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NEURONES IN THE BRAIN STEM OF THE CAT EXCITED BY VAGAL AFFERENT FIBRES FROM THE HEART AND LUNGS

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SUMMARY

1. Extracellular recordings were made from 164 neurones in the nucleus tractus solitarius and dorsal motor vagal nucleus of the chloralose-anaesthetized cat.

2. 139 neurones were excited synaptically and 25 non-synaptically by electrical stimulation of cardiac and pulmonary vagal branches.

3. Synaptically excited neurones fall into two populations, one activated solely by myelinated afferent fibres and a second activated solely by non-myelinated afferent fibres.

4. 94 neurones were synaptically excited by afferent fibres in a single vagal branch while 45 were excited by stimulation of two or three branches.

5. Neurones responding to volleys in myelinated afferent fibres were located in both medial and lateral regions of the nucleus tractus solitarius whilst those excited by non-myelinated afferent fibres were restricted to the medial region. Consistent differences in the locations of neurones excited by stimulation of either cardiac or pulmonary or by single or several branches could not be distinguished.

INTRODUCTION

The vagal afferent innervation of the heart and lungs is known to be extensive and numerous studies have defined the location of the receptors, their physiological characteristics and reflex responses (e.g. Hainsworth, Kidd & Linden, 1979), however, knowledge of the organization of the central nervous pathways involved in such reflexes remains scanty.

Histological studies have shown that the nucleus tractus solitarius (n.t.s.) is the major site of termination of vagal afferent fibres including those from the heart and lungs (e.g. Cottle, 1964; Kalia & Mesulam, 1980*a*, *b*).

In electrophysiological studies, neurones with a 'cardiovascular' pattern of activity evoked by vagal afferent fibres have been identified in the n.t.s. (Fussey, Kidd & Whitwam, 1967; Stroh-Werz, Langhorst & Camerer, 1977) but the receptors involved were not defined. Neurones, probably activated by myelinated vagal afferent fibres attached to atrial receptors, have been reported in the medial region

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of the n.t.s., the dorsal motor vagal nucleus, the parahypoglossal area and the reticular formation ventral to the n.t.s. in the dog (Keith, Kidd, Linden & Snow, 1973; Kidd, 1979), whereas activity during lung inflation, probably from neurones activated by myelinated afferent fibres from the lungs, has been described in the ventrolateral region of the n.t.s. (e.g. von Euler, Hayward, Martilla & Wyman, 1973).

However, most vagal afferent fibres from the heart and lungs are non-myelinated (Agostoni, Chinnock, de Burgh Daly & Murray, 1957) and yet to date there is only one specific study of neurones in the brain stem activated by such fibres (Donoghue, Fox, Kidd & Koley, 1981*a*). Neurones in or close to the n.t.s. were synaptically activated following electrical stimulation of afferent fibres in a cardiac vagal branch and, while a few were activated by myelinated afferent fibres, the majority were activated by non-myelinated afferent fibres. Convergent excitatory effects of afferent fibres in several cardiac and pulmonary vagal branches onto individual neurones were demonstrated, but the extent of this was not fully investigated.

The aim of the present study was to define the characteristics and the extent to which neurones in the n.t.s. are excited by afferent fibres in both cardiac and pulmonary vagal branches, and, to determine whether myelinated and non-myelinated cardiac and pulmonary afferent fibres activate separate populations of neurones within this nucleus. A preliminary account has been presented to the Physiological Society (Bennett, Goodchild, Kidd & McWilliam, 1981).

METHODS

The experiments were performed on thirty-six cats (1·0–4·25 kg) anaesthetized with either sodium thiopentone (Intraval, May and Baker, 40 mg/kg, I.P., six animals) or halothane (Fluothane, I.C.I., 5% in O_2, thirty animals). Anaesthesia was then maintained with intravenous α-chloralose (Cambrian Chemicals Ltd., 10–20 mg) when required or as a constant infusion (10–20 mg/kg . h). Catheters were placed in a femoral artery and vein, the trachea cannulated and the animal ventilated using a Starling 'Ideal' pump. Oxygen was added to the inspired air to bring the F_{I,O_2} to 0·4. The physiological condition of the animals, as assessed by P_{a,CO_2}, P_{a,O_2}, arterial pH and P_{A,CO_2}, blood pressure, heart rate, and oesophageal temperature, was maintained within normal limits.

