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THE BRAIN-STEM PROJECTIONS OF PULMONARY STRETCH AFFERENT NEURONES IN CATS AND RABBITS

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SUMMARY

1. Micro-electrode recordings were made from slowly adapting pulmonary stretch afferents within the nodose ganglia of cats and rabbits. Recording sites were distributed throughout the ganglia.

2. The projections of these afferents to the medulla oblongata were studied by antidromic stimulation. 'Point' and 'Field' type depth–threshold curves were interpreted as corresponding to stimulation of the main afferent axon and its branches, respectively. Increases in antidromic latency in conjunction with 'field' contours was additional evidence in support of this interpretation.

3. In cats, most (six out of seven) afferents had extensive branches, and probably also terminations, within the medial subnucleus of the ipsilateral nucleus tractus solitarius (n.t.s.). Two of these, plus one other afferent, also had projections to the lateral and ventrolateral subnuclei.

4. In rabbits the projections of such afferents were similar, i.e. mainly to the medial subnucleus of the n.t.s. (eight out of eleven) but also extending into the nucleus alaris, and occasionally to lateral and ventrolateral subnuclei (two out of eleven) or to both regions (one out of eleven).

5. Branching of single afferents was seen to occur over up to 3 mm of the rostro-caudal extent of the intermediate region of the n.t.s. The significance of the observations is discussed.

INTRODUCTION

Although many studies have been made of afferent receptors in the lungs with axons in the vagal nerves, and of their reflex effects (Paintal, 1973 for review), few studies have been made of the projections of these afferents to the brain stem. Anatomical studies of terminal degeneration following intracranial section of the vagal nerve have shown that vagal nerve afferents project to the intermediate and caudal regions of the nucleus tractus solitarius (n.t.s.) in the cat (e.g. Kerr, 1962; Cottle, 1964). However this technique does not allow the projections of specific groups of afferents,

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e.g. pulmonary, cardiac or gastric, to be defined. Recently, therefore, horseradish peroxidase (HRP) has been used to provide more specific information. After injection of HRP in the lungs of cats, Kalia & Mesulam (1980*b*) demonstrated extraperikaryal HRP mainly in dorsal and ventrolateral subnuclei of the n.t.s., with some additional projection to the medial subnucleus and commissural nucleus. However with this technique it is not possible to study projections of only pulmonary stretch afferents since it is likely that all classes of pulmonary afferent will take up the enzyme.

The technique of recording compound action potentials evoked in whole nerves by antidromic activation at their sites of projection has been used to define sites of termination of aortic and carotid sinus nerve afferents; it also allows distinction to be made between myelinated and non-myelinated fibres (e.g. Lipski, McAllen & Spyer, 1975; Jordan & Spyer, 1977; Donoghue, Fox, Kidd & McWilliam, 1981). This technique is not directly applicable to thoracic vagal branches, however, since these contain significant numbers of efferent axons. To study brain-stem projections of pulmonary afferents, therefore, we have used a technique recently described by us for studying aortic baroreceptor afferents (Donoghue, Garcia, Jordan & Spyer, 1981), i.e. recording from single afferents in the nodose ganglia, identifying them by their spontaneous discharge and determining the projection to the brain-stem by antidromic activation. Preliminary results have been communicated to the Physiological Society (Garcia, Jordan & Spyer, 1979).

METHODS

Experiments were performed on twelve adult cats (2·5–3·2 kg body weight) and eighteen New Zealand White or Red rabbits (2·0–3·2 kg body weight), anaesthetized with α-chloralose (B.D.H., 70 mg/kg I.V.) and ethyl carbamate (Urethane, Fisons Ltd., 1·5 g/kg I.V.) respectively. All animals were paralysed with gallamine triethiodide (Flaxedil, May and Baker Ltd., 1–4 mg/kg I.V.) and ventilated artificially with room air (end-tidal CO_2 was maintained close to 4 %). Rectal temperature was monitored and maintained at $37·5 \pm 0·5$ °C.

