of breathing also participate in the production of the cough motor pattern and that during coughing the drive to spinal motoneurons is transmitted via the same bulbospinal neurons that provide the descending drive during eupnea. These findings support the existence of multifunctional neural networks in the mammalian brainstem (e.g., [4, 34, 90] also for further Refs.) and, accordingly, of neurons that contribute to different functions, such as respiration, coughing, vomiting, and sneezing (see Fig. 3.3). When triggered by cough-related inputs to the NTS, the respiratory network appears to change configuration to generate the cough motor pattern. This hypothesis was advanced by Shannon et al. [94] who devised a neuronal respiratory network that undergoes a

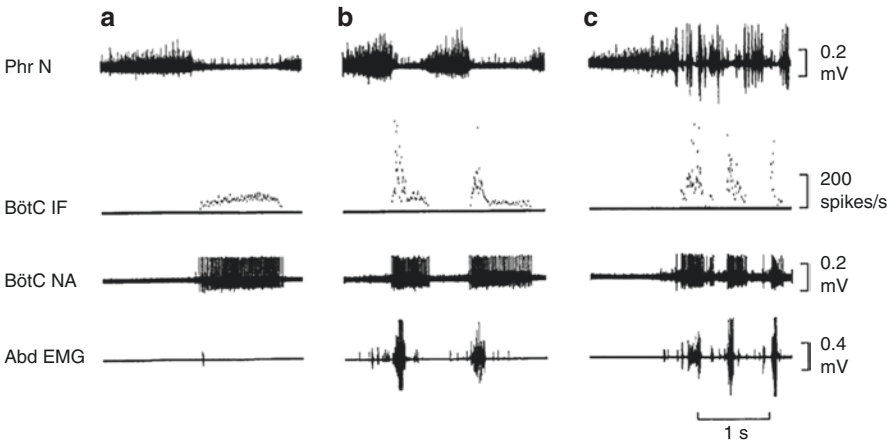

Fig. 3.3 Discharge pattern of one E-Aug neuron of the Bötzinger complex (BötC) displaying excitatory responses during coughing and sneezing induced by mechanical stimulation of the tracheobronchial tree and nasal mucosa, respectively. Pentobarbitone-anesthetized, spontaneously breathing cat. (**a**) Control discharge pattern. (**b**) Discharge pattern during coughing. (**c**) Discharge pattern during sneezing. *PhrN* phrenic neurogram; *BötC IF* instantaneous frequency of Bötzinger complex neuronal discharge; *BötC NA* Bötzinger complex neuronal activity; *Abd EMG* abdominal electromyogram. (Modified from Pantaleo et al. [34])

process of "reconfiguration" to produce coughing. A tentative computational model of coughing has more recently been described by Pitts et al. [95].

Interestingly, recordings from respiration-related neurons have been performed also in the RTN/pFRG region of guinea pigs during coughing and swallowing [96]. The majority of recorded neurons change activity in synchrony with coughing and swallowing. However, on the basis of brainstem transection experiments, it was concluded that RTN/pFRG neurons may modulate expiratory activity during eucapnic breathing, but are not essential components of the neural circuit underlying coughing and swallowing.

The results of recording experiments have been confirmed by studies on the expression of the immediate early gene c-fos during coughing [97, 98] and by lesion experiments ([91, 99–104] also for further Refs.). It has been shown that several brainstem regions either with or without respiration-related neuronal activities may contribute to generate or modulate the cough motor pattern. These regions include the BötC, the raphe nuclei and other midline structures, the periaqueductal gray, the lateral tegmental field, the PRG, and the reticular nuclei. The periaqueductal gray and the nucleus raphe magnus have also been reported to have suppressive influences on both coughing and swallowing ([105]; see also [106]).

3.5 Additional Neural Mechanisms Controlling or Modulating the Cough Reflex

Modulation or, in some instances, even generation of the cough reflex depend on the cerebellar nucleus interpositus [80], nasal mucosa trigeminal afferents [107, 108], esophageal vagal afferents [109], afferents from the external acoustic meatus (auricular branch of the vagus nerve, Arnold's or Alderman's nerve) that mediates the Arnold's ear-cough reflex (e.g., [110–112]), pharyngeal afferents (for review see [110]), chemoreceptors [18, 113–115] and baroreceptor [116] afferents. Of note, pharyngeal afferents from receptors probably traveling in vagal and glossopharyngeal nerves or in trigeminal branches can activate the gag reflex [117] and may also evoke coughing or the "urge-to-cough" [110].

Experiments performed in rodents (a species that lacks the cough reflex; see also [49]) have identified subcircuits in the brainstem and forebrain that receive relayed airway sensory inputs not only via the NTS but also via the paratrigeminal nucleus [118]. The NTS projects to several neural structures of the brainstem as well as to hypothalamic nuclei, well known components of autonomic and limbic/paralimbic central pathways. The central projections of the paratrigeminal nucleus are characterized by substantial input to the ventrobasal and submedius thalamus, which are important components subserving somatosensations, including those related to nociception. Interestingly, the paratrigeminal nucleus receives primary afferent projections from the pharynx, larynx, and tracheobronchial tree. In addition, visceral and somatic primary afferent inputs may converge in the paratrigeminal nucleus that has been suggested to be involved in the mediation of viscerovisceral and somatovisceral reflexes through efferent connections with autonomic centers in the brainstem. The paratrigeminal nucleus is certainly involved in some respiratory reflexes, but its possible role in cough production remains to be ascertained (see, e.g., [49, 119]).

Central and peripheral mechanisms involved in exercise and voluntary isocapnic hyperventilation may downregulate cough reflex responses [120, 121]. However, exercise (electrically induced hind limb muscular contractions) in ovalbumin-sensitized rabbits fails to produce similar effects [122]. An important characteristic of the cough reflex is its very strong dependency on the sleep-wakefulness state as well as on anesthesia ([123, 124]; see also Chap. 1 in this book). This reflex, in addition to a potent voluntary control, has also sensory, affective, and cognitive components. In fact, as revealed mainly by functional imaging studies, cough afferent pathways extend beyond a simple pontomedullary reflex to impinge on neuronal networks widely distributed throughout subcortical and cortical brain areas. The involvement of higher brain areas in the generation or modulation of coughing has been the subject of recent reviews (see e.g. [49, 125–128]). The main central and peripheral neural mechanisms that generate or modulate the cough reflex are schematically summarized in Fig. 3.4.

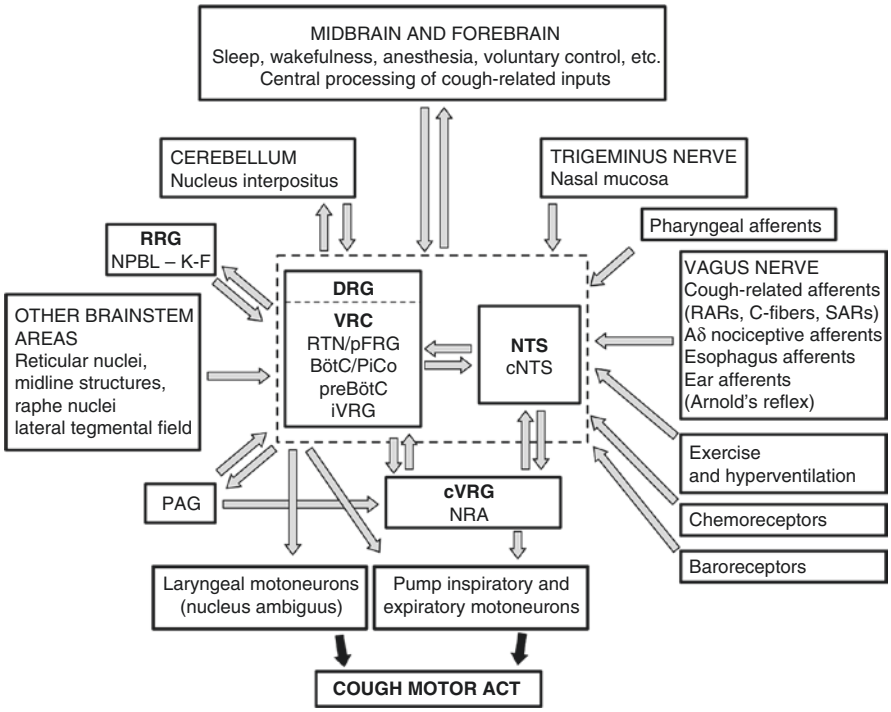

Fig. 3.4 Block diagram summarizing the main central and peripheral neural mechanisms involved in the production and modulation of the cough reflex. Possible connections between brainstem structures subserving this reflex have also been reported. Abbreviations: *BötC* Bötzinger complex; *cNTS* caudal aspect of the nucleus tractus solitarii; *cVRG* caudal ventral respiratory group; *DRG* dorsal respiratory group; *iVRG* inspiratory portion of the ventral respiratory group; *K-F* Kölliker-Fuse nucleus; *NPBL* nucleus parabrachialis lateralis; *NRA* nucleus retroambigualis; *NTS* nucleus tractus solitarii; *PAG* periaqueductal gray; *PiCo* Postinspiratory complex; *preBötC* preBötzinger complex; *PRG* pontine respiratory group; *RARs* rapidly adapting receptors; *SARs* slowly adapting receptors; *RTN/pFRG* retrotrapezoid nucleus/parafacial respiratory group; *VRC* ventral respiratory column. (Derived from Mutolo [4])