The thorax was opened on the right side by removing portions of ribs 2–7 and the expiratory outlet of the pump placed at a depth of 3·0 cm in water. The upper lobe of the right lung was removed and the azygos vein divided to expose the caudal cardiac and pulmonary branches of the right vagus. Further pulmonary vagal branches were found caudal to the root of the excised lung lobe. A cranial cardiac branch was sometimes present (18/36 animals) between the superior vena cava and the trachea. The number of branches varied but there was always at least one caudal cardiac branch and usually two, sometimes up to four, pulmonary branches.

Each of the vagal branches and the thoracic vagus rostral to the branches were placed on bipolar silver wire electrodes and embedded in silicone rubber (Silgel 604; Donoghue, Fox & Kidd, 1977); electrodes were connected to separate stimulators allowing each branch to be stimulated independently with rectangular pulses (0·1–1·0 ms, 1·0–2·0 V). The head was placed in a stereotaxic head holder (La Précision Cinématographique, Ansières, Paris) and the dorsal surface of the brain stem exposed. The cerebellum was not removed or retracted in any experiment.

Extracellular recordings were made from single neurones in the ipsilateral medulla oblongata using tungsten or gold micro-electrodes (Donoghue & Kidd, 1979; Crane, Goodchild, Kidd & McWilliam, 1982). Some recording sites were marked by the ionophoretic ejection of Pontamine Sky Blue (Hellon, 1971). Other sites were identified by reference to electrode tracks in histological sections and the characteristic patterns of spontaneous and evoked activity recorded during the penetration. When this was not possible, the position was estimated: sites less than 600 μm below

the nucleus gracilis were judged to be in the medial region of the n.t.s. whilst those less than 600 μm below the nucleus cuneatus were judged to be in the lateral region of the n.t.s. The accuracy of these estimates was established by applying these same criteria to twenty-two recording sites marked by Pontamine spots; for eighteen neurones the estimated location was correct.

Recording procedure

Penetrations were aimed at the medial region of the n.t.s. and when the electrode tip was judged to be in this nucleus the vagal branches were stimulated simultaneously (twin pulses at a 10 ms interval, 1·0 ms, 10·0 V, 0·3 Hz). Those vagal branches which excited a neurone and the nature of the excitation, i.e. synaptic or non-synaptic, were determined using established criteria (Fussey, Kidd & Whitwam, 1970; Donoghue *et al.* 1981*a*). At least twenty consecutive single or twin pulse stimuli were used to assess the probability of a response. In some cases, the conduction velocity of the afferent fibres was determined by stimulating the vagus at two points. Condition-test procedures were used to demonstrate subthreshold effects and to define whether both myelinated and non-myelinated afferent fibres influenced individual neurones. All values quoted are mean and standard deviation.

RESULTS

Recordings were made from 164 neurones which responded to stimulation of cardiac or pulmonary vagal branches; most with one or two action potentials but some gave up to six. Most (139) were synaptically excited by afferent fibres in these branches; ninety-four from one vagal branch and forty-five from fibres in two or three vagal branches (Fig. 1). The remainder, twenty-five, were excited non-synaptically.

Neurones excited synaptically

Latency. The minimum latency of the first action potential evoked from each neurone by supramaximal single or twin pulse stimuli ranged from 3 to 510 ms (Fig. 2*A* and *B*); the distribution of latencies shows peaks at approximately 20 ms and from 140 to 200 ms. The conduction distances from the cranial cardiac, caudal cardiac and pulmonary branches to the medulla were 130 ± 12 mm, 140 ± 18 mm, and 149 ± 18 mm respectively. With such distances some neurones must have been activated by myelinated afferent fibres while others (latency > 60 ms) were likely to be excited by non-myelinated afferent fibres. This was confirmed for sixteen neurones which responded at latencies of 65–235 ms to stimulation of a caudal cardiac (6) or pulmonary branch (10); two point stimulation revealed that the afferent fibres involved were non-myelinated (conduction velocity 0·7–2·1 m/s, mean $0{\cdot}9 \pm 0{\cdot}4$ m/s).