The dorsal surface of the medulla oblongata was exposed for stimulation and the right or left nodose ganglion prepared for recording as described previously (Donoghue, Garcia, Jordan & Spyer, 1981). The ongoing activity of neurones recorded in the ganglion was analysed and a cell selected that showed rhythmic activity related to the rate and depth of lung inflation (pulmonary stretch afferents). The medulla was then explored for sites from which the cell could be antidromically activated by electrical stimulation through tungsten–glass micro-electrodes (tip = 10 μm). These sites were subsequently determined histologically (Donoghue, Garcia, Jordan & Spyer, 1981).

RESULTS

The activity was recorded of eighteen pulmonary stretch afferents in rabbits and of twenty-two such afferents in cats. These cells showed characteristics typical of lung stretch afferents (see Paintal, 1973), i.e. they showed the highest frequency of action potentials during lung inflation, the number of spikes/cycle being determined by respiratory pump stroke volume (Fig. 1 *A* and *B*). The spike frequency was unaffected by fluctuations in systemic arterial blood pressure produced by 30 sec of haemorrhage or saline infusion (10–20 ml.).

Recording sites

In both the cat and rabbit, recording sites were distributed throughout the nodose ganglion, most frequently in the medial zone where the ganglion is of greatest diameter and contains a relatively large proportion of the cell bodies, but also extending into the most rostral and caudal extents of the ganglion.

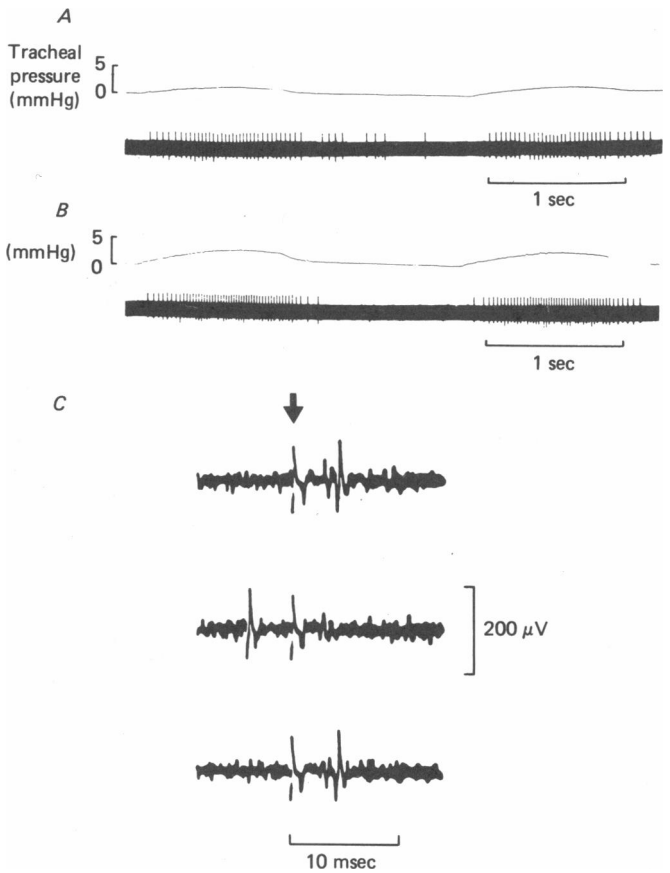

Fig. 1. Recordings from a pulmonary afferent in the nodose ganglion of a cat, at respiratory stroke volumes of 25 ml. (*A*) and 35 ml. (*B*). The higher stroke volume produces greater tracheal pressure during inspiration and a greater number of action potentials per respiratory cycle. In *C*, a stimulus was applied to the medulla at the times marked by the arrow. In three successive tests, the stimulus elicited an antidromic action potential (top and bottom) except when preceded by a spontaneous action potential (middle).

Projections to the medulla oblongata

The medullary projections of seven cells in the cat and eleven cells in the rabbit were studied using antidromic stimulation. The collision test (Fig. 1*C*) was always applied to ensure that the antidromically activated cell was the same as that exhibiting spontaneous activity. Each afferent that fulfilled this criterion also followed high frequencies of stimulation (500–1000 Hz). For each cell a map was then

made illustrating the sites in the medulla at which depth–threshold contours were of the 'point' or 'field' type, thus, we believe, demonstrating the course of the afferent axon and its branches within the tractus and nucleus tractus solitarius (Donoghue, Garcia, Jordan & Spyer, 1982).