3.6 Insights into the Brainstem Mechanisms by the Action of Antitussive or Protussive Drugs

Centrally acting antitussive drugs have already been extensively reviewed (e.g., [4, 129–134]). Although the possibility exists that, following systemic administration, centrally acting antitussive drugs have sites of action at suprapontine and/or spinal levels, different lines of evidence have led to the general assumption that they act in the brainstem to suppress the cough reflex (see, e.g., [130]). According to earlier suggestions [14, 135], at least two medullary structures proved to play a prominent role in cough production and in the mediation of the central action of cough-related drugs, i.e., the first relay medullary station of the reflex pathway and the medullary neuronal aggregate responsible for the expiratory drive component of the reflex. These two neural substrates are the cNTS and the cVRG, respectively (see Fig. 3.5). This latter region corresponds, to a great extent, to the nucleus retroambigualis (NRA). Intracerebroventricular or intravertebral artery administration of antitussive drugs have demonstrated the central activity of some drugs. However, the main drawback of these methods is that they lack anatomical specificity. For antitussive drugs that may act in the brainstem, the specific site of action and the receptors involved are important issues. Microinjection techniques may well localize the drug into a given brain region, although with some limits (see, e.g., [138–140]). The interpretation of studies performed with these techniques has some difficulties since the employed drugs may act pre- or postsynaptically as well as on multiple subpopulations of neurons within the injected area. Furthermore, with these methods it is somewhat difficult to relate the dose of a given drug microinjected to the actual concentration reached when the drug is systematically administered.

The following presentation is mainly focused on the cNTS and the cVRG and deals largely with results obtained by our research group making use of bilateral microinjections of neuroactive agents in pentobarbitone anesthetized, spontaneously breathing rabbits. The localization of injections sites is diagrammatically represented in Fig. 3.5. Cough was induced either by mechanical or chemical (citric acid inhalation) stimulation of the tracheobronchial tree. Investigations on the role of drugs and, particularly, neurotransmitters or neuromodulators within different cough-related brainstem regions may primarily provide insights into the basic neural mechanisms subserving the genesis of the cough motor pattern and, in addition, hints for further studies on antitussive or protussive agents and for novel therapeutic approaches.

3.6.1 Caudal Nucleus Tractus Solitarii

Codeine and dextromethorphan, microinjected in large amounts and volumes into the NTS and the nucleus reticularis parvocellularis (lateral tegmental field), have been shown (see e.g. [129, 130]) to cause suppressant effects on cough in cats and guinea pigs. Recently, it has been reported that microinjections of relatively small amounts of codeine into the rostral NTS and the lateral tegmental field, but not in

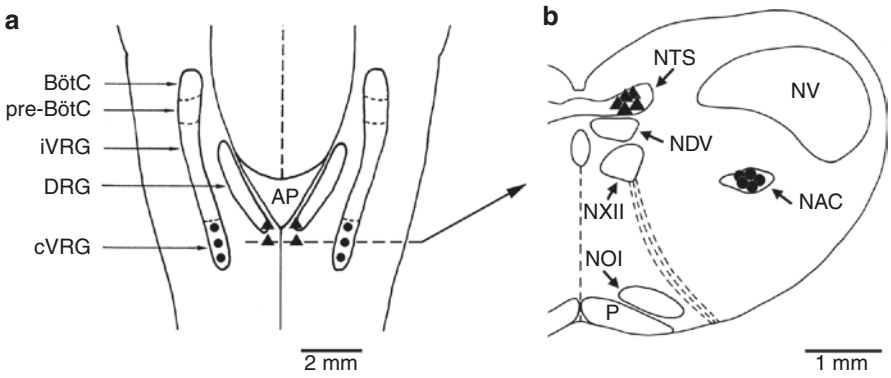

Fig. 3.5 Localization of injection sites. (**a**) A diagrammatic representation of a dorsal view of the medulla oblongata of the rabbit showing where bilateral microinjections of neuroactive agents have been performed into the cNTS (▲) and the cVRG (●), respectively. Abbreviations: *AP* area postrema; *BötC* Bötzinger complex; *cVRG* caudal ventral respiratory group; *DRG* dorsal respiratory group; *iVRG* inspiratory portion of the ventral respiratory group; *preBötC* preBötzinger complex. (**b**) Diagram of a coronal section of the medulla oblongata at the levels indicated in panel **a** (dashed lines) showing the location of representative sites where the microinjections have been performed. *NAC* nucleus ambiguous caudalis; *NDV* nucleus dorsalis nervi vagi; *NOI* nucleus olivaris inferior; *NTS* nucleus tractus solitarii; *NV* nucleus tractus spinalis nervi trigemini; *NXII* nucleus nervi hypoglossi; *P* tractus pyramidalis. (Outlines of some relevant structures derived from the atlas of Meessen and Olszewski [136] and the atlas of Shek et al. [137])

the cNTS, reduce cough in the cat [141]. In this connection, it seems appropriate to mention that codeine inhibits glutamatergic excitatory neurotransmission from primary cough-related afferents to second-order neurons of the NTS [142].

Blockade of non-*N*-methyl-D-aspartate (NMDA) receptors within the rabbit cNTS abolishes the cough reflex in response to mechanical or chemical stimulation, while only cough-depressant effects are induced by NMDA receptor blockade [13, 14]. An essential contribution of EAA receptors in the mediation of cough afferent inputs within the NTS has been observed in guinea pigs, but in regions more rostral and lateral to the cNTS and with a predominant role of NMDA receptors [16]. Recently, Poliacek et al. [143] have reported that in the cat bilateral microinjections of kynurenic acid, a broad spectrum EAA antagonist, into the cNTS are without effects, while similar microinjections into the rostral NTS cause marked alterations in both baseline respiratory activity and cough motor pattern. They have suggested the existence of important cough control mechanisms within the rostral NTS distinct from those processing the primary cough afferent signals located, according to the bulk of available literature, in caudal aspects of the NTS. Further investigations are needed to elucidate the involvement of different NTS subnuclei in the cough reflex. Interestingly, in accordance with previous findings by Mazzone et al. [45], the local application of substance P (NK1 receptor agonist) to the rabbit cNTS [14] potentiates or sensitizes cough responses by increasing both the amplitude of expiratory thrusts and the cough number, i.e., the number of coughs following each stimulation. The effects of C-fiber activation could be mimicked by microinjections

of capsaicin or substance P into the commissural NTS, and could be reversed by centrally administered NK receptor antagonists ([45]; see also [16]).

Local applications of the μ-opioid receptor agonist [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) and the $GABA_B$ receptor agonist baclofen downregulate (decreases in the cough number and peak abdominal activity) or completely suppress the cough reflex [42]. Microinjections of the NK1 receptor antagonist CP-99,994 abolish cough responses while those of the NK2 receptor antagonist MEN 10376 are without effects [42]. These results are consistent with previous findings showing that tachykinin NK1 antagonists delivered to the brainstem circulation depress cough [144]. The antitussive action of baclofen microinjected into the NTS has been confirmed by Canning and Mori [16] in anesthetized guinea pigs. Furthermore, recently Kotmanova et al. [145] have reported that GABA, muscimol, and baclofen microinjected into the rostral NTS cause suppressant effects on the cough reflex, while at the cNTS level only GABA can suppress cough reflex responses. The reasons of the discrepancy with previous experiments are unknown and further comparative studies in different animal species are necessary to clarify the role of NTS subnuclei in the cough reflex.

Since cough can be considered a defensive response to nociceptive stimulation, it is not surprising that peripheral and central mechanisms underlying nociception and cough share similar features, including central stations in the afferent pathways and descending control mechanisms (e.g., [1–3, 49, 106, 131, 146–153]). Some important similarities between cough and pain have been reported in Table 3.1.

In humans, hypersensitivity in response to inhaled capsaicin has been found to coincide with elevated neural activity in the midbrain (nucleus cuneiformis and

Table 3.1 Some of the main similarities between cough and pain

Afferent fibers	
Aδ, C	Cough, pain
Receptors on sensory afferents	
$TRPV_1$,$TRPA_1$, ASICs	Cough, pain
ATP and adenosine receptors	Cough, pain
Bradykinin and prostaglandin receptors	Cough, pain
Histamine receptors	Cough, pain
Serotonin receptors	Cough, pain
Peripheral sensitization	Cough, pain
Central sensitization	Cough, pain
Corresponding clinical features	
Upper airway tickling sensation	Pain
Paresthesia	Cough
Hypertussia	Pain
Hyperalgesia	Cough
Allotussia	Pain
Allodynia	Cough

