

Blessing, Chapter 4. Breathing, part 1

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Given the brain's continuous need for oxygen, we might expect the to and fro passage of air to be as involuntary as the circulation of blood. Probably because air movement is so useful for functions other than gas exchange, evolution has produced a unique combination of voluntary and involuntary mechanisms for respiratory control. Sniffing a rose, blowing out a candle, taking an extra breath—all are as voluntary as moving a limb. Yet voluntary failure to breathe is constrained by automatic, nonvoluntary mechanisms. Not even the opera singer can hold the note beyond the limits set by the brainstem respiratory control system.

Early studies of the neural regulation of respiration have been described by Gesell et al. (1936) and by Mitchell and Berger (1975). In 1812, Legallois found that breathing continues relatively normally in the rabbit when the hemispheres, the upper brainstem, and the cerebellum have been removed, but it ceases after transections through the upper medulla. Normal breathing is also terminated in otherwise intact animals by upper cervical cord transection. Primary regulation of respiratory activity is thus vested in the lower brainstem so that an *in vitro* brainstem-spinal cord preparation maintains a type of respiratory rhythm (Suzue, 1984). Brainstem respiratory control was originally interpreted in terms of the action of pneumotaxic, apneustic, inspiratory, and expiratory centers, as detailed by Lumsden, by Pitts, and by Wang and their colleagues (Lumsden, 1922, 1923a,b; Pitts et al., 1939a,b; Pitts, 1940; Wang et al., 1957). However, these centers remained poorly characterized hypothetical constructs, only vaguely related to actual brainstem regions. They did not provide an adequate theoretical framework for modern studies.

The intrathoracic pressures responsible for the to and fro passage of air are generated by the respiratory pump muscles. Other respiratory muscles, with a valve-like function, regulate the configuration of the upper airways, coordinating movements of the jaw, lips, tongue, pharynx, and larynx to ensure airway patency at the appropriate time. During normal respiration the vocal cords begin to separate slightly before the diaphragm begins to contract. The exquisite timing is illustrated by the coordination of valve and pump muscles during purring in the cat. The glottis opens and closes every 35-50 ms, yet peaceful breathing is maintained because the diaphragm contracts only when the glottis is open (Bartlett, 1989). Many of the valve muscles are also involved in nonrespiratory functions, such as ingestion, and both pump and valve muscles are involved in adapted respiratory functions, such as speaking. The valve muscles are controlled by motoneurons in cranial nuclei V, VII, IX, X, and XII. Motoneurons in XI control the sternomastoids, which function as accessory pump muscles as well as turning the head. Thus individual cranial nerve motoneurons, with both respiratory and nonrespiratory functions, are likely to display respiratory rhythms.

In contrast, since the phrenic nerve projects only to the diaphragm, the principal pump muscle, neural activity recorded in the proximal portion of the cut phrenic nerve is a very useful index of the discharge of inspiratory motoneurons in the spinal cord. These cells, like other somatic motoneurons, lack the capacity for spontaneous discharge. Their activity depends on descending excitatory and

inhibitory inputs and on local segmental influences. The ability of spinal respiratory motoneurons to integrate different inputs (excitatory [EPSPs] and inhibitory [IPSPs] postsynaptic potentials) was first demonstrated for intercostal respiratory motoneurons by the intracellular analysis of Eccles et al. (1962). In subsequent studies, occurrence of intracellularly recorded membrane potentials demonstrated that phrenic motoneurons are also depolarized during inspiration and hyperpolarized during expiration, as the neurons integrate supraspinal inputs from pontomedullary respiratory control centers (Gill and Kuno, 1963; Berger, 1979a; Monteau and Hilaire, 1991). Inputs to the pontomedullary respiratory control centers include those originating in forebrain and more rostral brainstem regions. The ventilatory response to increased PCO_2 , for example, is less when an individual is reading aloud than during silence (Phillipson et al., 1978). Other inputs to pontomedullary respiratory circuits originate in the periphery, in the upper airways and the lungs, in peripheral chemoreceptors, and in afferents from respiratory muscles. Information is conveyed to the CNS via afferents in relevant cranial and spinal nerves, resulting in respiratory modulation by hypoxemia, by airway obstruction, by submersion in water, and by exposure to smoke and other noxious gases. Coughing and sneezing occur in response to airway irritation. Various mechanical and chemical stimuli reflexly alter airway caliber and respiratory secretions. Cardiovascular and other visceral functions, via appropriate afferents, also affect respiratory rhythms and patterns. Pulmonary and systemic blood flows, for example, are closely linked with pulmonary ventilation. This chapter examines the brainstem neural circuitry responsible for the rhythms and patterns of respiration, together with the inputs to these neurons originating in receptors in the airways and in the peripheral chemoreceptors. Integration of breathing with cardiovascular and other homeostatic functions is considered in the chapters dealing with these functions. Since the different pictures of respiratory control systems have been shaped by the investigatory methods, these methods are considered in the following section.

Species and Methods Used in Studies of Central Respiratory Control

Soon after the introduction, in the 1930s, of microelectrodes for extracellular recordings, a search was made for lower brainstem neurons discharging in phase with respiration.

[With graded penetration there was an ever changing medley of action sounds—some weak, others loud; some discrete, others indiscrete, i.e. out of phase with one another; some of regular frequency, others of irregular sequence; some of high frequency and some of low frequency; and some fluctuating with the respiratory rhythm. (Gesell et al., 1936).]

The original work of Gesell et al. placed a healthy emphasis on anatomical localization of recording sites. Many subsequent studies neglected to document the brainstem sites from which the recordings were made; neurons were conceptualized predominantly in electrophysiological terms, with insufficient attention being paid to full neuroanatomical and neurochemical characterization. This omission is also sometimes evident in those neural network theories that see the generation of respiratory rhythms as reflecting complex interactions between ordinary neurons, with no particular

neuroanatomically defined cell group generating the rhythm. As might be expected, alternative theories relating respiratory rhythms to the activity of special pacemaker cells have placed more emphasis on fully characterizing these neurons.

Most early electrophysiological studies were performed on the cat. However, the neuroanatomy of the cat brainstem is still relatively poorly documented. The two frequently cited recovery intramedullary HRP-tracing studies in the cat (Bystrzycka, 1980; Kalia et al., 1979) omit detailed documentation of injection sites and distributions of labeled neurons. This lack of an adequate neuroanatomical theoretical framework may never be adequately remedied for the cat. Many electrophysiological studies are now being performed in the rat. Fortunately there is a wealth of careful neuroanatomical studies available for the rat. Few modern studies are being performed on the rabbit, a neglected opportunity given the suitability of this species for both functional and anatomical investigations. Thus the work of Jiang and colleagues (1991) in rabbits is particularly welcome. Functional localization of respiratory neurons and their synaptic connectivity can be investigated by purely electrophysiological methods. In orthodromic activation studies, a short latency between stimulation of an input pathway and consequent neuronal excitation or inhibition is used to infer monosynaptic or possibly disynaptic inputs to a particular neuron. However, this latency criterion is sometimes unreliable, since synaptic delay is only approximately 1 ms and latencies greater than 10 ms may be recorded, especially in studies of peripheral inputs to respiratory neurons. The requirement for short latencies in orthodromic studies is presumably a major factor underlying the paucity of studies directed at the role of unmyelinated fibers in peripheral afferents.

The presence of possible monosynaptic inputs from the brainstem to phrenic and thoracic motoneurons has also been assessed by cross correlations between extracellularly recorded medullary units and either phrenic or thoracic motoneuron activity or discharges in the intercostal or phrenic nerves (Cohen et al., 1974; Hilaire and Monteau, 1976; Kirkwood, 1995). The complexity of the assumptions underlying the cross-correlation procedure are evident in the discussion section of the paper by Kirkwood (1995), and some authors have questioned the validity of the procedure as a means of establishing monosynaptic connections between respiratory neurons (Fedorko et al., 1983, 1989b; Duffin and Lipski, 1987).