(*a*) *Cat.* Figs. 2 and 3 show the projections of one pulmonary stretch afferent in a cat. Fig. 2 contains a series of cross-sections of the dorsomedial region of the medulla oblongata at and rostral to the obex, and the locations of representative electrode penetrations. The corresponding depth–threshold curves for these penetrations are

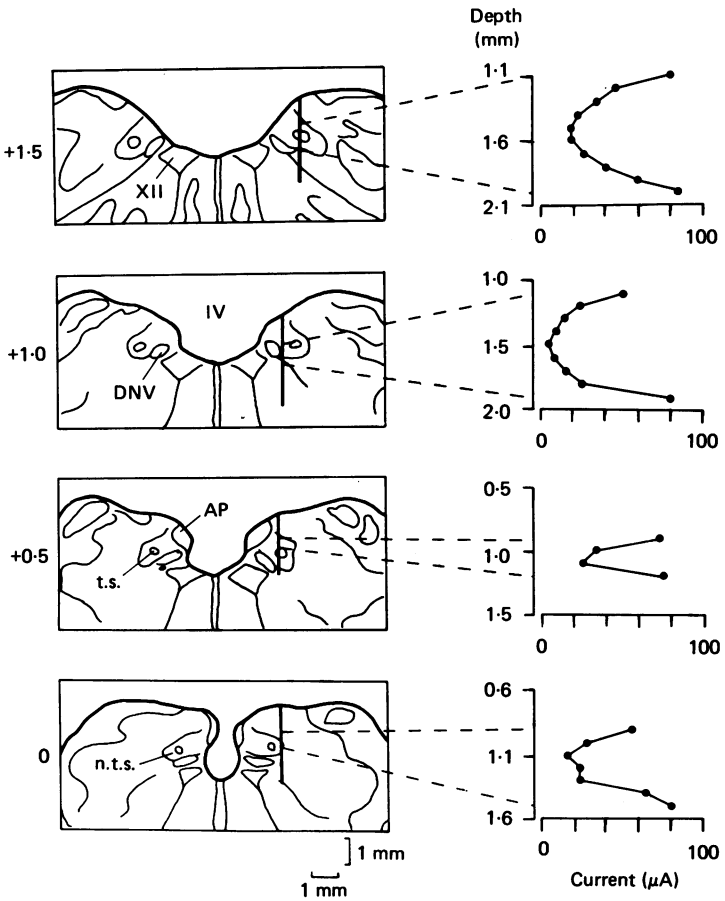

Fig. 2. Cat. This shows a series of cross-sections of the dorsomedial regions of the medulla oblongata at 0·5 mm intervals from the level of the obex (0) to 1·5 mm rostral to the obex (+1·5) (left). The thick vertical line on each section indicates the position of an electrode tract, determined histologically. On the right are shown depth–current threshold curves corresponding to each penetration. Depths are from the dorsal surface. The dashed lines indicate the region of the penetration from which an antidromic action potential was evoked. Curves at 0, +1 and +1·5 are of the 'field' type; that at +0·5 of the 'point' type. Abbreviations: Area postrema (AP), dorsal motor nucleus of vagus (DNV) nucleus tractus solitarius (n.t.s.), tractus solitarius (t.s.), hypoglossal nucleus (XII) and IVth ventricle (IV).

also shown. It can be seen that a penetration passing through the tractus solitarius produced a contour of the 'point' type, whilst those through the medial subnuclei of the n.t.s. rostral to obex, or through the lateral subnuclei of the n.t.s. at the level of the obex, produced contours of the 'field' type. In Fig. 3 this, together with additional information, has been transposed on to a map representing the extent of the n.t.s. in relation to a view of the dorsal surface of the medulla. Penetrations from