The population of synaptically excited neurones was divided according to whether their latency was less or greater than 60 ms on the basis that afferent volleys in myelinated afferent fibres, conducted over a distance of 150 mm, would take less than 60 ms to reach the brain stem while those in non-myelinated fibres (conduction velocity $< 2{\cdot}5$ m/s) would take more than 60 ms. This division of the population was unaffected when latencies were corrected for variations in conduction distance. Neurones in the short latency group ($n = 44$) never responded with a latency greater than 60 ms; those in the long latency group ($n = 92$) never responded with a latency of less than 60 ms.

Only three neurones could not be placed in either group. Two had mean latencies of 65 and 69 ms but had minimum latencies of 35 and 52 ms respectively. A third

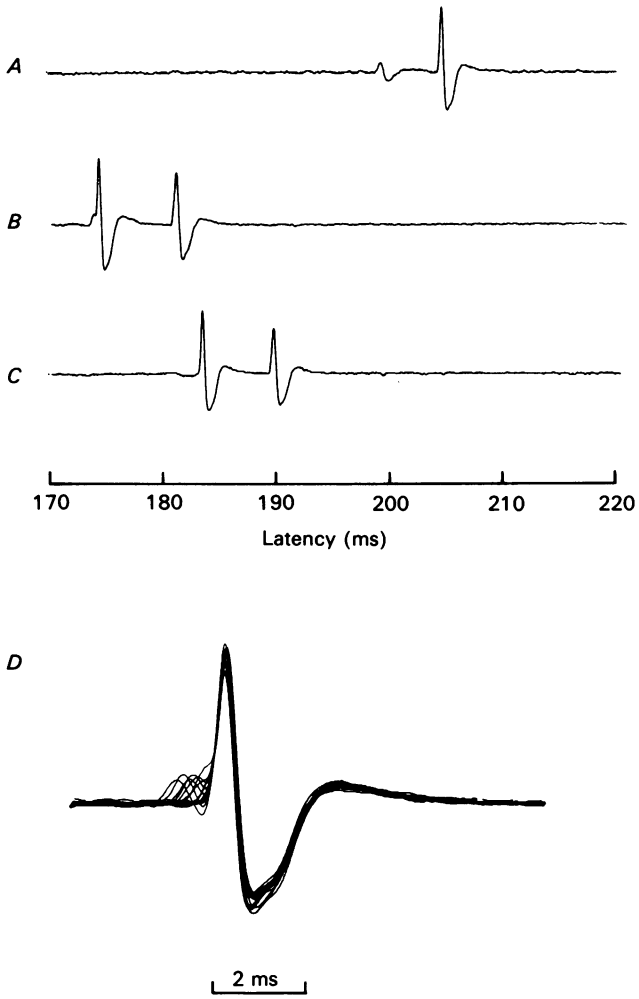

Fig. 1. Evoked action potentials from a single neurone, note the trace begins 170 ms after the stimulus: *A*, stimulation of a pulmonary branch (twin pulse) evoked a single action potential (large spike); *B*, stimulation of a cranial cardiac branch (twin pulse) evoked two action potentials from the neurone; *C*, stimulation of a second pulmonary branch (single pulse) also evoked two action potentials; *D*, fifteen superimposed action potentials, five evoked by stimulation of each branch, confirm that they are from the same neurone.

responded to stimulation of a caudal cardiac and a pulmonary vagal branch with latencies of 36 and 34 ms respectively while stimulation of a second pulmonary branch consistently evoked two action potentials: one with a latency of 34 ms (variability 4 ms) and another with a latency of 160 ms (variability 84 ms).