An alternative orthodromic electrophysiological method for establishing monosynaptic connections is that of spike-triggered averaging of membrane potentials. The spike of a trigger neuron is used, over many trials, to obtain a timed average of the intracellularly recorded membrane potentials in a target neuron, so that EPSPs or IPSPs can be distinguished from noise. Like all procedures, the technique has potential limitations. The amplitude of the potentials depends on how close the synaptic contacts are to the cell soma, so that inputs to dendrites may be difficult to detect. Each neuron in a large presynaptic population may provide weak inputs to each of many neurons in a postsynaptic population. When such divergence occurs, spike-triggered averaging may fail to detect synaptic inputs, even though sparse inputs to many neurons with a common output function may be of great physiological importance. The spike-triggered averaging method can produce false-positive results if the averaged membrane potentials actually reflect activity of a nontrigger neuron whose discharge is closely synchronized with the trigger neuron. Such synchronization is not rare in medullary systems

controlling breathing. All of these limitations notwithstanding, spike-triggered averaging studies are extremely valuable in determining the connections between the various types of respiratory neurons. Because of the technique, much progress has been made since Merrill (1981) noted the absence of direct evidence establishing synaptic interactions between any two medullary neurons.

Antidromic activation procedures are also used in electrophysiological studies, especially extracellular studies, so that neurons can be identified in terms of their axonal projections (Lipski, 1981). Since electrical stimulation readily activates axons of passage, antidromic activation of a neuron from a particular region does not establish that the axonal projection synapses in that region.

Introduction of neuroanatomical anterograde intraaxonal transport studies made it possible to identify inputs making close contacts with respiratory motoneurons. Appropriate ultrastructural procedures can document true synaptic relationships. However, a weakness with conventional anterograde studies is the impossibility of specific physiological or anatomical identification of the neuron that takes up the tracer. Major contributions are now emerging from laboratories with the skills to combine intracellular electrophysiological analysis with neuroanatomical techniques. Valuable studies of respiratory neuronal circuitry first used intracellular recordings to characterize particular neurons electrophysiologically and then used intracellular injections of anatomical tracers to determine the morphology and connectivity of the cell. Although this technique was used by von Euler (1973a) and by Kreuter and colleagues (1977), it was largely neglected by respiratory physiologists until revived in Berger's laboratory (Cameron et al., 1983; Berger et al., 1984). As will be apparent in the course of this chapter, subsequent utilization of this technique has made a major contribution to our understanding of the neural circuitry underlying brainstem respiratory control.

Events readily analyzed with electrophysiological recordings usually occur within milliseconds and, in the case of respiratory neurons, usually require anesthetized preparations. This has made it difficult to study longer term nonphasic events such as coughing and sneezing. Introduction of the fos neuroanatomical procedure has now provided an opportunity to investigate the central pathways mediating such events (Wallois et al., 1995).

Respiratory neurons can also be classified according to whether they excite or inhibit other respiratory neurons discharging in similar or different phases of the respiratory cycle. It is important to remind ourselves that individual respiratory neurons presumably have the same direct synaptic effects, either excitatory or inhibitory, on all neurons with which they synapse. Whether a neuron excites or inhibits its postsynaptic targets depends on the neurotransmitter substances it contains and on the postsynaptic receptors. Our present understanding is that it is unlikely that a given neuron causes EPSPs in one postsynaptic cell and IPSPs in another. This understanding may change as more is learned of the significance of the presence of more than one neurotransmitter agent in a given cell. At present it is wisest to presume that at least one phase of a biphasic (excitatory/inhibitory) respiratory effect produced by activation of a particular neuron must reflect an effect mediated by an interneuron rather than a direct synaptic connection with the target neuron being studied. This may not always be evident in the discussion sections of complex electrophysiological papers (Anders et al., 1991). It is only in recent years that respiratory electrophysiologists have begun to emphasize the importance of neurotransmitter identification in the subclassification of respiratory neurons.

Respiration continues after induction of general anesthesia. For unknown reasons, it is not until the so-called stage 4 of anesthesia, the stage of "medullary depression," that anesthetics abolish spontaneous breathing. This has proven fortunate for experimental investigation of brainstem regulation of respiration. Nevertheless, there are important differences in respiratory control between conscious and anesthetized subjects and between anesthetized and decerebrate animals. Brainstem excitation of the phrenic nucleus in anesthetized subjects ceases when CO₂ is reduced by hyperventilation and intensifies when CO₂ increases. However, in unanesthetized humans, apnea does not occur even when voluntary or mechanical hyperventilation reduces PCO₂ as low as 16 mm Hg (Comroe, 1974). The respiratory pattern also depends on body posture, sleep-waking state, emotional arousal, and exercise; these complexities are difficult or impossible to study in anesthetized animals.

Thus the same neuron could have different respiratory rhythms at different times, and some neurons with a respiratory function might have no obvious respiratory rhythm, or they might be silent in the preparations studied. This emphasizes the need for caution in the interpretation of the numerous rhythm studies that fail to identify the neurons by criteria other than their discharge patterns. A complete description of respiratory neurons must also include precise neuroanatomical location, connectivity, neurotransmitter content, membrane channel properties, and structural and metabolic characteristics.

Classifications of Lower Brainstem and Spinal Respiratory Neurons

Neurons with respiratory rhythms can be classified according to the relationship between neuronal discharge and phrenic nerve activity. Since phrenic discharge usually continues into the expiratory phase of respiration, inspiration (strictly defined as the time during which air is flowing into the lung) does not correspond exactly with phrenic nerve discharge. There is, therefore, some ambiguity concerning the definition of the firing patterns of respiratory neurons during the transition from inspiration to expiration. One influential theory, that of Richter and colleagues, envisages the respiratory cycle as consisting of three basic neural phases, one inspiratory and two expiratory (Richter, 1982; Schwarzacher et al., 1991). Phrenic nerve activity displays steadily increasing ramp activity during inspiration, followed by a rapid decline, a short period of silence, and then a resumption of weaker activity, steadily declining during the period of expiration from the passively recoiling lung. Discharge of other spinal motoneurons then produces active expiration (which may not occur during quiet shallow breathing), a process divisible into two phases. Stage 1 (postinspiration) occurs as phrenic nerve activity subsides, and stage 2 occurs shortly after cessation of phrenic discharge. Ezure's simple and helpful classification (1990) of respiratory neurons, based on their discharge pattern, is shown in Figure 4.1.

Figure 4.1 Electrophysiological classification of respiratory neurons. Inspiration is properly defined as the period during which air flows into the lung. For convenience in electrophysiological studies, inspiration is defined as the period of phrenic nerve discharge so that respiratory neurons are named according to the relationship of their discharge to this variable. Both the inspiratory (I) and the

expiratory (E) groups are divided into DEC (decrementing), CON (constant), and AUG (augmenting) subgroups. (Modified from Ezure, 1990.)