Derived from Mutolo [4]

periaqueductal gray). This enhanced activity in the midbrain is similar to that occurring in patients with chronic pain, thus supporting the notion that cough and pain share neurobiological similarities [106]. Of note, the periaqueductal gray is the source of one of the major descending pain controlling pathways (see, e.g., [149]). Since itch closely recalls the tickling sensation in the upper airways that leads to the "urge-to-cough" (e.g., [154, 155]), it seems relevant to recall that an interesting parallel has been made between pain and itch as well as between itch and cough [148, 156, 157]. Cough, pain, and itch obviously have important protective functions, but also characterize debilitating diseases under chronic pathological conditions ([147, 152, 153, 156, 157]; for further details and Refs. see [4]). Conceivably, neuroactive agents involved in the central control of reflex responses to nociceptive stimuli and associated pain sensation may also be relevant to the regulation of the cough reflex. Accordingly, attempts to downregulate the cough reflex have been made by using drugs suitable for pain control. Local applications of U0126, an inhibitor of ERK1/2 activation, have shown for the first time that the mitogen-activated protein kinase (MAPK) contributes to the processing of tussive inputs [150]. Bilateral microinjections of U0126 into the cNTS suppress cough responses without affecting the Breuer-Hering inflation reflex, the pulmonary chemoreflex, and the sneeze reflex. These results are coherent with those of previous studies showing that ERK1/2 has central effects on both acute pain behavior and neuronal plasticity underlying pain hypersensitivity (e.g., [158–161]). Similarly, the activation of α_2-adrenergic receptors by microinjections of clonidine and tizanidine, two agonists that may have analgesic effects (for review see [162–164]), has strong suppressant effects on cough reflex responses [165]. These α_2-adrenergic receptor agonists mainly act through the presynaptic inhibition of glutamate release, but also other mechanisms could be involved (see [165]). Another study [151] has been devoted to investigate the regulation of the cough reflex by galanin (a neuropeptide implicated in pain control) at the level of the NTS, where galanin receptors are known to be present [166]. Bilateral microinjections of galanin or galnon (a nonpeptide agonist at galanin receptors) into the cNTS markedly affect cough responses not only by decreasing the cough number and peak abdominal activity, but also by increasing the duration of the entire cycle of cough motor response. Galanin antitussive effects are possibly related to its interaction with substance P, opioids, and NMDA receptors [151]. Acetylcholine (ACh) applied to the cNTS has recently been shown to have depressant effects on the cough reflex mediated by muscarinic ACh receptors [167]. On the other hand, ACh is widely distributed in NTS and is a neurotransmitter profoundly involved in pain perception through both nicotinic and muscarinic receptors [168].

RAR cells located in the cNTS receive both phasic glycinergic and tonic $GABA_A$ receptor-mediated inhibitory inputs ([8, 25, 30, 169]; see also Fig. 3.1). Accordingly, evidence has been provided [31] that both $GABA_A$ and glycine receptors mediate a potent inhibitory control of the pattern of breathing and cough reflex responses. Bilateral microinjections of bicuculline and strychnine cause strong decreases in expiratory activity, marked increases in respiratory frequency, and potentiate the cough reflex mainly via increases in the cough number. Muscimol and glycine cause

opposite effects. Of note, an impairment of the activation of $GABA_A$ and glycine receptors can be the neural substrate of neuropathic pain ([170]; for review see [171, 172]). Taken together, these results strongly suggest that inhibition and disinhibition are prominent regulatory mechanisms of ongoing respiratory activity and cough reflex responses.

Noticeably, some drugs display a central protussive action. We have already mentioned that substance P potentiates the cough reflex by increasing expiratory drive and cough number. Consistently with the hypothesis of a central action of angiotensin-converting enzyme (ACE) inhibitors, that are known to cross the blood-brain barrier, microinjections of the ACE inhibitor lisinopril into the cNTS cause changes in the cough motor pattern characterized by increases in the cough number ([173] also for further Refs.). The complete blockade of lisinopril-induced cough potentiation was obtained either by bradykinin B_2 or NK1 receptor antagonism, thus suggesting a lisinopril-induced central accumulation of bradykinin and substance P. Accordingly, bradykinin microinjections into the cNTS induce a clear cough sensitization (increase in cough number), which is completely abolished by preceding microinjections of the NK1 receptor antagonist CP-99,994. In conclusion, the protussive effect of ACE inhibitors appears to be related to an action on NTS sensory neurons due to a bradykinin-induced release of substance P (see [173]).

Bolser and colleagues ([174, 175]; see also [15]) have proposed that in the brainstem is present a cough-gating mechanism. Their hypothesis is based on evidence derived from studies on the differential effects of antitussive drugs on the cough reflex and the breathing pattern. A cough-gating mechanism (probably constituted by a neural circuit including the population of second-order neurons in the cough-related pathways) may account for the fact that antitussive drugs generally do not alter breathing at doses that inhibit cough, thus implying that there exists a neural component important for cough that does not participate in breathing pattern generation. Furthermore, the finding that most antitussive drugs do not exert a generalized cough suppression, but specifically affect some components of the cough motor pattern, i.e., the cough number and the intensity of expiratory thrusts, is consistent with this hypothesis. The above reported results obtained in rabbits agree, to a large extent, with the assumptions of Bolser et al. [174, 175]. However, at variance with their hypothesis, some antitussive drugs have been found to change the timing component of cough probably affecting NTS neurons unrelated to vagal tussigenic inputs, but implicated in the control of respiratory timing and intensity [29, 50, 176] probably through ascending projections to neural circuits responsible for respiratory pattern formation (see [177] also for further Refs.). It seems possible that the cNTS is not a simple relay station, but plays more extensive functions in cough motor pattern generation and, in addition, that it may be an important location of the cough-gating neurons. At present, it cannot be excluded that different NTS subnuclei or other brainstem respiration-related regions may contribute to the cough-gating mechanism. On the other hand, species differences should be also taken into consideration (see [82, 95, 178], also for further Refs.). Examples of antitussive and protussive effects of some selected drugs have been reported in Fig. 3.6.

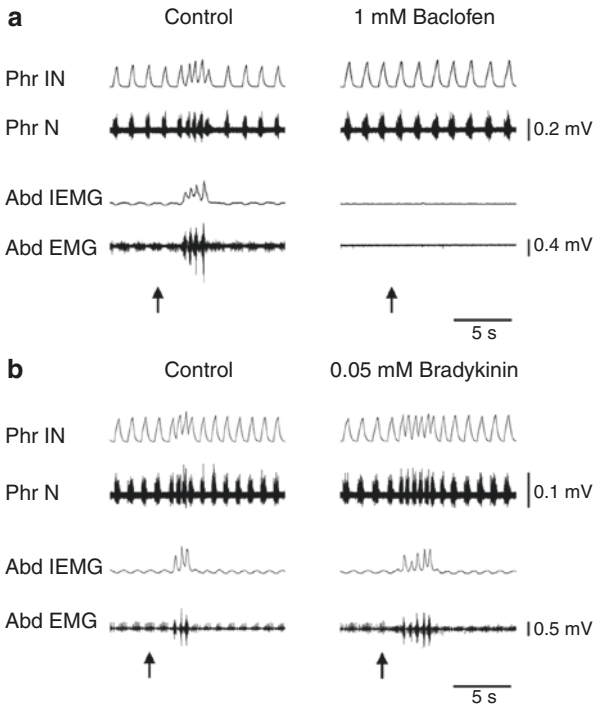

Fig. 3.6 Antitussive and protussive effects of selected drugs into the cNTS in anesthetized, spontaneously breathing rabbits. (**a**) Changes induced by bilateral microinjections (30 nl) of 1 mM baclofen into the cNTS. (**b**) Changes induced by bilateral microinjections (30 nl) of 0.05 mM bradykinin into the cNTS. Cough was induced by mechanical stimulation of the tracheobronchial tree (arrows). Traces are: *Phr IN* integrated phrenic neurogram; *Phr N* phrenic neurogram; *Abd IEMG* abdominal integrated electromyographic activity; *Abd EMG* abdominal electromyographic activity. (Modified from Mutolo et al. [42] and Cinelli et al. [173])

3.6.2 Caudal Ventral Respiratory Group (Nucleus Retroambigualis)

The cVRG is the region where bulbospinal expiratory neurons are located intermingled with other types of respiratory and non-respiratory neurons (e.g. [59, 179]) and, therefore, is strongly involved in the control of the cough reflex since the expulsive expiratory phase probably represents its most important component (see [177] also for further Refs.). Morphological and electrophysiological lines of evidence suggest that cVRG expiratory neurons probably are not involved in the respiratory rhythmogenesis since they seem to lack axon collaterals and therefore connections with other medullary respiratory neurons [29, 50, 59, 180, 181]. However, the activation of cVRG neurons causes transient inhibition of inspiratory activity in cats [182], rats [183, 184], and rabbits [135]. For instance, microinjections of the broad-spectrum EAA receptor agonist D,L-homocysteic acid (DLH) into the cVRG of the cat cause the activation of expiratory motoneurons and a corresponding silent period in phrenic nerve activity ([182]; see also [185]). Respiratory modulation triggered

by EAA receptor stimulation of the NRA region, comprising the cVRG, has been confirmed and analyzed in more detail by more recent studies [177, 186]. Available data support the possibility that caudal expiratory neurons can alter or shape the pattern of breathing via axon collaterals when strongly activated and that this could be relevant to some physiological conditions, such as airway defensive reflexes including coughing and sneezing [59, 182]. Anatomical studies showing cVRG projections to other brainstem respiration-related regions, such as the rostral VRG, the parabrachialis medialis/Kölliker-Fuse nuclei, and the NTS, are consistent with this view ([59, 177, 187–189]; for review see [4]).