The possibility that both myelinated and non-myelinated vagal afferent fibres exerted excitatory effects on the same neurone was investigated using condition-test procedures. Six neurones in the short latency group were unaffected, either in the probability or latency of their responses, when conditioning pulses were applied to several other vagal branches 50–500 ms before the test pulse. Similarly, thirty

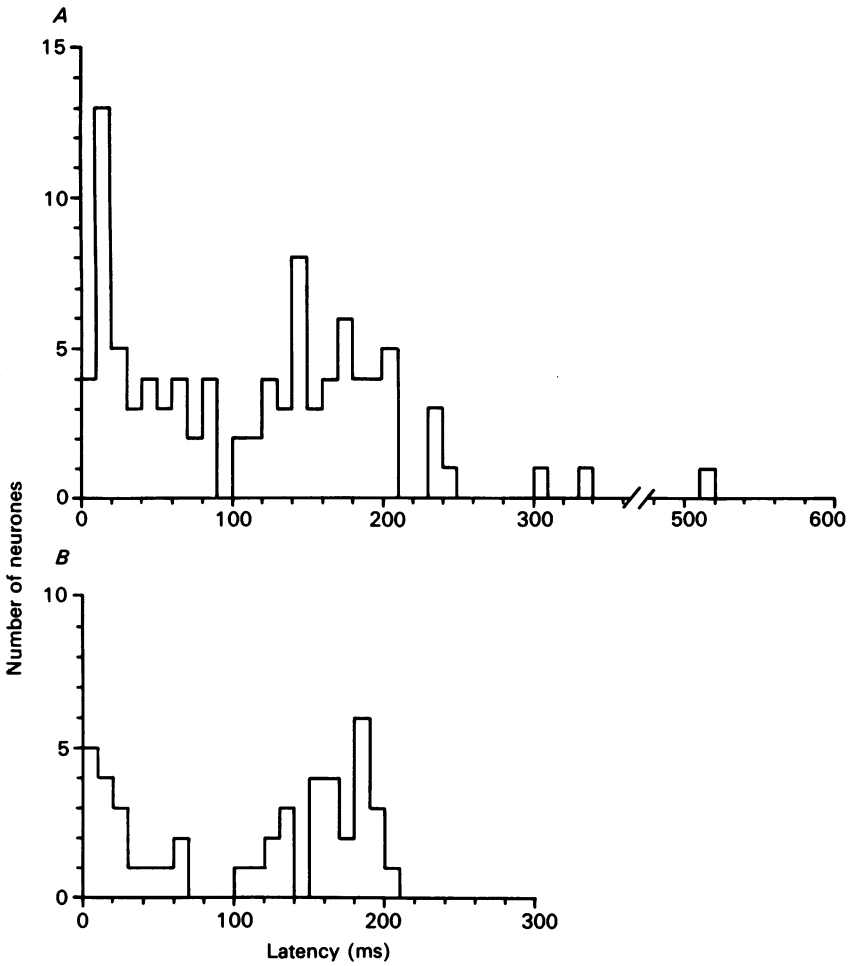

Fig. 2 The minimum latency of the first evoked action potential from each synaptically activated neurone. *A*, neurones activated from only one vagal branch (94). *B*, neurones activated from two or three vagal branches (44). The one neurone activated at both short and long latencies (see text) has been omitted.

neurones in the long latency group were unaffected when conditioning pulses were applied to several other vagal branches 0–60 ms before the evoked responses.

Convergent effects from vagal branches. Tests were performed on the 139 synaptically activated neurones to determine whether they were activated by more than one vagal branch.

Ninety-four neurones responded with one to six action potentials following single or twin pulse stimulation of a single vagal branch (Table 1) and for most (80), stimulation of other cardiac and pulmonary vagal branches did not evoke action potentials; tests on the remainder were incomplete. Thirty-nine of these neurones (eight short latency and thirty-one long latency) were tested for the presence of subthreshold excitatory effects exerted by afferent fibres in other cardiac and pulmonary vagal branches but in no case was there an increase in either probability

TABLE 1. The characteristics of neurones activated synaptically from only one branch with (*A*) short latencies or (*B*) long latencies (values are mean ±1 S.D. and range). Two neurones which could not be placed in either the short or long latency group have been omitted

Branch	No.	Latency (ms)	Latency variability (ms)	Maximum no. of evoked spikes	Maximum probability
(*A*) Cranial Cardiac	1	22	2	1	0·73
Caudal cardiac	20	23±14	8±5	2±1·5	0·82±0·2
		5–51	2–20	1–6	0·16–1·0
Pulmonary	8	20±16	7±15	2±1·6	0·9±0·2
		6–50	2–17	1–6	0·5–1·0
(*B*) Cranial cardiac	10	180±84	29±21	1·6±0·9	0·69±0·2
		68–338	4–65	1–4	0·25–1·0
Caudal cardiac	36	150±50	24±17	1·5±0·5	0·79±0·22
		62–250	1–60	1–3	0·13–1·0
Pulmonary	17	183±94	27±16	1·3±0·47	0·83±0·2
		71–510	1–65	1–2	0·25–1·0