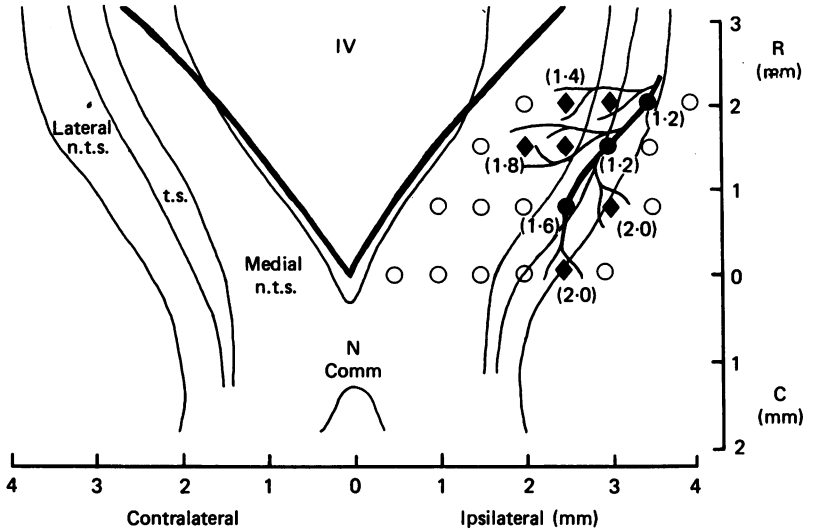

Fig. 3. Cat. Dorsal view of the medial regions of the medulla oblongata. The thick 'V' indicates the edge of the IVth ventricle. The lateral and medial boundaries of the tractus and nucleus tractus solitarius have been superimposed on this view. The position of stimulating micro-electrode penetrations are shown by symbols, according to the type of depth–threshold contour obtained, i.e. point (●), field (◆) or no response (○). Some of these penetrations correspond to those shown in Fig. 2. The likely course of the axon (heavy line) and some possible branches (thin line) is shown. Scales indicate mm rostral (R), caudal (C) and lateral to the obex (O). Abbreviations as Fig. 2, plus nucleus commissuralis (N Comm). Figures in brackets indicate antidromic latency (in msec) following stimulation at the relevant site.

which contours of the 'point' or 'field' type were produced, or during which no antidromic response was evoked, are shown and a likely course of the axon and its branches has been drawn in.

In this example the main axon, within the tractus solitarius, gives branches to the medial subnucleus of the n.t.s. rostral to the obex and other branches to lateral and ventrolateral subnuclei at and just rostral to the obex. Also shown in Fig. 3 is the latency of the antidromic action potential evoked from each site. It can be seen that the latency increases significantly at sites corresponding to regions of branching (1·4–2·0 msec) compared to stimulation of the main axon (1·2–1·6 msec). An increase in latency along the course of the axon from rostral (1·2 msec) to caudal (1·6 msec) can also be seen.

Similar patterns of projection to that described above were seen for six out of seven pulmonary stretch afferents studied, i.e. the predominant projection was to the

medial subnucleus of the n.t.s. rostral to the obex. Two of these also showed projections to lateral and ventrolateral subnuclei at the level of the obex. Only one neurone projected solely to lateral subnuclei. It is illustrated in Fig. 4. The axon in the tractus solitarius gives branches to lateral and ventrolateral subnuclei only as far caudal as 2 mm rostral to the obex. An increase in latency in the area of branching was again seen. No projections to the medial subnucleus were found.