The afferent drive inputs to the NRA are not completely unraveled. They may arise from more rostral expiration-related regions such as the BötC ([90, 93]; for review see also [34, 36, 59]) or the RTN/pFRG (e.g., [54, 61, 64, 66, 190–192]) as well as from the limbic system and the periaqueductal gray [177, 186].

Excitatory drive transmission to cVRG expiratory neurons appears to be mediated by glutamate with a major involvement of non-NMDA receptors. Bilateral microinjections of the non-NMDA receptor antagonist CNQX completely suppress spontaneous rhythmic and reflex abdominal activity [135]. More interestingly, they also suppress both the inspiratory and expiratory components of the cough reflex. This shows that neurons located in the cVRG are not merely elements of the expiratory output system, but are crucial for the production of all the components of the cough motor pattern. In fact, CNQX-induced effects on the inspiratory component of the cough reflex cannot be justified by the suppression of the excitatory output from cVRG bulbospinal neurons since Newsom Davis and Plum [193] have demonstrated that bilateral lesions of descending bulbospinal expiratory pathways deteriorate spontaneous rhythmic abdominal activity and block only the expiratory components of the cough reflex. It is worth noting that the existence of a cough-suppressant neuronal circuit within the cVRG has been shown by using DLH microinjections [185]. At variance with the suggestions arising from previous studies [90, 91], a cough-suppressant neuronal circuit has also been found within the BötC of the cat by means of similar microinjections [194]. The reasons for the discrepancy with previous findings are not clear and could, in part, be ascribed to differences in the preparation and microinjection procedures as well as to the nonhomogeneous composition of the BötC neuronal population.

Some antitussive drugs, already tested at the cNTS level, are active also within the cVRG mainly affecting cough number and expiratory thrusts. DAMGO and baclofen display inhibitory effects on the cough reflex [195]. Similar effects are also shown by the NK1 receptor antagonist CP-99,994, while the NK2 receptor antagonist MEN 10376 is ineffective. Furthermore, the cough reflex is reduced by tizanidine and completely suppressed by clonidine, thus supporting the notion of the essential role of this region in the production of the cough motor pattern [165].

Caudal expiratory neurons receive a potent bicuculline-sensitive GABAergic inhibitory input [196–198]. Accordingly, bilateral microinjections of bicuculline into the cVRG affect the ongoing pattern of breathing by increasing abdominal bursts and respiratory frequency, with a concomitant upregulation of the cough reflex. On the contrary, muscimol not only abolishes expiratory activity and decreases respiratory frequency, but also, like clonidine, induces the complete suppression of the cough

reflex [199]. These results show that $GABA_A$ receptors within the cVRG exert a very strong inhibitory control not only on the pattern of breathing, but also on airway defensive reflexes involving intense expiratory efforts. They further underline the role of inhibition and disinhibition phenomena in the central regulation of both breathing and coughing. Codeine and nicotine microinjected into the cVRG also cause depressant effects on cough responses in cats [200, 201].

3.7 Concluding Remarks

The central organization of the cough reflex is fairly complex and involves the brainstem respiratory network ("reconfiguration" hypothesis) and many modulatory influences that may contribute to the cough motor pattern formation. Several brainstem structures contribute to the regulation of this reflex, but at least two of them, i.e., the cNTS and the cVRG, are important sites of action of antitussive or protussive drugs. Interestingly, the results of drug microinjections suggest an essential role not only of the cNTS but also of the cVRG in the genesis of the overall cough motor pattern. Further investigations on the basic physiological and pathophysiological mechanisms underlying cough, pain, and itch, and the analysis of their similarities and differences, could suggest novel therapeutic strategies. Other studies are needed on the different brainstem areas subserving cough motor pattern formation and particularly on the RTN/pFRG that may be important in the generation of the expiratory thrusts, and on the PiCo that may have an essential role in the generation of postinspiratory behaviors which include coughing and swallowing. Moreover, the periaqueductal gray appears to be relevant to cough researches as indicated by some already mentioned lines of evidence (see [105, 106, 149]) and by the finding that it is the source of one of the major afferent input to the cVRG (e.g., [59, 177, 186]). Interestingly, also reciprocal connections between the periaqueductal gray and the preBötC have been reported [54, 202]. Although the preBötC is the core of the central mechanism generating the inspiratory rhythm, its contribution to the production of the cough motor pattern has not yet been completely unraveled. A recent aspect of the physiology of the central nervous system is the great contribution of glial cells, especially astrocytes, to the functional characteristics of neuronal activities (e.g., [203]). It has been proposed that astrocytes in the respiratory network may contribute to the characteristics of inspiratory activity [204–207] and play a crucial role in central chemoreception within the RTN [190, 208]. The finding that ozone-induced pulmonary inflammation results in a specific activation of vagal afferents that induces astroglial cellular alterations in the NTS ([209] also for further Refs.) suggests a possible involvement of astrocytes in cough regulation. However, the contribution of astrocytes to the modulation of the motor pattern of reflex cough remains to be investigated. Finally, it should be also remembered that many neuroimmune interactions can occur at different sites of the peripheral and central nervous systems in the development and maintenance of chronic cough, and that they could be interesting targets for studies aimed at developing novel effective antitussive therapies [210].

References

1. De Felice M, Ossipov MH. Cortical and subcortical modulation of pain. Pain Manag. 2016;6:111–20.
2. Groh A, Krieger P, Mease RA, Henderson L. Acute and chronic pain processing in the thalamocortical system of humans and animal models. Neuroscience. 2018;387:58–71.
3. Moayedi M, Davis KD. Theories of pain: from specificity to gate control. J Neurophysiol. 2013;109:5–12.
4. Mutolo D. Brainstem mechanisms underlying the cough reflex and its regulation. Respir Physiol Neurobiol. 2017;243:60–76.
5. Eccles R. Central mechanisms IV: conscious control of cough and the placebo effect. Handb Exp Pharmacol. 2009:241–62.
6. Leech J, Mazzone SB, Farrell MJ. Brain activity associated with placebo suppression of the urge-to-cough in humans. Am J Respir Crit Care Med. 2013;188:1069–75.
7. Ezure K, Otake K, Lipski J, She RB. Efferent projections of pulmonary rapidly adapting receptor relay neurons in the cat. Brain Res. 1991;564:268–78.
8. Ezure K, Tanaka I, Miyazaki M. Electrophysiological and pharmacological analysis of synaptic inputs to pulmonary rapidly adapting receptor relay neurons in the rat. Exp Brain Res. 1999;128:471–80.
9. Kubin L, Davies RO. Sites of termination and relay of pulmonary rapidly adapting receptors as studied by spike-triggered averaging. Brain Res. 1988;443:215–21.
10. Kubin L, Davies RO. Regulation of breathing. In: Hornbein TF, editor. Central pathways of pulmonary and airway vagal afferents. New York: Dekker; 1995. p. 219–84.
11. Lipski J, Ezure K, Wong She RB. Identification of neurons receiving input from pulmonary rapidly adapting receptors in the cat. J Physiol. 1991;443:55–77.
12. Kubin L, Alheid GF, Zuperku EJ, McCrimmon DR. Central pathways of pulmonary and lower airway vagal afferents. J Appl Physiol. 2006;101:618–27.
13. 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. 2009;80:22–9.
14. Mutolo D, Bongianni F, Fontana GA, Pantaleo T. The role of excitatory amino acids and substance P in the mediation of the cough reflex within the nucleus tractus solitarii of the rabbit. Brain Res Bull. 2007;74:284–93.
15. Canning BJ, Mori N. An essential component to brainstem cough gating identified in anesthetized guinea pigs. FASEB J. 2010;24:3916–26.
16. Canning BJ, Mori N. Encoding of the cough reflex in anesthetized Guinea pigs. Am J Physiol Regul Integr Comp Physiol. 2011;300:R369–77.
17. Hanacek J, Davies A, Widdicombe JG. Influence of lung stretch receptors on the cough reflex in rabbits. Respiration. 1984;45:161–8.
18. Hanacek J, Tatar M, Widdicombe J. Regulation of cough by secondary sensory inputs. Respir Physiol Neurobiol. 2006;152:282–97.
19. Sant'Ambrogio G. Information arising from the tracheobronchial tree of mammals. Physiol Rev. 1982;62:531–69.
20. Bolser DC, Davenport PW. Volume-timing relationships during cough and resistive loading in the cat. J Appl Physiol. 2000;89:785–90.
21. Javorka K, Kulisek V, Calkovska A. Defensive reflexes of the respiratory system in anaesthetized rabbits during high frequency jet ventilation. Exp Physiol. 1994;79:967–73.
22. Nishino T, Sugimori K, Hiraga K, Hond Y. Influence of CPAP on reflex responses to tracheal irritation in anesthetized humans. J Appl Physiol. 1989;67:954–8.
23. Poliacek I, Simera M, Veternik M, Kotmanova Z, Pitts T, Hanacek J, Plevkova J, Machac P, Visnovcova N, Misek J, Jakus J. The course of lung inflation alters the central pattern of tracheobronchial cough in cat-the evidence for volume feedback during cough. Respir Physiol Neurobiol. 2016;229:43–50.