TABLE 2. The combination of various vagal branches that synaptically activated forty-four neurones. The neurone responding at both short and long latencies (see text) has not been included in this table

	Number of neurones	
	Short latency	Long latency
Two branches		
Caudal cardiac + pulmonary	9	13
Pulmonary + pulmonary	1	7
Caudal cardiac + cranial cardiac	1	1
Cranial cardiac + pulmonary	—	1
Caudal cardiac + caudal cardiac	—	1
Total	11	23
Three branches		
Caudal cardiac + pulmonary + pulmonary	4	4
Caudal cardiac + cranial cardiac + pulmonary	—	1
Cranial cardiac + pulmonary + pulmonary	—	1
Total	4	6

or the number of evoked action potentials when all branches were stimulated simultaneously.

An additional forty-five neurones were excited by afferent fibres contained in two or three vagal branches (Table 2). Thirty-four responded with up to six action potentials to stimulation of a single branch and there was a further increase, either in probability of response or number of action potentials to single or twin pulses, when other branches were stimulated simultaneously (Fig. 1). Subthreshold spatial summation could also be demonstrated on another eleven neurones either in the form of an enhancement of the probability of evoked responses by stimulation of another vagal branch, which, by itself, did not evoke action potentials, or that it was necessary to stimulate several branches simultaneously in order to obtain a response.

Neurones responding at short latency to volleys in afferent fibres of individual caudal cardiac or pulmonary vagal branches were not significantly different in terms of latency, latency variability, maximum number of evoked action potentials or their probability of response ($P < 0{\cdot}05$, Table 1). Similarly, there were no significant differences between responses of neurones activated with long latencies by afferent fibres in cardiac or pulmonary branches. Overall, the mean latencies of neurones excited by afferent fibres in two or three vagal branches were not significantly different to those of neurones excited by a single branch (Fig. 2), although the maximum probability of those neurones excited by two or three branches was significantly lower ($P > 0{\cdot}05$) (Tables 1 and 3).

TABLE 3. The latency of response, latency variability, the maximum number of evoked spikes and the maximum probability to stimulation of each branch (or for subthreshold effects, pairs of branches) that activated the forty-four neurones shown in Table 2 (mean ± S.D. and range)

	No. of neurones	Latency (ms)	Latency variability	Maximum no of evoked spikes	Maximum probability
Short latency	14	17 ± 13 3–45	16 ± 13 1–40	1·35 ± 0·79 1–6	0·59 ± 0·36 0·05–1·00
Long latency	30	154 ± 40 60–202	31 ± 22 2–84	1·6 ± 1·0 1–4	0·53 ± 0·36 0·02–1·00

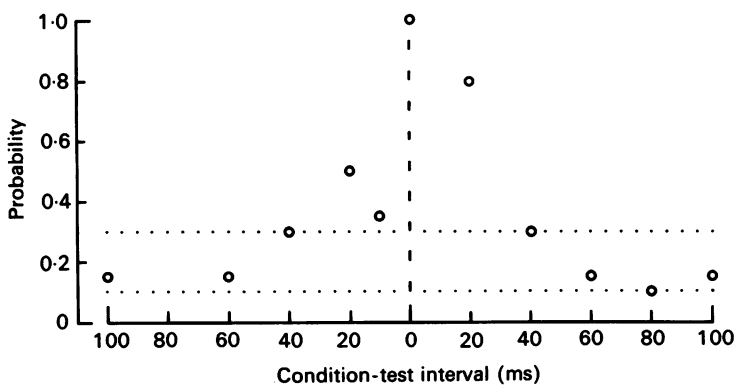

Fig. 3. The probability of a response from a neurone to stimulation of a caudal cardiac and pulmonary branch at various condition-test intervals (pulmonary branch stimulated at time zero). The neurone responded to stimulation of the cardiac branch alone with a probability of 0·1–0·3 (shown by dotted lines); this probability was enhanced by stimulation of the pulmonary branch although the neurone would not respond to stimulation of the pulmonary branch alone.