Unfortunately, different discharge patterns seem to be encountered by different laboratories, and the different terminologies are not always easily translatable from one laboratory to another. Experimental conditions have a marked affect on respiratory rhythms, particularly the intactness or otherwise of the cervical vagi and the degree to which neuronal discharge is entrained to the rhythm of the mechanical ventilator. Augmenting inspiratory neurons are also called late-peak neurons. Decrementing inspiratory neurons may also be called early-burst inspiratory neurons or pre-inspiratory neurons. Schwarzacher, Smith, and Richter (1995), who note the rather confusing way the term pre-inspiratory is used in different laboratories, reserve the term for a specific type of neuron that, "in addition to its discharge during stage 2 expiration and inspiration, shows a characteristic high frequency burst of action potentials during the transition from the expiratory to the inspiratory phase." Complexity in terminology is also illustrated by the explanatory note of Richter et al. (1992:791) concerning postinspiratory neurons that are said to correspond with decrementing expiratory neurons except that "postinspiratory neurons are characterized by a biphasic discharge before and after inspiration, i.e. postinspiration [sic]." Species differences may also affect terminology. As an example, Parkes and colleagues (1994) consider that rats lack a pronounced postinspiratory discharge in the phrenic nerve; thus for this species the definition of stage I expiratory neurons (postinspiratory) requires modification to include cells discharging immediately after the cessation of phrenic discharge.

Neurons with a primary respiratory function have also been classified according to the medullary region in which they are located. A word of caution is necessary, since many early studies failed to appreciate that cranial nerve motoneurons may display marked respiratory rhythms (Fig. 4.2), as might be expected given their prominent valve-like functions.

Figure 4.2 Many cranial motoneurons have marked respiratory rhythms, as exemplified by this augmenting inspiratory neuron antidromically activated from the glossopharyngeal nerve and identified by intracellular electrophysiological recording and subsequent HRP filling. (Modified from Bianchi et al., 1988.) Abbreviations listed on pages xiii-xiv.

Such rhythms are particularly prominent when recordings are made in decerebrated but otherwise unanesthetized preparations (Bianchi et al., 1988; Barillot et al., 1990; Zheng et al., 1991a,b; 1992a,b; Bryant et al., 1993). Antidromic activation from the peripheral cranial nerves identifies the neuron as a motoneuron. Antidromic activation from a spinal or brainstem site identifies the neuron as a primary respiratory cell. However, failure to activate a neuron antidromically is relatively weak evidence that the neuron does not project to the stimulation site. In the periphery, the stimulating electrode may not be around the appropriate branch of the appropriate cranial nerve. Pharyngeal branches of the vagus and glossopharyngeal nerves are not easily accessible. When careful investigators have used intracellular fill techniques to identify ventrolateral neurons with marked

respiratory rhythms histologically, many filled cells have unexpectedly proven to be motoneurons (Pilowsky et al., 1990b; Zheng et al., 1991a,b; Bryant et al., 1993; Parkes et al., 1994). In the ventrolateral medullary region encompassing the compact and loose subdivisions of the nucleus ambiguus (Bieger and Hopkins, 1987), esophageal motoneurons are among the few cranial motoneurons that do not have a respiratory rhythm (Kruszewska et al., 1994).

Classification of neurons with obvious respiratory rhythms according to their anatomical location was first formulated in the cat (Bianchi, 1971), and the numerous studies in this species are referenced by Ezure (1990) and later in this chapter. With some variation, the same groups are also present in the rat (Saether et al., 1987; Ezure et al., 1988; Pilowsky et al., 1990b; Núñez-Abades et al., 1991; Schwarzacher et al., 1991; Zheng et al., 1992a,b; DeCastro et al., 1994), the rabbit (Jiang et al., 1986, 1987; Ellenberger et al., 1990c; Jiang and Shen, 1991) and the guinea pig (Richerson and Getting, 1992). The different groups are summarized below and discussed in detail in appropriate portions of this chapter. Their anatomical locations in the rat are summarized in Figure 4.3, from a neuroanatomical study using transneuronal transport of pseudorabies virus from the phrenic nerve (see later in this chapter).

Figure 4.3 Transverse sections and horizontal hemisection through the medulla and lower pons summarizing the successive distribution of virus-containing neurons after application of pseudorabies virus to one phrenic nerve. The different respiratory groups are explained in the text. (Modified from Dobbins and Feldman, 1994.) Neuroanatomical abbreviations listed on pages xiii-xiv.

The dorsal respiratory group (DRG), in the nucleus tractus solitarius (nTS) and just ventrolateral to the tractus solitarius, contains bulbospinal inspiratory neurons in the cat. The corresponding region in the rabbit contains both inspiratory and expiratory bulbospinal neurons (Jiang et al., 1987). Rat DRG respiratory neurons were thought to be absent or sparse (Saether et al., 1987; Ezure et al., 1988; Zheng et al., 1991a), but a careful study confirms the presence of a group of principally inspiratory neurons in the ventrolateral nTS in this species (DeCastro et al., 1994). The caudal ventrolateral respiratory group (cVRG), in the ventrolateral medulla generally caudal to the level of the area postrema, contains bulbospinal expiratory neurons that excite internal intercostal and abdominal motoneurons in the ventral horn of the thoracolumbar cord. The rostral ventrolateral respiratory group (rVRG), in the ventrolateral medulla at the level of the area postrema and rostral to this structure, contains bulbospinal inspiratory neurons projecting to the phrenic nucleus and to the thoracic ventral horn. Further rostrally, in the rostral ventrolateral medulla, ventral and ventromedial to the compact division of the nucleus ambiguus, is the Böttinger complex (BötC) group of principally expiratory bulbospinal premotoneurons and the expiratory interneurons. The retrotrapezoid nucleus, ventral to the facial nerve nucleus in the most rostral medulla and caudal pons, contains inspiratory and expiratory neurons with projections to the cells in rVRG. Finally, the pre-BötC group, just caudal to the BötC group and rostral to the rVRG group, has been proposed to contain pacemaker neurons that generate respiratory rhythms.

A More Anatomical Classification of Respiratory Neurons

Given that many of the properties of respiratory neurons are still undiscovered, it is probably wise to keep in mind the following basic classes of respiratory cells, acknowledging the possibility of functional overlap between neurons in classes 2, 3, and 4. Some interneurons may be premotor neurons or secondary sensory neurons.

Motoneurons of lower cranial, phrenic, and intercostal nerves

These motoneurons vary in the extent of their respiratory and nonrespiratory functions. As already indicated, the discharge of many lower cranial motoneurons is important in the regulation of valve muscle respiratory function. Monteau and Hilaire (1991) have written a very useful review of spinal respiratory motoneurons; these are not considered in any detail in this book.

Respiratory premotor neurons (including bulbospinal neurons)

All lower brainstem neurons with direct inputs to spinal cord and/or brainstem respiratory motoneurons can be classified as premotor respiratory neurons. Neurons with obvious respiratory rhythms such as those in the rVRG region have been extensively studied, but other respiratory premotor neurons (defined in the present manner) are located outside the recognized brainstem respiratory subregions, and they may lack obvious respiratory rhythms (e.g., Lindsey et al., 1992). Neurons in the pontomedullary raphe nuclei fall into this category. Such neurons may also be involved in control of nonrespiratory functions. Respiratory premotor neurons distribute axon collaterals to neurons other than motoneurons (Ezure and Manabe, 1989), and in this respect they presumably also function as respiratory interneurons. Obviously a great deal of experimental attention is directed to neurons with obvious respiratory rhythms, in recognized respiratory subregions, and defined as premotor by antidromic activation from the spinal cord.

Respiratory interneurons (propriobulbar, segmental, and propriospinal neurons)

These neurons include medullary or spinal cells with a marked respiratory rhythm not belonging to the class of cranial nerve motoneurons and not directly innervating motoneurons. Respiratory interneurons and respiratory premotor neurons may be difficult to distinguish experimentally, since it can sometimes be difficult to prove the absence of an axonal projection to a motoneuron. As noted in the introduction to this section, the distinction between interneurons and premotor neurons need not be overemphasized. Medullary pacemaker neurons presumably belong to the class of respiratory interneurons, although they could project directly to motoneurons. Presumably, brainstem neurons primarily involved in functions other than respiration, and lacking a marked respiratory rhythm, also project to respiratory premotor neurons.