24. Romaniuk JR, Kowalski KE, Dick TE. The role of pulmonary stretch receptor activation during cough in dogs. Acta Neurobiol Exp (Wars). 1997;57:21–9.
25. Ezure K, Tanaka I. Lung inflation inhibits rapidly adapting receptor relay neurons in the rat. Neuroreport. 2000;11:1709–12.
26. Berger AJ. Dorsal respiratory group neurons in the medulla of cat: spinal projections, responses to lung inflation and superior laryngeal nerve stimulation. Brain Res. 1977;135:231–54.
27. Davies RO, Kubin L, Pack AI. Pulmonary stretch receptor relay neurones of the cat: location and contralateral medullary projections. J Physiol. 1987;383:571–85.
28. Pantaleo T, Corda M. Respiration-related neurons in the medial nuclear complex of the solitary tract of the cat. Respir Physiol. 1986;64:135–48.
29. Von Euler C. Handbook of physiology. The respiratory system. Control of breathing. In: Cherniack NS, Widdicombe JG, editors. Brain stem mechanisms for generation and control of breathing pattern. Bethesda, MA: American Physiological Society; 1986. p. 1–67.
30. Ezure K, Tanaka I. GABA, in some cases together with glycine, is used as the inhibitory transmitter by pump cells in the Hering-Breuer reflex pathway of the rat. Neuroscience. 2004;127:409–17.
31. Cinelli E, Iovino L, Bongianni F, Pantaleo T, Mutolo D. GABAA- and glycine-mediated inhibitory modulation of the cough reflex in the caudal nucleus tractus solitarii of the rabbit. Am J Physiol Lung Cell Mol Physiol. 2016;311:L570–80.
32. Haji A, Ohi Y, Kimura S. Cough-related neurons in the nucleus tractus solitarius of decerebrate cats. Neuroscience. 2012;218:100–9.
33. Otake K, Ezure K, Lipski J, Wong She RB. Projections from the commissural subnucleus of the nucleus of the solitary tract: an anterograde tracing study in the cat. J Comp Neurol. 1992;324:365–78.
34. Pantaleo T, Bongianni F, Mutolo D. Central nervous mechanisms of cough. Pulm Pharmacol Ther. 2002;15:227–33.
35. Shannon R, Baekey DM, Morris KF, Lindsey BG. Brainstem respiratory networks and cough. Pulm Pharmacol. 1996;9:343–7.
36. Shannon R, Baekey DM, Morris KF, Nuding SC, Segers LS, Lindsey BG. Production of reflex cough by brainstem respiratory networks. Pulm Pharmacol Ther. 2004;17:369–76.
37. Gatti PJ, Shirahata M, Johnson TA, Massari VJ. Synaptic interactions of substance P immunoreactive nerve terminals in the baro- and chemoreceptor reflexes of the cat. Brain Res. 1995;693:133–47.
38. Lindefors N, Yamamoto Y, Pantaleo T, Lagercrantz H, Brodin E, Ungerstedt U. In vivo release of substance P in the nucleus tractus solitarii increases during hypoxia. Neurosci Lett. 1986;69:94–7.
39. Potts JT, Fuchs IE, Li J, Leshnower B, Mitchell JH. Skeletal muscle afferent fibres release substance P in the nucleus tractus solitarii of anaesthetized cats. J Physiol. 1999;514:829–41.
40. Thor KB, Helke CJ. Serotonin- and substance P-containing projections to the nucleus tractus solitarii of the rat. J Comp Neurol. 1987;265:275–93.
41. Zhang WB, Li JS, Li HM. SP-like immunoreactivity in the primary trigeminal neurons projecting to the nucleus tractus solitarii. Brain Res. 1991;558:87–9.
42. 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. 2008;295:R243–51.
43. Mazzone SB, Canning BJ. Central nervous system control of the airways: pharmacological implications. Curr Opin Pharmacol. 2002;2:220–8.
44. Mazzone SB, Canning BJ. Synergistic interactions between airway afferent nerve subtypes mediating reflex bronchospasm in guinea pigs. Am J Physiol Regul Integr Comp Physiol. 2002;283:R86–98.
45. Mazzone SB, Mori N, Canning BJ. Synergistic interactions between airway afferent nerve subtypes regulating the cough reflex in guinea-pigs. J Physiol. 2005;569:559–73.

46. Bonham AC, Sekizawa S, Chen CY, Joad JP. Plasticity of brainstem mechanisms of cough. Respir Physiol Neurobiol. 2006;152:312–9.
47. Sekizawa S, Joad JP, Bonham AC. Substance P presynaptically depresses the transmission of sensory input to bronchopulmonary neurons in the guinea pig nucleus tractus solitarii. J Physiol. 2003;552:547–59.
48. Colin I, Blondeau C, Baude A. Neurokinin release in the rat nucleus of the solitary tract via NMDA and AMPA receptors. Neuroscience. 2002;115:1023–33.
49. Mazzone SB, Undem BJ. Vagal afferent innervation of the airways in health and disease. Physiol Rev. 2016;96:975–1024.
50. Bianchi AL, Denavit-Saubie M, Champagnat J. Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. Physiol Rev. 1995;75:1–45.
51. Cui Y, Kam K, Sherman D, Janczewski WA, Zheng Y, Feldman JL. Defining pre Botzinger complex rhythm- and pattern-generating neural microcircuits in vivo. Neuron. 2016;91:602–14.
52. Del Negro CA, Funk GD, Feldman JL. Breathing matters. Nat Rev Neurosci. 2018;19:351–67.
53. Feldman JL, Del Negro CA, Gray PA. Understanding the rhythm of breathing: so near, yet so far. Annu Rev Physiol. 2013;75:423–52.
54. Ikeda K, Kawakami K, Onimaru H, Okada Y, Yokota S, Koshiya N, Oku Y, Iizuka M, Koizumi H. The respiratory control mechanisms in the brainstem and spinal cord: integrative views of the neuroanatomy and neurophysiology. J Physiol Sci. 2017;67:45–62.
55. Oku Y, Okabe A, Hayakawa T, Okada Y. Respiratory neuron group in the high cervical spinal cord discovered by optical imaging. Neuroreport. 2008;19:1739–43.
56. Aoki M, Mori S, Kawahara K, Watanabe H, Ebata N. Generation of spontaneous respiratory rhythm in high spinal cats. Brain Res. 1980;202:51–63.
57. Alheid GF, McCrimmon DR. The chemical neuroanatomy of breathing. Respir Physiol Neurobiol. 2008;164:3–11.
58. Smith JC, Abdala AP, Koizumi H, Rybak IA, Paton JF. Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms. J Neurophysiol. 2007;98:3370–87.
59. Iscoe S. Control of abdominal muscles. Prog Neurobiol. 1998;56:433–506.
60. Smith JC, Ellenberger HH, Ballanyi K, Richter DW, Feldman JL. Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science. 1991;254:726–9.
61. Onimaru H, Homma I. A novel functional neuron group for respiratory rhythm generation in the ventral medulla. J Neurosci. 2003;23:1478–86.
62. Huckstepp RT, Cardoza KP, Henderson LE, Feldman JL. Role of parafacial nuclei in control of breathing in adult rats. J Neurosci. 2015;35:1052–67.
63. Huckstepp RT, Henderson LE, Cardoza KP, Feldman JL. Interactions between respiratory oscillators in adult rats. elife. 2016;5
64. Janczewski WA, Feldman JL. Distinct rhythm generators for inspiration and expiration in the juvenile rat. J Physiol. 2006;570:407–20.
65. Janczewski WA, Onimaru H, Homma I, Feldman JL. Opioid-resistant respiratory pathway from the preinspiratory neurones to abdominal muscles: in vivo and in vitro study in the newborn rat. J Physiol. 2002;545:1017–26.
66. Pagliardini S, Janczewski WA, Tan WB, Dickson CT, Deisseroth K, Feldman JL. Active expiration induced by excitation of ventral medulla in adult anesthetized rats. J Neurosci. 2011;31:2895–905.
67. Abdala AP, Rybak IA, Smith JC, Zoccal DB, Machado BH, St John WM, Paton JF. Multiple pontomedullary mechanisms of respiratory rhythmogenesis. Respir Physiol Neurobiol. 2009;168:19–25.
68. Moraes DJ, Dias MB, Cavalcanti-Kwiatkoski R, Machado BH, Zoccal DB. Contribution of the retrotrapezoid nucleus/parafacial respiratory region to the expiratory-sympathetic coupling in response to peripheral chemoreflex in rats. J Neurophysiol. 2012;108:882–90.
69. Boutin RC, Alsahafi Z, Pagliardini S. Cholinergic modulation of the parafacial respiratory group. J Physiol. 2017;595:1377–92.