The time course of spatial summation effects from the different branches was examined ($n = 4$). In each case the enhanced probability was maximal when two branches were stimulated simultaneously and intervals of 10–20 ms between the stimulus pulses to different branches markedly reduced or abolished this enhancement (Fig. 3).

During examination of these neurones, inhibitory effects were not observed,

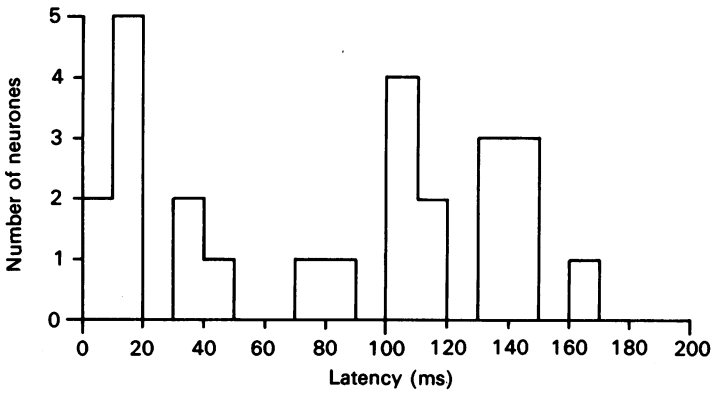

Fig. 4 The distribution of the latencies of all non-synaptically activated neurones.

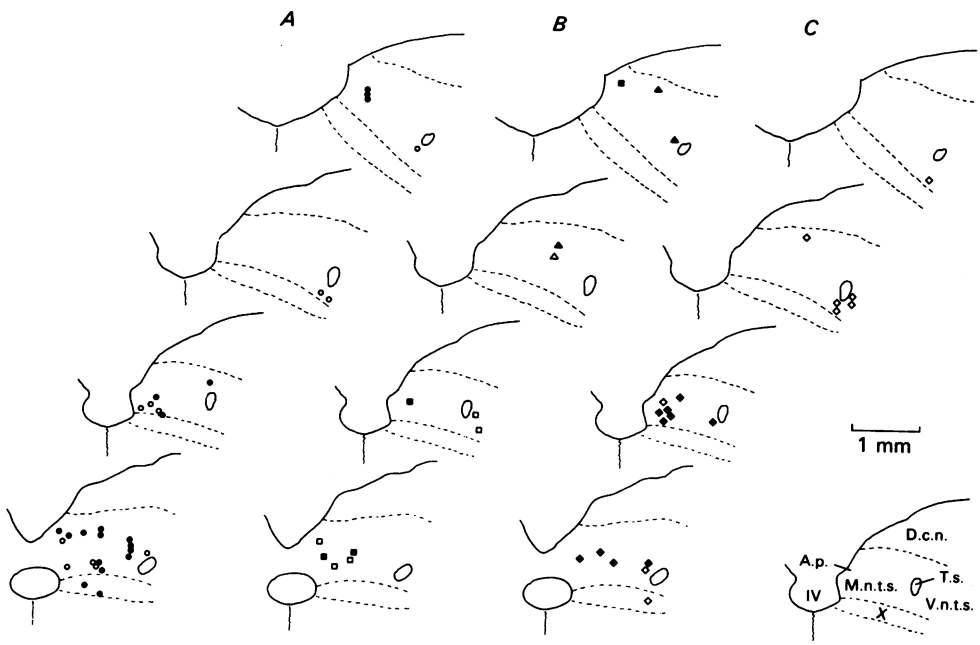

Fig. 5. Positively identified locations of neurones activated synaptically from vagal branches. They are shown on representative hemisections of the dorsomedial brain stem at 1 mm intervals rostral and caudal to the obex. Neurones activated from: *A*, ○, a caudal cardiac branch only at short latency; ●, a caudal cardiac branch only at long latency. *B*, △, a cranial cardiac branch only at short latency; ▲, a cranial cardiac branch only at long latency; □, a pulmonary branch only at short latency; ■, a pulmonary branch only at long latency. *C*, ◇, two or three branches at short latency; ◆, two or three branches at long latency; t.s., tractus solitarius; X, dorsal motor vagal nucleus; a.p., area postrema; IV, fourth ventricle; d.c.n., dorsal column nuclei; m.n.t.s., v.n.t.s., medial and ventrolateral regions of the nucleus tractus solitarius.