The upper cervical spinal cord also contains respiratory interneurons (Lipski et al., 1993). Their functional role and precise synaptic targets have not yet been identified. In the cat the neurons seem to have only a limited projection to the phrenic nucleus.

Medullary neurons directly innervated by respiratory primary afferents

These spinal and medullary neurons receive direct inputs from respiratory primary afferent neurons, and they are therefore located in medullary regions in which primary afferents terminate, principally the nTS, but also, in the case of chemoreceptor afferents, in the caudal ventrolateral medulla. Some respiratory interneurons may also be secondary afferent respiratory neurons.

Primary afferent neurons with respiratory functions

These peripheral neurons, with cell bodies in trigeminal, petrosal, nodose, and dorsal root ganglia, convey information to the CNS concerning the contraction state of striated respiratory muscles, the PO₂ and PCO₂/acidity of the blood, the presence of mechanical/thermal/chemical/nociceptive stimuli in the upper and lower airway passages, and the contraction state of airway smooth muscle.

Neuroanatomical Studies in Respiratory Regions of the Lower Brainstem and Spinal Cord

Central pathways that could underlie various respiratory functions have been the subject of conventional neuroanatomical studies, sometimes conducted without reference to similar studies of nonrespiratory neurons in similar brainstem regions. Investigators interested in different physiological functions have given different functionally committed names to the same medullary regions. Neuroanatomical studies utilizing intracellular or intra-axonal injection of tracing agents are considered later in this chapter. Other neuroanatomical studies are reviewed in this section.

Projections to motor respiratory nuclei in the spinal cord studied with conventional neuroanatomical procedures

Ellenberger and Feldman (1990b) and Ellenberger and colleagues (1990b) injected fluorogold into the rat phrenic nucleus and related the labeled neurons in the ventrolateral medulla to vagal preganglionic cells in the nucleus ambiguus, to A1 and C1 catecholamine neurons, and to neurons, presumably respiratory interneurons, projecting to the cVRG. Within the ventrolateral medulla these neuronal populations were intermingled but independent. Other multilabel retrograde transport studies have also been carried out in the rat (Núñez-Abades et al., 1991; Portillo and Núñez-Abades, 1992). Onai and colleagues (1987) studied all medullary and pontine neurons retrogradely labeled when HRP was injected into the region of the phrenic nucleus in the rat. Extremely few HRP-containing neurons were present in the nTS region. Most labeled neurons were present in the ventrolateral medulla, principally ipsilaterally, most dense at the level of the obex, but extending from 2 mm caudal to the obex up to the level of the facial nucleus. Unfortunately maps of the distribution of the labeled neurons are not provided. Efforts were made to confine the HRP to the phrenic nucleus. However, HRP is avidly taken up by fibers of passage, complicating the interpretation of this study.

Although the cat is frequently used in physiological studies of respiration, the species has not been as intensively studied with conventional neuroanatomical procedures. Retrograde studies of neocortical and brainstem projections to the region of the phrenic nucleus were completed by Rikard-Bell and colleagues (1984, 1985), by Holtman and colleagues (1984), by Onai and Miura (1986), and by

Portillo and colleagues (1994). In the medulla, retrogradely labeled neurons were found in the DRG region ventrolateral to the tractus solitarius, bilaterally with marked contralateral predominance. Few or no spinally projecting neurons were found in the nTS dorsal or medial to the tractus solitarius. The studies differ in their account of phrenic-region projections of cVRG neurons. Rickard-Bell and colleagues found many labeled neurons in the cVRG region, but Onai and Miura noted the absence of labeled neurons in this region. Both studies report many retrogradely labeled neurons in the rVRG and probably in the BötC. Descending cells were also found in raphe nuclei and in the parabrachial (Kölliker-Fuse) region of the pons.

In the rabbit, after injection of tracer into the phrenic region, retrogradely labeled neurons are found in medullary and pontine regions generally similar to those observed in rat and cat except that the projections have ipsilateral predominance, and neurons in the presumed DRG are ventral, rather than ventrolateral, to the tractus solitarius.

Conventional anterograde anatomical studies have also been carried out in cats, rats, and rabbits, with electrophysiological identification of the respiratory areas. After injection of tritiated amino acids into the cVRG and rVRG in cats, autoradiographic analysis reveals silver grains concentrated within phrenic and intercostal nuclei, suggesting direct inputs to spinal motoneurons from the medullary respiratory cells (Feldman et al., 1985). After ipsilateral injection of Phaseolus vulgaris leucoagglutinin (PHA-L) into either the rVRG or the BötC, bulbospinal axons are labeled bilaterally (Ellenberger and Feldman, 1988; Feldman et al., 1985; Ellenberger et al., 1990c; Yamada et al., 1988; Goshgarian et al., 1991). Axons descend in the lateral and ventral funiculi of the contralateral spinal cord, after passing dorsomedially and decussating dorsal to the central canal. Other axons first ascend, decussate in the more rostral medulla, and descend through the contralateral cVRG into the lateral and ventral funiculi of the spinal cord. A small proportion of fibers descend through the cVRG without decussating. Some axons decussate at the level of the phrenic nucleus (Goshgarian et al., 1991). As the descending axons reach the C3-C5 segments, entire axons, or their local branches, then pass medially to form close contacts with the perikarya and dendritic trees of phrenic motoneurons. The pattern of axonal arborization within the phrenic nucleus suggests that individual medullary premotor neurons contribute only a sparse input to individual phrenic motoneurons, but each phrenic motoneuron is innervated by many premotor neurons. As a consequence, individual premotor neurons presumably have minimal effects on individual phrenic motoneurons. This means that both correlational analysis and spike-triggered averaging techniques are likely to underestimate the direct synaptic input from the medullary respiratory neurons.

Ultrastructural studies of phrenic neurons after wheat germ agglutinin-HRP injections into rat rVRG have established the presence of direct synapses between neurons in this region and phrenic motoneurons, with contacts on the perikaryon and on the dendritic tree (Ellenberger et al., 1990a). Other ultrastructural studies of identified phrenic motoneurons demonstrate synaptic inputs from 5-HT-containing neurons, suggesting an input from 5-HT groups in the medulla or lower pons (Pilowsky et al., 1990a).

Studies based on transneuronal transport of viruses

Conventional anatomical studies have been supplemented by a transneuronal viral tracing study in the rat (Dobbins and Feldman, 1994). The results of this important study are summarized in Figure 4.3. Application of virus to the phrenic nerve labeled phrenic motoneurons (first-order labeling), respiratory premotor neurons (second-order labeling), and respiratory interneurons (third-order labeling) in the medulla and the pons. Lower brainstem neurons projecting to the phrenic nucleus were found in the ventrolateral medulla in the rVRG and the BötC, but not in the cVRG. Other second-order neurons were found in the gigantocellular reticular nucleus lateral to the raphe in the rostral medulla, in the ventrolateral nTS, in the caudal raphe (especially raphe obscurus), and in the Kölliker-Fuse-parabrachial complex. Labeled premotor neurons appeared bilaterally in the ventrolateral nTS at the level of the area postrema, with slight contralateral predominance. The number of labeled nTS neurons was about 10% of the number of labeled rVRG neurons. The viral tracing procedure therefore suggests that the dominant pathway from the brainstem to the phrenic nucleus in the rat is a monosynaptic projection from rVRG, with approximately equal ipsilateral and contralateral contributions. No second-order labeling was observed in cVRG, and the anatomical study thus indicates that cVRG bulbospinal neurons do not directly innervate the phrenic nucleus. Second-order labeling in the BötC region, bilateral with strong ipsilateral predominance, was more extensive than expected on the basis of electrophysiological identification of bulbospinal expiratory neurons in this region of the rat medulla (Bryant et al., 1993).