70. Lemes EV, Colombari E, Zoccal DB. Generation of active expiration by serotoninergic mechanisms of the ventral medulla of rats. J Appl Physiol. 2016;121:1135–44.
71. Guyenet PG, Bayliss DA. Neural control of breathing and CO2 homeostasis. Neuron. 2015;87:946–61.
72. Nattie EE, Li A. Retrotrapezoid nucleus lesions decrease phrenic activity and CO2 sensitivity in rats. Respir Physiol. 1994;97:63–77.
73. Nattie EE, Li A. Rat retrotrapezoid nucleus iono- and metabotropic glutamate receptors and the control of breathing. J Appl Physiol. 1995;78:153–63.
74. Nattie EE, Li AH, St John WM. Lesions in retrotrapezoid nucleus decrease ventilatory output in anesthetized or decerebrate cats. J Appl Physiol. 1991;71:1364–75.
75. Amiel J, Laudier B, Attie-Bitach T, Trang H, de Pontual L, Gener B, Trochet D, Etchevers H, Ray P, Simonneau M, Vekemans M, Munnich A, Gaultier C, Lyonnet S. Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. Nat Genet. 2003;33:459–61.
76. Moreira TS, Takakura AC, Czeisler C, Otero JJ. Respiratory and autonomic dysfunction in congenital central hypoventilation syndrome. J Neurophysiol. 2016;116:742–52.
77. Anderson TM, Garcia AJ III, Baertsch NA, Pollak J, Bloom JC, Wei AD, Rai KG, Ramirez JM. A novel excitatory network for the control of breathing. Nature. 2016;536:76–80.
78. Toor R, Sun QJ, Kumar NN, Le S, Hildreth CM, Phillips JK, McMullan S.Neurons in the intermediate reticular nucleus coordinate postinspiratory activity, swallowing, and respiratory-sympathetic coupling in the rat. J Neurosci. 2019;39:9757-66.
79. Jakus J, Tomori Z, Bosel'ova L, Nagyova B, Kubinec V. Respiration and airway reflexes after transversal brain stem lesions in cats. Physiol Bohemoslov. 1987;36:329–40.
80. Xu F, Frazier DT, Zhang Z, Baekey DM, Shannon R. Cerebellar modulation of cough motor pattern in cats. J Appl Physiol. 1997;83:391–7.
81. Fontana G, Bongianni F, Lavorini F, Pantaleo T. Experimental and clinical pharmacology of gastroesophageal reflux-induced asthma. In: Dal Negro RW, Geppetti P, Morice AH, editors. Neurobiology and mechanics of cough. Pisa: Pacini Editore; 2002. p. 34–46.
82. Haji A, Kimura S, Ohi Y. A model of the central regulatory system for cough reflex. Biol Pharm Bull. 2013;36:501–8.
83. Engelhorn R, Weller E. Zentrale representation hustenwirksamer afferenzen in der medulla oblongata der katze. Pflugers Arch. 1965;284:224–39.
84. Price WM, Batsel HL. Respiratory neurons participating in sneeze and in response to resistance to expiration. Exp Neurol. 1970;29:554–70.
85. Dawid-Milner MS, Lara JP, Milan A, Gonzalez-Baron S. Activity of inspiratory neurones of the ambiguus complex during cough in the spontaneously breathing decerebrate cat. Exp Physiol. 1993;78:835–8.
86. Jakuš J, Tomori Z, Stránsky A. Activity of bulbar respiratory neurones during cough and other respiratory tract reflexes in cats. Physiol Bohemoslov. 1985;34:127–36.
87. Jakuš J, Tomori Z, Stránsky A, Bošel'ová L. Bulbar respiratory activity during defensive airways reflexes in cats. Acta Physiol Hung. 1987;70:245–54.
88. Oku Y, Tanaka I, Ezure K. Activity of bulbar respiratory neurons during fictive coughing and swallowing in the decerebrate cat. J Physiol. 1994;480:309–24.
89. Gestreau C, Milano S, Bianchi AL, Grelot L. Activity of dorsal respiratory group inspiratory neurons during laryngeal-induced fictive coughing and swallowing in decerebrate cats. Exp Brain Res. 1996;108:247–56.
90. Bongianni F, Mutolo D, Fontana GA, Pantaleo T. Discharge patterns of Bötzinger complex neurons during cough in the cat. Am J Physiol Regul Integr Comp Physiol. 1998;274:R1015–24.
91. Mutolo D, Bongianni F, Pantaleo T. Effects of lignocaine blockades and kainic acid lesions in the Bötzinger complex on spontaneous expiratory activity and cough reflex responses in the rabbit. Neurosci Lett. 2002;332:175–9.
92. Baekey D, Morris K, Gestreau C, Li Z, Lindsey B, Shannon R. Medullary respiratory neurones and control of laryngeal motoneurones during fictive eupnoea and cough in the cat. J Physiol. 2001;534:565–81.

93. Shannon R, Baekey DM, Morris KF, Li Z, Lindsey BG. Functional connectivity among ventrolateral medullary respiratory neurones and responses during fictive cough in the cat. J Physiol. 2000;525:207–24.
94. Shannon R, Baekey DM, Morris KF, Lindsey BG. Ventrolateral medullary respiratory network and a model of cough motor pattern generation. J Appl Physiol. 1998;84:2020–35.
95. Pitts T, Morris KF, Segers LS, Poliacek I, Rose MJ, Lindsey BG, Davenport PW, Howland DR, Bolser DC. Feed-forward and reciprocal inhibition for gain and phase timing control in a computational model of repetitive cough. J Appl Physiol. 2016;121:268–78.
96. Sugiyama Y, Shiba K, Mukudai S, Umezaki T, Sakaguchi H, Hisa Y. Role of the retrotrapezoid nucleus/parafacial respiratory group in coughing and swallowing in guinea pigs. J Neurophysiol. 2015;114:1792–805.
97. Gestreau C, Bianchi AL, Grelot L. Differential brainstem Fos-like immunoreactivity after laryngeal- induced coughing and its reduction by codeine. J Neurosci. 1997;17:9340–52.
98. Jakus J, Poliacek I, Halasova E, Murin P, Knocikova J, Tomori Z, Bolser DC. Brainstem circuitry of tracheal-bronchial cough: c-fos study in anesthetized cats. Respir Physiol Neurobiol. 2008;160:289–300.
99. Jakuš J, Stránsky A, Poliacek I, Barani H, Bošel'ová L. Effects of medullary midline lesions on cough and other airway reflexes in anaesthetized cats. Physiol Res. 1998;47:203–13.
100. Jakuš J, Stránsky A, Poliacek I, Barani H, Bošel'ová L. Kainic acid lesions to the lateral tegmental field of medulla: effects on cough, expiration and aspiration reflexes in anesthetized cats. Physiol Res. 2000;49:387–98.
101. Poliacek I, Jakus J, Simera M, Veternik M, Plevkova J. Control of coughing by medullary raphe. Prog Brain Res. 2014;212:277–95.
102. Poliacek I, Jakuš J, Stránsky A, Baráni H, Halašová E, Tomori Z. Cough, expiration and aspiration reflexes following kainic acid lesions to the pontine respiratory group in anesthetized cats. Physiol Res. 2004;53:155–63.
103. Poliacek I, Stransky A, Szereda-Przestaszewska M, Jakus J, Barani H, Tomori Z, Halasova E. Cough and laryngeal muscle discharges in brainstem lesioned anaesthetized cats. Physiol Res. 2005;54:645–54.
104. Simera M, Poliacek I, Veternik M, Dobrolubov B, Cibulka M, Barani H, Visnovcova N, Jakus J. Differential effects of kainic acid lesions in medullary raphe on cough and sneeze in anesthetized rabbits. Adv Exp Med Biol. 2013;788:39–45.
105. Sessle BJ, Ball GJ, Lucier GE. Suppressive influences from periaqueductal gray and nucleus raphe magnus on respiration and related reflex activities and on solitary tract neurons, and effect of naloxone. Brain Res. 1981;216:145–61.
106. Ando A, Smallwood D, McMahon M, Irving L, Mazzone SB, Farrell MJ. Neural correlates of cough hypersensitivity in humans: evidence for central sensitisation and dysfunctional inhibitory control. Thorax. 2016;71:323–9.
107. Plevkova J, Antosiewicz J, Varechova S, Poliacek I, Jakus J, Tatar M, Pokorski M. Convergence of nasal and tracheal neural pathways in modulating the cough response in Guinea pigs. J Physiol Pharmacol. 2009;60:89–93.
108. Poussel M, Varechova S, Demoulin B, Chalon B, Schweitzer C, Marchal F, Chenuel B. Nasal stimulation by water down-regulates cough in anesthetized rabbits. Respir Physiol Neurobiol. 2012;183:20–5.
109. Hennel M, Brozmanova M, Kollarik M. Cough reflex sensitization from esophagus and nose. Pulm Pharmacol Ther. 2015;35:117–21.
110. 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;146:1633–48.
111. Murray AR, Atkinson L, Mahadi MK, Deuchars SA, Deuchars J. The strange case of the ear and the heart: the auricular vagus nerve and its influence on cardiac control. Auton Neurosci. 2016;199:48–53.
112. Todisco T. The oto-respiratory reflex. Respiration. 1982;43:354–8.
113. Nishino T, Hiraga K, Honda Y. Inhibitory effects of CO2 on airway defensive reflexes in enflurane-anesthetized humans. J Appl Physiol. 1989;66:2642–6.