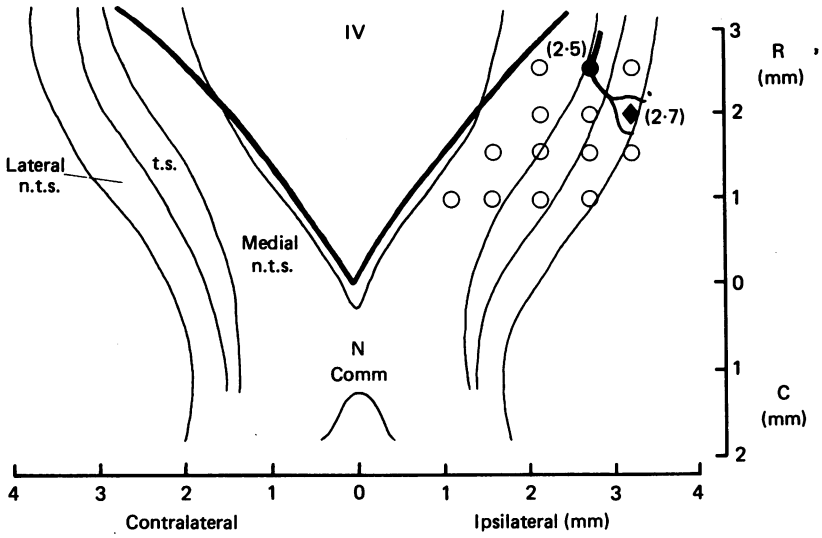

Fig. 4. Cat. The projections of another pulmonary stretch afferent exhibiting a limited pattern of distribution. Explanation and abbreviations as Fig. 3.

For all neurones antidromic action potentials could only be evoked by stimulation of the ipsilateral tractus and nucleus tractus solitarius. Projections to the contralateral side of the medulla were not found.

(*b*) *Rabbit*. The projections of lung stretch afferents in the rabbit were very similar to those described above for the cat. Fig. 5 illustrates the pattern of projection of one afferent derived in the same way as those for the cat (above). The axon passed close to, but not within, the tractus solitarius, giving branches to the medial subnucleus of the n.t.s. and to the nucleus alaris between 0·5 mm caudal and 2·5 mm rostral to the obex, with no branches to lateral subnuclei. Nine (of eleven) afferents showed similar patterns of projection, i.e. an axon in the n.t.s. giving branches to the medial subnucleus of the n.t.s. rostral to the obex. One of these also have branches to the lateral and ventrolateral subnuclei rostral to the obex. Two further axons gave branches only to the lateral and ventrolateral subnuclei; the projections of one of these are shown in Fig. 6. The terminations of all afferents were restricted to the ipsilateral n.t.s. between 0·5 mm caudal and 2·5 mm rostral to obex, although in some cases the axon could be followed up to 3·5 mm rostral. Projections outside the n.t.s. and nucleus alaris or to the contralateral medulla were not found.

Latencies

Latencies to stimulation of the axons were in the range 1·2–2·5 msec (cat) and 0·9–2·0 msec (rabbit), corresponding to conduction velocities of 16–33 msec and 17·5–39 msec, respectively, which is within the range previously reported for pulmonary stretch afferents (Paintal, 1973). As stated above, stimulation in areas giving 'field' type contours, i.e. in areas of branching and probable termination, gave significant