Intramedullary Connections Between Respiratory Neurons Studied With Conventional Neuroanatomical Methods

Numerous studies of intramedullary neural connections are now available. As already stressed, functional interpretation of these studies often depends on the point of view of the investigator. The same applies to the names given to the various regions. Respiratory neurons in the rVRG, for example, are intermingled with motoneurons of the caudal nucleus ambiguus, with A1 catecholamine neurons, and with vasodepressor neurons (see Chapters 3 and 5). When neuroanatomical tracing agents are injected into this region they will presumably be taken up by neurons in all three classes, by axons innervating all three classes of cells, and possibly by fibers of passage. The best that conventional neuroanatomical studies of intramedullary pathways can achieve is the documentation of the neural connections postulated to underlie particular functions. Summaries of these connections, emphasizing a respiratory viewpoint of medullary anatomy, can be found in Ellenberger and Feldman (1990a) and Smith et al. (1989).

Parasympathetic and Sympathetic Innervation of Lower Airways

Airway resistance from the trachea down to the small bronchi is affected by tone in smooth muscle in these airways. Muscarinic cholinergic receptors in the muscle are innervated by postganglionic parasympathetic neurons with cell bodies in the muscle itself. These neurons, in turn, are innervated by vagal efferents. Preganglionic cell bodies are located in the medulla, in the rostral dorsal motor nucleus of the vagus, and in the nucleus ambiguus (see Chapter 3). Inputs to these neurons in the rat

have been defined by a retrograde transneuronal study after injection of pseudorabies virus into the tracheal wall in spinalized rats (Haxhiu et al., 1993).

Airways smooth muscle is also regulated by sympathetic postganglionic fibers, which increases tone via stimulation of α -adrenergic receptors and decreases tone via stimulation of β -adrenergic receptors. Activation of neurons in the ventrolateral medulla can affect the tone of bronchial smooth musculature (Connelly et al., 1987; Haselton et al., 1991), possibly via both parasympathetic and sympathetic pathways.

Receptors in Airways and Lungs: Reflexes to Which They Give Rise

The airways and the lungs, from the nose to the alveoli, contain diverse receptors whose activation gives rise to neuronal impulses in afferent cranial nerves I, V, IX, and X, thereby initiating a variety of centrally mediated responses including specific respiratory responses such as sniffing, sneezing, and coughing; behavioral arousal; and cardiovascular and visceral reflexes associated with changes in respiratory activity. The receptors and their nerve fibers, as well as the axon reflexes and the centrally mediated responses to which their activation gives rise, have been studied over the years, especially by Breuer, Hering, von Bezhold, Brodie, Jarisch, Paintal, Widdicombe, and Coleridge and Coleridge, (for reviews, see Fillenz and Widdicombe, 1971; Paintal, 1973; Comroe, 1974; Coleridge and Coleridge, 1984, 1986, 1995; Widdicombe, 1986; Coleridge et al., 1989; Kubin and Davies, 1995). It is only in recent years that detailed attention has been directed to the central pathways mediating the reflex changes.

Most receptors were first studied by electrophysiological recordings from their afferent nerves. This naturally led to classification by adequate stimulus, by conduction velocity in the afferents, and by degree of adaptation in axonal discharge with continued stimulation of the receptors. Many studies of centrally mediated responses are based on electrical stimulation of the various afferent nerves, sometimes with stimulation parameters, or blocking procedures, designed to differentially activate myelinated or unmyelinated fibers. Stimulation studies present the usual problem of functional identification of the stimulated axons, but they have the advantage that a reliable response may be elicited, and the precise timing of the stimulus means that effects on identified CNS neurons can be established.

In most cases little is known about the structure or the transduction mechanism of the various receptors or about the disposition of afferent nerves in the airways and lungs. Previously it was difficult to distinguish these afferents from intrinsic parasympathetic axons. Now it should be possible to identify the afferents by anterograde transport of agents such as PHA-L from ganglia such as the nodose ganglion of the vagus. However, such studies are not yet available. In this chapter the types of receptors and the reflexes to which they give rise are briefly described so that evidence concerning the relevant central pathways can be evaluated in a proper context.

Mechanoreceptors

The epithelium lining of the nasal passages, the pharynx, larynx, and major airways, is very sensitive to light touch, giving rise to impulses in myelinated fibers of the relevant cranial nerves. In the awake

individual these impulses result in conscious awareness of the stimulus as well as vigorous reflex responses, generally interpretable as protective in the absence of disease states. Mechanical stimulation of the nose may cause apnea or sneezing. Misdirected food particles cause intense laryngeal discomfort, together with closure of the glottis, and coughing. In experimental work, the particular response elicited is often difficult to predict, depending a great deal on the state of the CNS. Sullivan and colleagues (1978) showed in dogs that an apparently unvarying laryngeal stimulus produced apnea, expiratory effort, coughing, or even swallowing, with some of the variability reflecting the effects of anesthesia or the sleep-wakefulness state of the unanesthetized animal. The various respiratory responses to stimulation of mechanical receptors are reviewed by Widdicombe (1986). In humans, dramatic cardiovascular responses, including hypotension in adults and hypertension in children, may occur during endotracheal suctioning, intubation or extubation, or during laryngoscopy or bronchoscopy (E.T. Cunningham et al., 1992).

Irritant receptors in the upper and lower airways, including pulmonary "J" receptors (previously called rapidly adapting receptors)

Any interchange with the environment is fraught with danger, and the process of breathing is no exception. Irritants and toxins must be kept out of the upper and lower airways. If they gain entry, the damage they cause must be minimized. The respiratory system contains numerous detection mechanisms for foreign irritants. Receptors in the nose and upper airways have been summarized by Fillenz and Widdicombe (1971) and by Widdicombe (1986). Stimulation of these receptors may evoke apnea, glottic closure, coughing, or sneezing. Cardiovascular changes including bradycardia and marked peripheral vasoconstriction may follow application of cold water to the face or inhalation of irritant agents such cigarette smoke, ammonia, or formaldehyde (see discussion in Chapter 5). Many reflexes elicited from the nasal mucosa are presumably dependent on activity in unmyelinated afferents, called by Widdicombe "the neglected afferent system of the upper respiratory tract." This neglect is part of a general under-estimation of the importance of visceral afferents in neuroscience, as discussed in Chapter 1.

Receptors in the lower airways and in the lung are also important; irritant receptors in these regions, and the responses to which they give rise, are reviewed by Coleridge and Coleridge (1984, 1986) and by Coleridge et al. (1989). Diverse responses include contraction of airways muscle and secretion from submucosal glands, changes in respiration and circulatory control, changes in arousal state, changes in behavior, and initiation of the sensation of dyspnea.

Application of nicotine (in cigarette smoke) to the lower trachea increases secretion from submucosal glands in the upper trachea (Schultz et al., 1991). This response depends on unmyelinated vagal pulmonary afferents, augmented after several seconds by activation of carotid sinus chemoreceptor afferents. Inhalation of capsaicin or nicotine causes cough and broncho-constriction in humans, via activation of airway afferents (Hansson et al., 1994).

Excessive deflation of the lung (by tracheal suction or by production of a sudden pneumothorax) or maintained hyperinflation of the lung increases the force and frequency of respiratory effort. The response is prevented by moderate cooling of the vagus and is thought to depend on myelinated

afferents (Coleridge and Coleridge, 1986), although this is controversial. These responses, studied mainly in the cat, were originally said to be mediated by rapidly adapting stretch receptors, and this term is still frequently employed. However, Widdicombe (see references above), noting that certain pulmonary receptors are vigorously, but transiently, excited by respiratory irritants such as ammonia, dust, and smoke, suggested that so-called rapidly adapting receptors are actually irritant receptors. By evoking cough and other protective reflexes, these receptors function to protect the respiratory tree from inhaled foreign bodies and other lung insults.