114. Tatar M, Korpas J, Polacek H, Zahradny V. Changes induced by severe hypoxia in respiratory defence reflexes in anaesthetized cats. Respiration. 1986;49:114–21.
115. Tatar M, Tarkanov IA, Korpas J, Kulik AM. Effect of hypoxia and hypercapina on the airways defence reflexes. Acta Physiol Hung. 1987;70:195–200.
116. Poliacek I, Morris KF, Lindsey BG, Segers LS, Rose MJ, Corrie LW, Wang C, Pitts TE, Davenport PW, Bolser DC. Blood pressure changes alter tracheobronchial cough: computational model of the respiratory-cough network and in vivo experiments in anesthetized cats. J Appl Physiol. 2011;111:861–73.
117. Bassi GS, Humphris GM, Longman LP. The etiology and management of gagging: a review of the literature. J Prosthet Dent. 2004;91:459–67.
118. McGovern AE, Driessen AK, Simmons DG, Powell J, Davis-Poynter N, Farrell MJ, Mazzone SB. Distinct brainstem and forebrain circuits receiving tracheal sensory neuron inputs revealed using a novel conditional anterograde transsynaptic viral tracing system. J Neurosci. 2015;35:7041–55.
119. Mazzone SB, McGovern AE, Koo K, Farrell MJ. Mapping supramedullary pathways involved in cough using functional brain imaging: comparison with pain. Pulm Pharmacol Ther. 2009;22:90–6.
120. 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. 2010;108:1061–8.
121. Poussel M, Bosser G, Varechova S, Demoulin B, Chalon B, Ruckebusch O, Tiotiu A, Renaud P, Schweitzer C, Chenuel B. Desensitization of the cough reflex during limb muscle contraction in anesthetized rabbits. Pulm Pharmacol Ther. 2014;27:96–101.
122. Tiotiu A, Chenuel B, Foucaud L, Demoulin B, Demoulin-Alexikova S, Christov C, Poussel M. Lack of desensitization of the cough reflex in ovalbumin-sensitized rabbits during exercise. PLoS One. 2017;12:e0171862.
123. Nishino T, Hiraga K, Mizuguchi T, Honda Y. Respiratory reflex responses to stimulation of tracheal mucosa in enflurane-anesthetized humans. J Appl Physiol. 1988;65:1069–74.
124. Widdicombe J, Singh V. Physiological and pathophysiological down-regulation of cough. Respir Physiol Neurobiol. 2006;150:105–17.
125. Ando A, Farrell MJ, Mazzone SB. Cough-related neural processing in the brain: a roadmap for cough dysfunction? Neurosci Biobehav Rev. 2014;47:457–68.
126. Driessen AK, Farrell MJ, Mazzone SB, McGovern AE. Multiple neural circuits mediating airway sensations: recent advances in the neurobiology of the urge-to-cough. Respir Physiol Neurobiol. 2016;226:115–20.
127. Mazzone SB, McGovern AE, Yang SK, Woo A, Phipps S, Ando A, Leech J, Farrell MJ. Sensorimotor circuitry involved in the higher brain control of coughing. Cough. 2013;9:7.
128. McGovern AE, Ajayi IE, Farrell MJ, Mazzone SB. A neuroanatomical framework for the central modulation of respiratory sensory processing and cough by the periaqueductal grey. J Thorac Dis. 2017;9:4098–107.
129. Bolser DC. Current and future centrally acting antitussives. Respir Physiol Neurobiol. 2006;152:349–55.
130. Bolser DC. Central mechanisms II: pharmacology of brainstem pathways. Handb Exp Pharmacol. 2009;187:203–17.
131. Canning BJ. Central regulation of the cough reflex: therapeutic implications. Pulm Pharmacol Ther. 2009;22:75–81.
132. Chung KF. Currently available cough suppressants for chronic cough. Lung. 2008;186(Suppl 1):S82–7.
133. Chung KF. NMDA and GABA receptors as potential targets in cough hypersensitivity syndrome. Curr Opin Pharmacol. 2015;22:29–36.
134. Mazzone SB, McGovern AE, Cole LJ, Farrell MJ. Central nervous system control of cough: pharmacological implications. Curr Opin Pharmacol. 2011;11:265–71.
135. Bongianni F, Mutolo D, Nardone F, Pantaleo T. Ionotropic glutamate receptors mediate excitatory drive to caudal medullary expiratory neurons in the rabbit. Brain Res. 2005;1056:145–57.

136. Meessen H, Olszewski JA. A cytoarchitectonic atlas of the rhomboencephalon of the rabbit. Basel: Karger; 1949.
137. Shek JW, Wen GY, Wisniewski HM. Atlas of the rabbit brain and spinal cord. Basel: Karger; 1986.
138. Lipski J, Bellingham MC, West MJ, Pilowsky P. Limitations of the technique of pressure microinjection of excitatory amino acids for evoking responses from localized regions of the CNS. J Neurosci Methods. 1988;26:169–79.
139. Nicholson C. Diffusion from an injected volume of a substance in brain tissue with arbitrary volume fraction and tortuosity. Brain Res. 1985;333:325–9.
140. Sykova E, Nicholson C. Diffusion in brain extracellular space. Physiol Rev. 2008;88:1277–340.
141. Poliacek I, Simera M, Veternik M, Kotmanova Z, Bolser DC, Machac P, Jakus J. Role of the dorsomedial medulla in suppression of cough by codeine in cats. Respir Physiol Neurobiol. 2017;246:59–66.
142. Ohi Y, Kato F, Haji A. Codeine presynaptically inhibits the glutamatergic synaptic transmission in the nucleus tractus solitarius of the guinea pig. Neuroscience. 2007;146:1425–33.
143. Poliacek I, Pitts T, Rose MJ, Davenport PW, Simera M, Veternik M, Kotmanova Z, Bolser DC. Microinjection of kynurenic acid in the rostral nucleus of the tractus solitarius disrupts spatiotemporal aspects of mechanically induced tracheobronchial cough. J Neurophysiol. 2017;117:2179–87.
144. Bolser DC, DeGennaro FC, O'Reilly S, McLeod RL, Hey JA. Central antitussive activity of the NK1 and NK2 tachykinin receptor antagonists, CP-99,994 and SR 48968, in the Guinea-pig and cat. Br J Pharmacol. 1997;121:165–70.
145. Kotmanova Z, Simera M, Veternik M, Martvon L, Misek J, Jakus J, Shen TY, Musselwhite MN, Pitts T, Bolser DC, Poliacek I. GABA-ergic neurotransmission in the nucleus of the solitary tract modulates cough in the cat. Respir Physiol Neurobiol. 2018;257:100–6.
146. Barnes PJ. The problem of cough and development of novel antitussives. Pulm Pharmacol Ther. 2007;20:416–22.
147. Dicpinigaitis PV, Morice AH, Birring SS, McGarvey L, Smith JA, Canning BJ, Page CP. Antitussive drugs—past, present, and future. Pharmacol Rev. 2014;66:468–512.
148. Lavinka PC, Dong X. Molecular signaling and targets from itch: lessons for cough. Cough. 2013;9:8.
149. Millan MJ. Descending control of pain. Prog Neurobiol. 2002;66:355–474.
150. 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. 2012;302:R976–83.
151. 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. 2014;307:R1358–67.
152. Niimi A, Chung KF. Evidence for neuropathic processes in chronic cough. Pulm Pharmacol Ther. 2015;35:100–4.
153. O'Neill J, McMahon SB, Undem BJ. Chronic cough and pain: Janus faces in sensory neurobiology? Pulm Pharmacol Ther. 2013;26:476–85.
154. Davenport PW. Urge-to-cough: what can it teach us about cough? Lung. 2008;186(Suppl 1):S107–11.
155. Muroi Y, Undem BJ. Targeting peripheral afferent nerve terminals for cough and dyspnea. Curr Opin Pharmacol. 2011;11:254–64.
156. Hoon MA. Molecular dissection of itch. Curr Opin Neurobiol. 2015;34C:61–6.
157. Liu T, Ji RR. New insights into the mechanisms of itch: are pain and itch controlled by distinct mechanisms? Pflugers Arch. 2013;465:1671–85.
158. Ji RR, Gereau RW, Malcangio M, Strichartz GR. MAP kinase and pain. Brain Res Rev. 2009;60:135–48.
159. Kawasaki Y, Kohno T, Zhuang ZY, Brenner GJ, Wang H, Van Der MC, Befort K, Woolf CJ, Ji RR. Ionotropic and metabotropic receptors, protein kinase A, protein kinase C, and Src contribute to C-fiber-induced ERK activation and cAMP response element-binding pro-