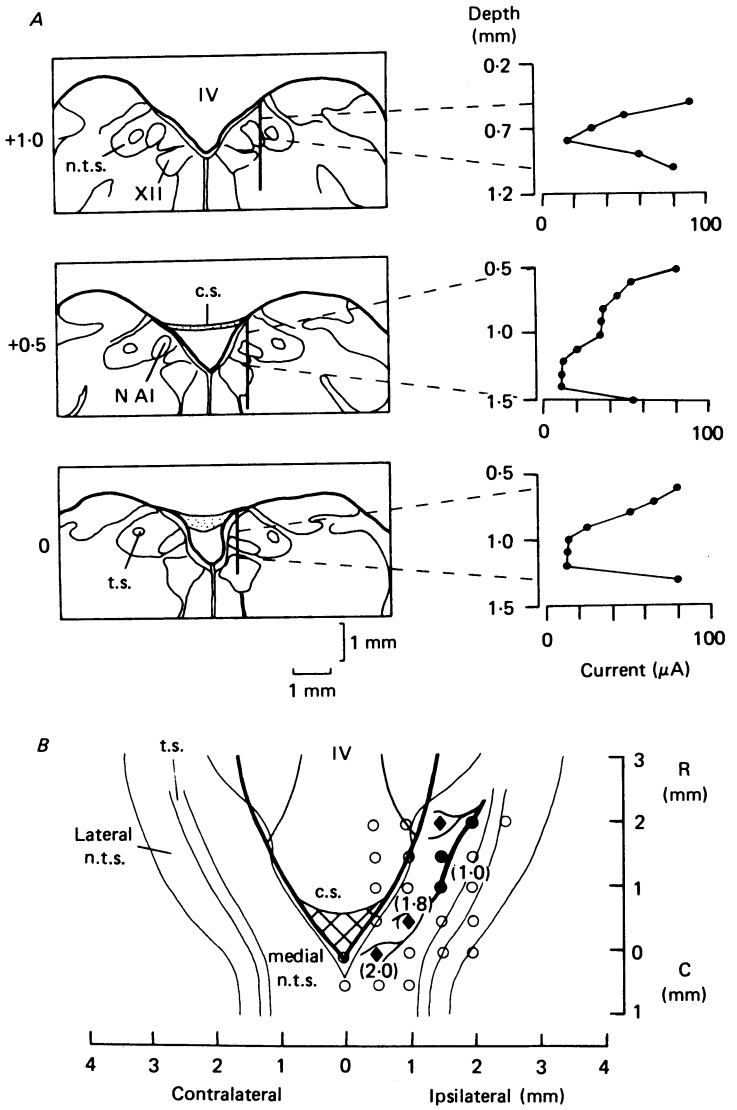

Fig. 5. Rabbit. The projections of a pulmonary stretch afferent shown on cross sections (*A*) and on the dorsal view (*B*). In (*A*) contours of the point (+1·0) and field (+0·5, 0) types can be seen. Explanation and abbreviations as Figs. 2 and 3 plus nucleus alaris (N Al) and calamus scriptorius (c.s.).

increases in antidromic latency. The maximum increases were in the range 0·2–1·3 msec (cat) and 0·2–1·3 msec (rabbit), corresponding to increases of 8–67% and 10–122%, respectively, in latency compared to stimulation of the main axon.

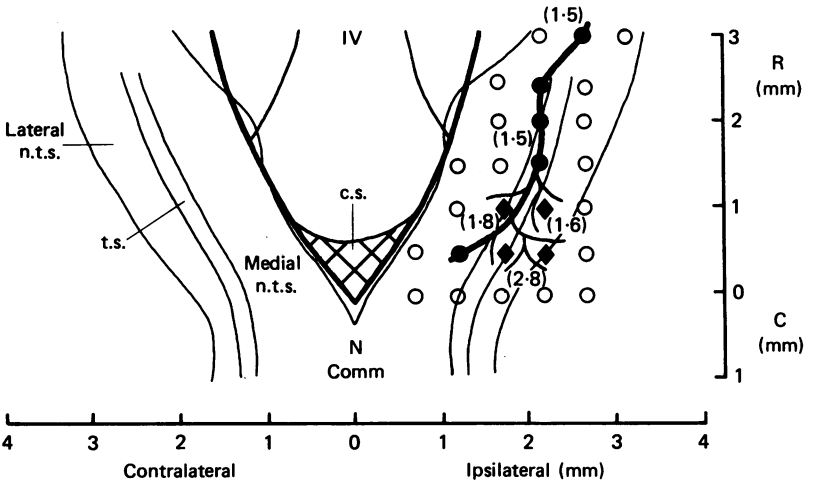

Fig. 6. Rabbit. Projections of another pulmonary stretch afferent. Explanation and abbreviations as Fig. 3.

DISCUSSION

The pulmonary stretch afferent neurones investigated in this study were identified by their spontaneous activity which was characteristic of slowly adapting receptors of this type, i.e. a prolonged burst of action potentials (typically ten or more) during each phase of lung inflation with relatively small volumes of air (30–35 ml.), the number of spikes/cycle depending on inflation volume (Mei, 1970; Paintal, 1973). Conduction velocities of the central part of the axons, measured in this study, were within the range of conduction velocities for such afferents measured peripherally (Mei, 1970; Paintal, 1973). Richter, Camerer & Röhrig (1979) have reported previously that there was no significant slowing of conduction along the intracephalic course of pulmonary stretch afferents, and our present observations are in agreement with this.