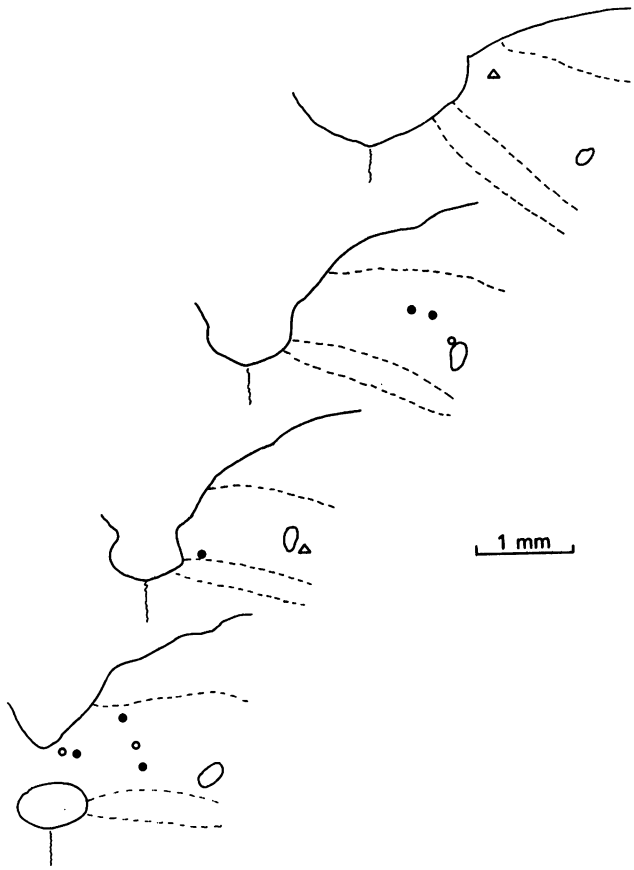

Fig. 6. Positively identified locations of neurones activated non-synaptically from: ○, a caudal cardiac branch at short latency; ●, a caudal cardiac branch at long latency; △, a pulmonary branch at short latency. Arrangement as in Fig. 5.

however, such effects have been observed on other neurones in the n.t.s. (Bennett, Goodchild, Kidd & McWilliam, 1982).

Spontaneous activity. Eleven neurones influenced by a single branch and sixteen neurones responding to stimulation of two or three branches were spontaneously active: the activity was usually irregular and ranged from 0·03 to 7 Hz. Two neurones excited from a pulmonary branch, located in the ventrolateral region of the n.t.s. had a pronounced inspiratory pattern of discharge. Neurones with spontaneous activity which was irregular or had a cardiovascular or respiratory rhythm were frequently encountered but they were not influenced by the cardiac and pulmonary vagal branches tested.

Neurones excited non-synaptically

Twenty-five neurones were excited non-synaptically by stimulation of cardiac and pulmonary vagal branches with latencies of 4–165 ms (Fig. 4). Ten responded with a short latency (see above), seven to stimulation of a caudal cardiac and three to stimulation of a pulmonary branch. Fifteen neurones formed the long latency group; eight were excited from a caudal cardiac and seven from a pulmonary branch.

Location of neurones. All recordings were made from the ipsilateral medulla oblongata from 2·0 mm caudal to 3·0 mm rostral to the obex. The identification of electrode tracks or dye spots enabled the locations of sixty-three synaptically activated neurones to be identified. Forty-six lay in the medial region of the n.t.s. and the remainder were in other areas of the n.t.s. (13), the dorsal motor vagal nucleus (3), or on the border between the dorsal motor vagal nucleus and the n.t.s. (1). (Fig. 5).

The locations of another sixty-six neurones, synaptically excited by vagal branches, were estimated: thirty-nine were in the medial region of the n.t.s. with the remainder being in other regions of the n.t.s. (10) or the dorsal motor vagal nucleus (17). Insufficient data were available to estimate the positions of ten neurones.