tein phosphorylation in dorsal horn neurons, leading to central sensitization. J Neurosci. 2004;24:8310–21.
160. Obata K, Noguchi K. MAPK activation in nociceptive neurons and pain hypersensitivity. Life Sci. 2004;74:2643–53.
161. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science. 2000;288:1765–9.
162. Chan AK, Cheung CW, Chong YK. Alpha-2 agonists in acute pain management. Expert Opin Pharmacother. 2010;11:2849–68.
163. Eisenach JC, De Kock M, Klimscha W. Alpha(2)-adrenergic agonists for regional anesthesia. A clinical review of clonidine (1984-1995). Anesthesiology. 1996;85:655–74.
164. Pertovaara A. Noradrenergic pain modulation. Prog Neurobiol. 2006;80:53–83.
165. Cinelli E, Bongianni F, Pantaleo T, Mutolo D. Suppression of the cough reflex by alpha 2-adrenergic receptor agonists in the rabbit. Physiol Rep. 2013;1:e00122.
166. Endoh T, Sato D, Wada Y, Shibukawa Y, Ishihara K, Hashimoto S, Yoshinari M, Matsuzaka K, Tazaki M, Inoue T. Galanin inhibits calcium channels via Galpha(i)-protein mediated by GalR1 in rat nucleus tractus solitarius. Brain Res. 2008;1229:37–46.
167. Cinelli E, Iovino L, Bongianni F, Pantaleo T, Mutolo D. Inhibitory modulation of the cough reflex by acetylcholine in the caudal nucleus tractus solitarii of the rabbit. Respir Physiol Neurobiol. 2018;257:93–9.
168. Naser PV, Kuner R. Molecular, cellular and circuit basis of cholinergic modulation of pain. Neuroscience. 2018;387:135–148.
169. Ezure K, Tanaka I, Miyazaki M. Inspiratory inhibition of pulmonary rapidly adapting receptor relay neurons in the rat. Neurosci Lett. 1998;258:49–52.
170. Kami K, Taguchi MS, Tajima F, Senba E. Improvements in impaired GABA and GAD65/67 production in the spinal dorsal horn contribute to exercise-induced hypoalgesia in a mouse model of neuropathic pain. Mol Pain. 2016;12:174480691662905.
171. Prescott SA. Synaptic inhibition and disinhibition in the spinal dorsal horn. Prog Mol Biol Transl Sci. 2015;131:359–83.
172. Zeilhofer HU, Ralvenius WT, Acuna MA. Restoring the spinal pain gate: GABA(A) receptors as targets for novel analgesics. Adv Pharmacol. 2015;73:71–96.
173. Cinelli E, Bongianni F, Pantaleo T, Mutolo D. The cough reflex is upregulated by lisinopril microinjected into the caudal nucleus tractus solitarii of the rabbit. Respir Physiol Neurobiol. 2015;219:9–17.
174. Bolser DC, Hey JA, Chapman RW. Influence of central antitussive drugs on the cough motor pattern. J Appl Physiol. 1999;86:1017–24.
175. 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;152:255–65.
176. Budzinska K, Von Euler C, Kao FF, Pantaleo T, Yamamoto Y. Effects of graded focal cold block in the solitary and para-ambigual regions of the medulla in the cat. Acta Physiol Scand. 1985;124:317–28.
177. Jones SE, Stanic D, Dutschmann M. Dorsal and ventral aspects of the most caudal medullary reticular formation have differential roles in modulation and formation of the respiratory motor pattern in rat. Brain Struct Funct. 2016;221:4353–68.
178. Bolser DC, Pitts TE, Davenport PW, Morris KF. Role of the dorsal medulla in the neurogenesis of airway protection. Pulm Pharmacol Ther. 2015;35:105–10.
179. Arita H, Kogo N, Koshiya N. Morphological and physiological properties of caudal medullary expiratory neurons of the cat. Brain Res. 1987;401:258–66.
180. Huang Q, St John WM. Respiratory neural activities after caudal-to-rostral ablation of medullary regions. J Appl Physiol. 1988;64:1405–11.
181. Merrill EG. Central nervous control mechanisms in breathing. In: Von Euler C, Lagercrantz H, editors. Is there reciprocal inhibition between medullary inspiratory and expiratory neurones? Oxford: Pergamon Press; 1979. p. 239–54.

182. Bongianni F, Corda M, Fontana GA, Pantaleo T. Chemical activation of caudal medullary expiratory neurones alters the pattern of breathing in the cat. J Physiol. 1994;474:497–507.
183. Bonham AC, Jeske I. Cardiorespiratory effects of DL-homocysteic acid in caudal ventrolateral medulla. Am J Phys. 1989;256:H688–96.
184. Chitravanshi VC, Sapru HN. Phrenic nerve responses to chemical stimulation of the subregions of ventral medullary respiratory neuronal group in the rat. Brain Res. 1999;821:443–60.
185. Poliacek I, Corrie LW, Wang C, Rose MJ, Bolser DC. Microinjection of DLH into the region of the caudal ventral respiratory column in the cat: evidence for an endogenous cough-suppressant mechanism. J Appl Physiol. 2007;102:1014–21.
186. Subramanian HH, Holstege G. The nucleus retroambiguus control of respiration. J Neurosci. 2009;29:3824–32.
187. Gerrits PO, Holstege G. Pontine and medullary projections to the nucleus retroambiguus: a wheat germ agglutinin-horseradish peroxidase and autoradiographic tracing study in the cat. J Comp Neurol. 1996;373:173–85.
188. Smith JC, Morrison DE, Ellenberger HH, Otto MR, Feldman JL. Brainstem projections to the major respiratory neuron populations in the medulla of the cat. J Comp Neurol. 1989;281:69–96.
189. Zheng Y, Riche D, Rekling JC, Foutz AS, Denavit-Saubie M. Brainstem neurons projecting to the rostral ventral respiratory group (VRG) in the medulla oblongata of the rat revealed by co-application of NMDA and biocytin. Brain Res. 1998;782:113–25.
190. Guyenet PG, Bayliss DA, Stornetta RL, Ludwig MG, Kumar NN, Shi Y, Burke PG, Kanbar R, Basting TM, Holloway BB, Wenker IC. Proton detection and breathing regulation by the retrotrapezoid nucleus. J Physiol. 2016;594:1529–51.
191. Guyenet PG, Mulkey DK. Retrotrapezoid nucleus and parafacial respiratory group. Respir Physiol Neurobiol. 2010;173:244–55.
192. Mulkey DK, Stornetta RL, Weston MC, Simmons JR, Parker A, Bayliss DA, Guyenet PG. Respiratory control by ventral surface chemoreceptor neurons in rats. Nat Neurosci. 2004;7:1360–9.
193. Newsom Davis J, Plum F. Separation of descending spinal pathways to respiratory motoneurons. Exp Neurol. 1972;34:78–94.
194. Poliacek I, Corrie LW, Rose MJ, Wang C, Bolser DC. Influence of microinjections of D,L-homocysteic acid into the Bötzinger complex area on the cough reflex in the cat. J Physiol Pharmacol. 2008;59:585–96.
195. 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. 2010;109:1002–10.
196. McCrimmon DR, Zuperku EJ, Hayashi F, Dogas Z, Hinrichsen CF, Stuth EA, Tonkovic-Capin M, Krolo M, Hopp FA. Modulation of the synaptic drive to respiratory premotor and motor neurons. Respir Physiol. 1997;110:161–76.
197. Tonkovic-Capin V, Stucke AG, Stuth EA, Tonkovic-Capin M, Hopp FA, McCrimmon DR, Zuperku EJ. Differential processing of excitation by GABAergic gain modulation in canine caudal ventral respiratory group neurons. J Neurophysiol. 2003;89:862–70.
198. Tonkovic-Capin V, Stucke AG, Stuth EA, Tonkovic-Capin M, Krolo M, Hopp FA, McCrimmon DR, Zuperku EJ. Differential modulation of respiratory neuronal discharge patterns by GABA(A) receptor and apamin-sensitive K(+) channel antagonism. J Neurophysiol. 2001;86:2363–73.
199. Cinelli E, Bongianni F, Pantaleo T, Mutolo D. Modulation of the cough reflex by GABA(A) receptors in the caudal ventral respiratory group of the rabbit. Front Physiol. 2012;3:403.
200. Poliacek I, Rose MJ, Pitts TE, Mortensen A, Corrie LW, Davenport PW, Bolser DC. Central administration of nicotine suppresses tracheobronchial cough in anesthetized cats. J Appl Physiol. 2015;118:265–72.

201. Poliacek I, Wang C, Corrie LW, Rose MJ, Bolser DC. Microinjection of codeine into the region of the caudal ventral respiratory column suppresses cough in anesthetized cats. J Appl Physiol. 2010;108:858–65.
202. Tan W, Pagliardini S, Yang P, Janczewski WA, Feldman JL. Projections of pre Botzinger complex neurons in adult rats. J Comp Neurol. 2010;518:1862–78.
203. Fellin T. Communication between neurons and astrocytes: relevance to the modulation of synaptic and network activity. J Neurochem. 2009;108:533–44.
204. Hulsmann S, Oku Y, Zhang W, Richter DW. Metabolic coupling between glia and neurons is necessary for maintaining respiratory activity in transverse medullary slices of neonatal mouse. Eur J Neurosci. 2000;12:856–62.
205. Okada Y, Sasaki T, Oku Y, Takahashi N, Seki M, Ujita S, Tanaka KF, Matsuki N, Ikegaya Y. Preinspiratory calcium rise in putative pre-Botzinger complex astrocytes. J Physiol. 2012;590:4933–44.
206. Oku Y, Fresemann J, Miwakeichi F, Hulsmann S. Respiratory calcium fluctuations in low-frequency oscillating astrocytes in the pre-Botzinger complex. Respir Physiol Neurobiol. 2016;226:11–7.
207. Schnell C, Fresemann J, Hulsmann S. Determinants of functional coupling between astrocytes and respiratory neurons in the pre-Botzinger complex. PLoS One. 2011;6:e26309.
208. Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K, Kasparov S. Astrocytes control breathing through pH-dependent release of ATP. Science. 2010;329:571–5.
209. Chounlamountry K, Boyer B, Penalba V, Francois-Bellan AM, Bosler O, Kessler JP, Strube C. Remodeling of glial coverage of glutamatergic synapses in the rat nucleus tractus solitarii after ozone inhalation. J Neurochem. 2015;134:857–64.
210. McGovern AE, Short KR, Kywe Moe AA, Mazzone SB. Translational review: Neuroimmune mechanisms in cough and emerging therapeutic targets. J Allergy Clin Immunol. 2018;142:1392–402.