Neuronal activity was recorded at sites throughout the nodose ganglia of both cats and rabbits, indicating that there is no discrete localization of the cell bodies of these afferents within the ganglia of either species. Previously, Mei (1970) had studied the distribution of various types of vagal afferent in the nodose ganglion of cats, by recording with micro-electrodes, and similarly concluded that in this species pulmonary afferents could be recorded at sites throughout the ganglion. Recently, Kalia & Mesulam (1980*a*) made injections of HRP into the trachea, bronchi or lungs of cats and subsequently described the locations of cell bodies in the nodose ganglia that were labelled by the enzyme. These cells again showed no specific localization.

As far as we are aware, there have been no previous reports using neurophysiological techniques on the projections of pulmonary stretch afferents to the medulla. In two

studies concerned with investigating the presynaptic influences on these afferents it was reported that they could be antidromically activated from the ipsilateral n.t.s. at about the level of the obex (Barillot, 1970; Sessle, 1973) but no attempt was made to determine whether stimulation was of axons or terminals, nor to determine the extent of the projection. After injection of HRP into the trachea, bronchi and lungs of cats Kalia & Mesulam (1980*b*) described extraperikaryal labelling within most subnuclei of the n.t.s., particularly within the dorsolateral and ventrolateral subnuclei, but also within the medial subnucleus, extending from 3 mm rostral to 2 mm caudal to the obex. In that study, however, HRP has probably been taken up by all classes of pulmonary afferent; the extent of extraperikaryal labelling does not therefore necessarily reflect the distribution of pulmonary stretch afferents.

In the present study the brain-stem projections of single pulmonary stretch afferents have been studied. From the depth–threshold curves and the observed antidromic latency during each penetration into the brain stem it is possible to follow the course of an axon along the tractus solitarius and n.t.s. and define areas where the axon gives branches and probably also terminations (Donoghue *et al.* 1981). Accordingly it was found that in the rabbit extensive branching occurred within the ipsilateral medial subnucleus of the n.t.s. around the level of obex, and in the nucleus alaris; only occasionally was there branching to the lateral subnuclei. In the cat, three of seven afferents projected to the lateral (including the ventrolateral) subnuclei, although the largest distribution was again to the medial subnucleus. All afferents terminated within an area of the n.t.s. extending for 2–5 mm rostral to 0·5 mm caudal to the obex.

Surprisingly the major projection of these afferents was not to the ventrolateral subnucleus of the n.t.s. where the dorsal group of respiratory neurones is located in the cat (e.g. von Euler, Hayward, Marttila & Wyman, 1973) and probably also in the rabbit (Fallert & Baum, 1976). This is where one class of these cells, R_{β} inspiratory neurones, has been shown to receive a short latency excitatory input from vagal afferents (von Euler *et al.* 1973) an influence which represents a monosynaptic input from pulmonary stretch afferents (Richter *et al.* 1979).

Some pulmonary stretch afferents may make contact with interneurones located in the medial subnuclei that have axons projecting to the n.t.s. or other respiratory regions, particularly to the pontine respiratory areas, i.e. the parabrachial nuclei, and the lateral groups of respiratory neurones in the nucleus ambiguus and nucleus retroambigualis in the medulla oblongata. Pathways between these groups of neurones have been demonstrated anatomically (Morest, 1967; Cottle & Calaresu, 1975; Kalia, 1977; Loewy & Burton, 1978; Norgren, 1978; King, 1980). In addition, respiratory neurones in pontine nuclei have been shown to be strongly inhibited by pulmonary afferents (Feldman, Cohen, & Wolotsky, 1976), although there is no evidence that this is a direct pathway from medulla to pons.

Projections to the medial subnucleus of the n.t.s. may alternatively or additionally represent the pathway for an effect of pulmonary stretch afferents on non-respiratory neurones; there is now evidence that activation of pulmonary stretch afferents produces reflex depressor effects on the cardiovascular system (see Shepherd, 1981, for review), and some n.t.s. neurones have been shown to receive a number of

excitatory inputs from different groups of afferent, e.g. from the cardiovascular and pulmonary systems (Stroh-Werz, Langhorst & Camerer, 1977), possibly accounting for some of the interactions between the two systems (see Spyer, 1981).

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