Whether all so-called rapidly adapting stretch receptors are irritant receptors in the airway epithelium is not established, particularly because irritant receptors may also be associated with unmyelinated nerve fibers, and these are more difficult to study. The continued use of rapid deflation and maintained hyperinflation to stimulate "rapidly adapting receptors" is confusing because it directs attention away from physiological function. More emphasis should be placed on distinguishing which receptors are being activated and whether information from the receptors travels in myelinated and/or unmyelinated vagal afferents. Careful analysis in the rat documents the importance of unmyelinated vagal afferents, while indicating that rapidly adapting receptors are rare in this species (Bergren and Peterson, 1993).

Receptors in the lung also respond during congestion of the pulmonary capillaries, as occurs with left ventricular failure, and in response to pulmonary embolism. Some of the bradycardia and hypotension observed in these clinical situations may therefore be secondary to vagally initiated reflexes rather than reflecting primary circulatory distress. This was initially suggested by Brodie (1900) and discussed by Whitteridge (1950). Vagal afferents originating in cardiac ventricular receptors may also be important (Bell et al., 1993). Injection of phenylbiguanide into the right atrium also causes marked hypotension and bradycardia (Bezhold-Jarisch reflex). Paintal observed in cats that this procedure evokes potentials in slowly conducting vagal afferents. The relevant receptors are located in the interstitial lung tissue, close to the pulmonary capillaries—thus the designation "J" receptors (juxtapulmonary capillary) coined by Paintal (see review by Paintal, 1977). Active chemical agents include phenyldiguanide (now recognised as a 5-HT₃ receptor agonist), lobeline, nicotine, capsaicin, and certain opiate agonists. There are species differences, and 5-HT does not produce the pulmonary chemoreflex in dogs or humans.

The functional significance of J receptor activation by chemical agents is not well understood. The relevant response, present in fish, stops circulation through the gills, thereby preventing absorption of toxic agents (see discussions by Comroe, 1974; Coleridge and colleagues, 1991). Central pathways mediating the Bezhold-Jarisch reflex are considered in Chapter 5.

Airway and respiratory sensations, including dyspnea

As noted in the previous section, Widdicombe (1986) emphasized how little study has been directed to upper airway afferents. Sensations mediated by such afferents include touch, pain, temperature, sensation of airflow, tickling, irritation, nasal rawness and stuffiness, and that associated with the desire to sneeze or cough.

Definition of sensations arising from the lung itself is complicated by the pleural innervation. Of major interest is the sensation of dyspnea. The dramatic distress associated with this sensation is frequently encountered by emergency room physicians managing patients with pulmonary edema, pulmonary embolism, pneumonia, as well as obstructive and restrictive airways disease. People in normal health experience dyspnea during vigorous exercise, a factor that helps to keep muscular activity within physiologically safe limits. Presumably in this situation the sensation occurs as a "psychical adjunct of an imperative protective reflex," the phrase used by Sherrington (see Chapters 1 and 6).

Complex mechanisms initiate the sensation of dyspnea. The first point to emphasize is that this sensation, like nausea (see Chapter 7), is usually initiated by activation of receptors located in the periphery rather than in the CNS. Previous theories stressed the relationship between dyspnea and increase in the level of central motor command necessary to drive the respiratory muscles, with much less emphasis being placed on the state of the blood gases or activation of airways and lung receptors; further studies, including work with ventilator-dependent quadriplegic patients, suggest that blood gas abnormalities may cause dyspnea (see discussion by Manning and Schwartzstein, 1995) and references below to the work of Gandevia and colleagues.

Nevertheless, the sensation of dyspnea is not directly related to the state of the blood gases or the blood acidity. In healthy subjects, voluntary breath holding can be prolonged beyond the normal breaking point if the subject is allowed a few breaths of a gas mixture that decreases arterial PO_2 and increases PCO_2 (Fowler, 1954). Chronic changes in the blood gases, such as the chronic hypercapnea that may accompany chronic obstructive airways disease, may not be associated with any sensation of dyspnea. Moderate hypoxia is not a very potent or common cause of dyspnea (see references in Manning and Schwartzstein, 1995).

The sensation of dyspnea induced by increasing PCO_2 via a rebreathing technique in healthy subjects is reduced when the vagus nerve is functionally inactivated. On the other hand, patients with high spinal transection still experience dyspnea with hypercapnea and with respiratory conditions such as pulmonary edema. Evidence for this is documented by Fillenz and Widdicombe (1971) and by Paintal (1977, 1995). These authors consider that dyspnea may result from impulses initiated by pulmonary J receptors. It may be that receptors also signal the CO_2 content of mixed venous blood, although this has never been proved.

Guz and colleagues (Guz et al., 1966a,b; Guz and Trenchard, 1971; Guz, 1977) found that anesthetizing their own vagi and glossopharyngeal nerves reduced the sensation of dyspnea and the reflex respiratory responses to increased PCO_2 (Fig. 4.4A). Arterial PO_2 was maintained at very high levels. Under this condition arterial chemoreceptors are very insensitive to PCO_2 , and Guz concluded that lung afferents, reaching the CNS via the vagus, are important for the sensation of dyspnea and for the drive to inspiration during breath holding. Gandevia and colleagues (1993) participated in a study in which they were paralyzed (atracurium) and ventilated, but not anesthetized. In this situation arterial PCO_2 could be increased without change in respiratory muscle effort, tension, or length. Progressive hypercapnia still caused progressive dyspnea (Fig. 4.4B), suggesting that afferent

activity from respiratory muscles signaling effort, tension, or length is not essential to the sensation of dyspnea.

Figure 4.4 A, Effect of hypercapnia on minute ventilation and respiratory rate in humans after bilateral blockade of vagus and glossopharyngeal nerves with lignocaine. (Modified from Guz et al., 1966a.) **B**, Dyspnea in a conscious paralyzed, ventilated human during increase in PCO_2 initiated by rebreathing. Oxygen saturation was maintained at greater than 98% at all times. (Modified from Gandevia et al., 1993.)

Slowly adapting stretch receptors in airway smooth muscle: Breuer-Hering lung inflation and deflation reflexes

The respiratory effect of acute bilateral vagotomy in anesthetized animals was described in the 1800s. Tidal volume increases, and the frequency of breathing slows, suggesting that vagal afferents normally inhibit a central inspiratory center, interrupting inspiration before maximal inflation occurs. The various respiratory responses to relatively physiological lung inflation and deflation are now collectively known as *Breuer-Hering reflexes*. Cajal (1909:614) presented a diagram summarizing the neuroanatomical pathways mediating lung inflation reflexes. Pitts (1940) explains that this reflex was one of the earliest forms of negative feedback in physiological thinking, dominating early theories of respiratory control even though it should have been obvious that pulmonary stretch reflexes, by themselves, could not regulate arterial O_2 and CO_2 , a physiological point also noted by Cajal (1909). Breuer-Hering reflexes were placed in a more realistic context after the discovery of peripheral chemoreceptors by Heymans in the 1920s. Now it is understood that inflation reflexes may function to increase the efficiency of breathing, ensuring the most alveolar ventilation for the least effort by the respiratory muscles.

The Breuer-Hering responses have been documented by measuring phrenic nerve activity and altering inflation. Inflating the lungs at reasonably physiological rates in anesthetized animals during inspiration prematurely terminates phrenic discharge. If the inflation is maintained into expiration, expiratory time is lengthened and frequency of respiration is reduced. Conversely, deflating the lungs at reasonable rates increases the frequency and amplitude of phrenic nerve activity. These reflexes depend on myelinated vagal afferents (conduction velocity 15-60 m/s in cat) originating in slowly adapting stretch receptors in smooth muscle within intrapulmonary airways. Activity of these receptors increases during normal inspiration, facilitating, at least in anesthetized animals, a respiratory off-switch. Their continuing discharge in expiration lengthens the respiratory pause. In addition to effects on respiration, activity of slowly adapting stretch receptors relaxes tracheo-bronchial smooth muscle, thus dilating the airways (Coleridge and Coleridge, 1986).

Airway stretch receptors with unmyelinated vagal afferents: Head's paradoxical inflation reflex

Head cooled the vagi in anesthetized rabbits and studied inflation reflexes. As the nerves warmed up, moderate inflation of the lung enhanced rather than inhibited inspiration. This paradoxical response is

sometimes ascribed to stimulation of rapidly adapting stretch receptors, but it probably reflects activation of unmyelinated vagal afferents, still functioning at temperatures too low for myelinated fibers to function (Coleridge and Coleridge, 1986). During normal breathing the response is not observed because myelinated stretch receptor fibers mediating inhibitory Breuer-Hering reflexes are active. The paradoxical response is important because it suggests that some afferent vagal C-fibers supplying the lower airways may normally be tonically active.

Arterial chemoreceptors and the reflexes to which they give rise

Chemoreceptors in the carotid and aortic bodies respond to decreases in oxygen supply (resulting either from decreased PO_2 , or from decreased blood supply) and to increases in PCO_2 and/or decreases in the pH of the arterial blood (Heymans and Neil, 1958). Impulses from carotid body chemoreceptors travel in the sinus nerve, together with baroreceptor afferents from the carotid sinus, joining the glossopharyngeal nerve just distal to the petrosal ganglion. Impulses from aortic body chemoreceptors travel in the aortic depressor nerve or in chemoreceptor fibers linked with the vagus nerve. Whether there are CO_2 receptors in the territory perfused by the pulmonary arteries is occasionally debated but seems unlikely. The brain itself also contains neurons directly sensitive to changes in PO_2 and to changes in PCO_2 and/or pH.

Activity in chemoreceptor afferents varies with the changes in blood gas indicators (particularly PCO_2 and pH) that accompany each respiratory cycle. The responsiveness of the system is sufficiently fast for chemoreceptors to modulate respiration on a breath to breath basis. The ventilatory effects of brief chemoreceptor inputs depend on the phase of respiration (Eldridge and Millhorn, 1986). More prolonged acute stimulation of the peripheral chemoreceptors by an increase in PCO_2 or a decrease in pH, or by moderate to severe hypoxia, increases tidal volume and frequency of respiration, with an associated increase in bronchiolar tone. Conversely, phrenic nerve discharge generally ceases in hyperventilated anesthetized animals (Heymans and Neil, 1958; Comroe, 1974). A wide range of other physiological and behavioral indices are also affected. Interactions make these effects quite complex. Increased chemoreceptor activity usually results in regionally selective vasoconstriction, with bradycardia, reduced cardiac output, and sometimes hypertension. Cerebral cortical rhythms become desynchronized, and the individual may move or struggle. Adrenal cortical and medullary secretions are increased. Marshall (1994) has written a comprehensive review on the physiology of peripheral arterial chemoreceptors and the effects that their stimulation evokes.

Neuroanatomical Studies of CNS Termination Sites of Afferents from Airways, Lungs, and Arterial Chemoreceptors

Upper airways afferents

The nasal mucosa is innervated by the ethmoidal branch of the first division of the trigeminal nerve. Anton and Peppel (1991) applied WGA-HRP to the nasal mucosa of the rat and studied the pontomedullary distribution of the primary afferents. Labeled terminals were observed ipsilaterally in the ventral spinal trigeminal nucleus (subnucleus interpolaris and subnucleus caudalis) from the level of the area postrema to the pyramidal decussation. No labeling was observed in the principal sensory

nucleus of the trigeminal nerve. The only other pontomedullary labeling was observed in the nTS, bilaterally in the interstitial subnucleus on the dorsolateral aspect of the tractus. When WGA-HRP is applied to the ethmoidal nerve in the cat there are labeled cell bodies in the mesencephalic nucleus of V, and labeled terminals in the spinal nucleus of the trigeminal nerve, but nTS labeling is not reported (Lucier and Egizii, 1986).

Superior laryngeal afferents

Studies of the central projections of nerves innervating the larynx have been studied in the rat by Hamilton and Norgren (1984), in the rabbit by Hanamori and Smith (1989), and in the cat by Kalia and Mesulam (1980b), Nomura and Misuno (1983a), Lucier and colleagues (1986), and Bellingham and Lipski (1992). The latter study demonstrated that the internal branch of the superior laryngeal nerve in the cat is a purely sensory nerve. Incoming medullary rootlets of this nerve traverse the spinal nucleus and tract of the trigeminal nerve from the level of the obex to approximately 4 mm rostral to the obex. Between 2 and 4 mm rostral to the obex some terminals were observed just ventral and ventrolateral to the tractus solitarius. Between 1 and 2 mm rostral to the obex most terminals are seen in medial, intermediate, dorsolateral, ventral, interstitial, and ventrolateral subnuclei. The terminals do not extend more than "one tractus solitarius diameter" ventrolateral to the tractus. More caudally there are many fewer terminals in the medial subnucleus. Most terminals at the level of the area postrema are found around the dorsal, lateral, and ventral aspects of the tractus solitarius. Some terminals are found in the commissural nucleus. The projection is bilateral with marked ipsilateral predominance.

Lung and lower airway afferents

These are considered below in the sections on secondary afferent neurons and the DRG.

Chemoreceptor afferents

WGA-HRP has been injected directly into the carotid body, with attempts made to confine the tracer to this organ. In the rat, labeled fibers enter the dorsolateral medulla at the level of the facial nucleus and terminate in the nTS, bilaterally with ipsilateral predominance, from the level just rostral to the area postrema, to the caudal commissural nTS (Housley et al., 1987; Finley and Katz, 1992). In the rostral portion the fibers leave the tractus and course dorsomedially, terminating in the dorsolateral portion of the nTS, near the border with the cuneate nucleus. Caudal to the area postrema the fibers terminate in the commissural nTS. In the nTS region rostral to and overlapping the area postrema there are very few fibers ventrolateral to the tractus solitarius. However, at the level of the commissural nucleus many fibers occur ventrolateral to the tractus solitarius, with quite a substantial projection right out to the ventrolateral medulla, toward the dorsomedial tip of the lateral reticular nucleus. A few fibers occur within the area postrema and the dorsal motor nucleus of the vagus. In the study by Finley and Katz no mention is made of any labeled fibers in the spinal tract and nucleus of the trigeminal nerve. The projection directly to the caudal ventrolateral medulla was also observed by Chen and colleagues (1992) after injections of tracer into the rat carotid sinus nerve. Their

photomicrographs are very convincing. Projections to the spinal trigeminal nucleus were also demonstrated.

In the cat the medullary connections of nerves innervating the carotid body have been studied after application of tracer directly into the carotid body itself (Claps and Torrealba, 1988), with control injections into nearby structures. No medullary efferents appear to project to the carotid body. Central termination sites were confined to the nTS. There were very few terminals ventrolateral to the tractus solitarius, no direct projections to the ventrolateral medulla, and no afferents in the spinal tract and nucleus of the trigeminal nerve. Panneton and Loewy (1980) and Davies and Kalia (1981) found that the cat carotid sinus nerve projects to the nTS, principally ipsilaterally, from approximately 2 mm rostral to the obex to 2 mm caudal to the obex. In the more rostral regions the fibers extend dorsally and medially from the tractus solitarius, being concentrated toward the lateral edge of the nTS where it borders the gracile nucleus. Some fibers are found ventrolateral to the tractus solitarius. Davies and Kalia found that some carotid sinus nerve fibers arch out toward the ventrolateral medulla, particularly in the more caudal regions of the medulla. No fibers were reported in spinal tract and nucleus of the trigeminal nerve. Figure 4.5 summarizes the central termination of the carotid sinus nerve in the cat.

Figure 4.5 Distribution of labeled terminals in medulla oblongata after unilateral injection of HRP into the carotid sinus nerve. (Modified from Davies and Kalia, 1981.) Abbreviations listed on pages xiii-xiv.

There also appears to be a projection of the carotid sinus nerve to the ventrolateral medulla in the dog (Ruiz-Pesini et al., 1995). This region also receives afferent input in the muskrat after application of tracer to the cervical trunk of the glossopharyngeal nerve (Panneton, 1991a).

Before appropriate neuroanatomical techniques were available, a number of studies used electrophysiological procedures to determine the central projections of carotid sinus and aortic nerves. Studies from Reis's laboratory (Miura and Reis, 1968, 1969, 1972; Crill and Reis, 1968; Homma et al., 1970) identified the nTS projections and suggested that in the cat both the carotid sinus and the aortic nerves also project directly to the reticular formation, particularly to the region designated as the paramedian reticular nucleus. This conclusion was later criticized on technical grounds (Spyer, 1975; Lipski et al., 1975). As discussed above, neuroanatomical studies suggest that the carotid sinus nerve does project directly to the ventrolateral medulla (but not to the paramedian region) in the cat and in the rat.

Taken together, the evidence suggests that chemoreceptor afferents in the carotid sinus nerve have a major projection to the more lateral regions of the nTS from just rostral to the obex to the caudal extent of the commissural nucleus. In the more caudal medullary regions the primary afferents project directly to the ventrolateral medulla. This latter projection is obviously very important because it suggests the possibility that chemoreceptor afferents may bypass the nTS and directly innervate respiratory neurons in the ventrolateral medulla. More attention needs to be paid to the question of whether chemoreceptor afferents terminate in the spinal nucleus of the trigeminal nerve.

Secondary Afferent Respiratory Neurons (Excluding DRG and Chemoreceptor Secondary Afferent Neurons)

Secondary afferent respiratory neurons are those receiving direct inputs from primary respiratory afferents. Not all of these neurons have an obvious respiratory rhythm. The early electrophysiological discovery of the DRG neurons focused attention on pulmonary stretch receptors and the ventrolateral portion of the nTS, even though anatomical studies of primary afferent termination sites emphasized major projections to more medial and dorsolateral nTS sites for both pulmonary stretch receptors and for other respiratory afferents. Neurons in primary termination sites outside the nTS, including the spinal nucleus of the trigeminal nerve, also need to be considered. Secondary afferent neurons responding to slowly adapting lung stretch receptors are considered in a later section dealing with the DRG neurons. A review of central pathways mediating pulmonary and airway vagal afferents has been provided by Kubin and Davies (1995). Central pathways mediating chemoreceptor reflexes are discussed later in this chapter.

Respiratory effects elicited by electrical stimulation of the ethmoidal nerve include apnea and sneezing. Brainstem neurons responding to nasal and ethmoidal nerve stimulation have been identified electrophysiologically in the cat (Batsel and Lines, 1975, 1978; Lucier and Egizii, 1989), but such studies are still at an early stage. Fos studies of medullary neurons activated by nasal irritation or airpuff stimulation have been carried out in rat, rabbit, and cat (Anton et al., 1991; Gieroba et al., 1994b; Wallois et al., 1995). In the rat, following application of mustard oil to the nasal mucosa, fos-positive neurons were observed in the spinal trigeminal nucleus, closely corresponding to the distribution of ethmoidal primary afferents. Neurons in the area postrema and the commissural and more medial regions of the nTS also contained fos. In the unanesthetized rabbit, puffs of formaldehyde vapor directed to the nostrils, repeatedly over 2 hours, causes appearance of fos in neurons in medullary regions likely to receive inputs from nociceptive primary afferents (Gieroba et al., 1994b), as shown in Figure 5.23 in Chapter 5. The formaldehyde stimulus causes apnea, bradycardia, and marked peripheral vasoconstriction. The apnea could be associated with activation of expiratory neurons in the BötC and the cVRG. Neurons in the cVRG region do contain fos, but they have not been specifically identified. No fos-positive neuron was observed in the rabbit BötC region after formaldehyde inhalation, but at present it is not clear whether BötC neurons ever express fos.

Secondary afferent neurons in the dorsomedial commissural portion of the nTS form part of the CNS pathway mediating the respiratory response to stimulation of pulmonary J receptors in the anesthetized rat (Bonham and Joad, 1991). The rapid shallow breathing, bradycardia, and hypotension that follow injection of phenyldiguanide into the right atrium also occur with injection of excitatory amino acid into the dorsomedial portion of the commissural nTS. Synaptic inactivation in this same region using cobalt chloride impairs the respiratory but not the cardiovascular responses to intra-atrial phenyldiguanide.

Secondary afferent neurons receiving inputs from bronchial or pulmonary C fibers in the cat appear to be situated similarly to those receiving the corresponding afferents in the rat. Davies and colleagues

(Kubin et al., 1991) identified cat vagal afferent nodose ganglion neurons with unmyelinated axons (C fibers or thinly myelinated afferents with peripheral conduction velocity less than 2.5 m/s) and selected cells responsive to injection of capsaicin or phenyldiguanide into the right atrium. They then mapped the dorsomedial medullary sites from which the cells could be antidromically activated. The positive sites included the commissural nTS and the dorsomedial nTS at the level of the area postrema (Fig. 4.6). The relevant nodose neurons could not be activated from the ventrolateral subnucleus, or from the region medial and ventromedial to the tractus solitarius.

Figure 4.6 Central termination of bronchopulmonary C-fibers in the cat determined by antidromic stimulation of the medulla and recording from physiologically identified nodose ganglion neurons. Modified from (Kubin et al., 1991.) Abbreviations listed on pages xiii- xiv.

Similar findings, also in the cat, were obtained by averaging nTS field potentials using as a trigger nodose ganglion cells with a rapidly adapting pattern in response to mechanically stimulating the lung with a plastic rod (Kubin and Davies, 1988). The termination site of the afferents was medial to the tractus solitarius, not ventrolateral to this structure. Lipski and colleagues (1991) identified nTS second-order neurons referred to as RAR cells, i.e., neurons activated by pulmonary rapidly adapting receptors. These neurons responded to stimuli commonly used to stimulate pulmonary rapidly adapting receptors in the cat (low-intensity electrical stimulation of the vagus, lung collapse, increase in tidal volume and maintained lung inflation, and inhalation of ammonia vapor). Vagal conduction velocity was about 5 m/s. Responding nTS neurons were found at the level of the obex and caudal to this level, medial to the tractus solitarius, and in the commissural nucleus, in agreement with the findings of Kubin and colleagues (1991) summarized in Figure 4.6. The finding that inputs to the nTS from unmyelinated axons terminate more medially than inputs from myelinated axons is consistent with an anatomical study indicating that the more medially terminating inputs arise from smaller nodose ganglion cells (Torrealba and Calderon, 1990).

Lesions of the commissural nTS in cats abolish respiratory responses to inhalation of ammonia vapor and to pulmonary hyperinflation (Ezure et al., 1991). In the same study, commissural nTS neurons responding to these stimuli (pulmonary irritant, rapidly adapting receptors) have been examined with respect to pontomedullary sites from which the neurons can be antidromically activated. There is a major projection of RAR cells, principally ipsilateral, to the ventral aspect of the lateral parabrachial nucleus. Very few projections to the DRG region, to the rVRG, or to the BötC nucleus were demonstrated.

Blessing, Chapter 4, Breathing (part 2), continued. p.128.