Consistent differences in the locations of neurones excited by cardiac or pulmonary vagal branches or those responding to a single or several branches were not apparent. All neurones in the lateral and ventrolateral regions of the n.t.s. responded with short latencies to vagal branch simulation.

The locations of eleven non-synaptic neurones, identified by electrode tracks or dye spots, are shown in Fig. 6; locations of a further thirteen neurones were estimated. Seven lay in the medial region of the n.t.s. Five neurones in the dorsal motor vagal nucleus were all excited at long latencies.

DISCUSSION

Recordings have been made from single neurones in the n.t.s. and the dorsal motor vagal nucleus excited by electrical stimulation of afferent fibres in the cardiac and pulmonary vagal branches.

Synaptically activated neurones responded with minimum latencies which ranged from 3 to 510 ms; such latencies are consistent with the results of the previous study (Donoghue *et al.* 1981*a*). In both investigations two point stimulation confirmed that neurones with latencies greater than 60 ms were activated by non-myelinated afferent fibres. In addition, condition-test procedures to examine potential sub-threshold effects show that, with a single exception, individual neurones were not influenced by both myelinated and non-myelinated afferent fibres. Clearly for the few neurones with latencies close to 60 ms, small errors in conduction distance estimates could influence the category in which they were placed or, they may be activated by the small proportion of afferent fibres which are myelinated only over a part of their course (B–C fibres, Duclaux, Mei & Ranieri, 1976). Whatever the explanation, such neurones were a small proportion of the total population. Only one neurone showed clear evidence of activation in both myelinated and non-myelinated afferent fibres. Overall, the results of this and our previous studies are consistent in showing that n.t.s. neurones activated by cardiac and pulmonary vagal afferent fibres comprise two populations: one activated solely by myelinated vagal afferent fibres while another responds solely to activity in non-myelinated vagal afferent fibres. In a previous study, several n.t.s. neurones in the cat, driven by aortic nerve fibres responded with latencies consistent with volleys in both myelinated and non-myelinated afferents (Donoghue *et al.* 1981*b*), this

difference needs further investigation since afferent fibres in the cat aortic nerve originate from baroreceptors and chemoreceptors.

These findings can be contrasted with dorsal horn interneurones in the spinal cord, where, in addition to selective excitation by myelinated or non-myelinated fibres a high proportion show convergent effects from both myelinated and non-myelinated afferent fibres (e.g. Christenson & Perl, 1970; Gregor & Zimmerman, 1972; Kumazawa & Perl, 1976).

This study has provided indirect evidence for the existence of polysynaptic pathways in the n.t.s. The conduction velocities of most myelinated cardiac and pulmonary vagal afferent fibres are greater than 10 m/s (Coleridge, Coleridge, Dangel, Kidd, Luck & Sleight, 1973; Paintal, 1973) and, evoked afferent volleys should influence brain stem neurones within 15 ms. However, a proportion of the present population responds with latencies substantially longer; similar delays were observed from n.t.s. neurones excited from the aortic nerve (Donoghue *et al.* 1981*b*). There it was argued that such delays were largely due to polysynaptic linkages since conduction delays in fine afferent terminals are unlikely to make a significant contribution. While the possibility that long central pathways outside the n.t.s. may be involved cannot be discounted, we think it unlikely. Similar arguments are more difficult when the latencies of neurones responding to non-myelinated fibres are related to the expected arrival time of the major volley; here, small differences in conduction distance and terminal conduction velocity make large differences to the latency. Nevertheless, we see no reason not to speculate that n.t.s. polysynaptic pathways are also excited by these afferent fibres.

Many neurones were only activated from either cardiac or pulmonary branches suggesting that afferent fibres from the heart and lungs influence separate populations. However, a significant number were activated by fibres in both cardiac and pulmonary branches and such neurones may be later in the central pathway 'down-stream' to the separate populations noted above. Whether or not afferent fibres from both the heart and the lungs activate the same neurone is difficult to assess. Cardiac vagal branches certainly contain afferent fibres from the atria and ventricles (Amann & Schaefer, 1943; Jarisch & Zotterman, 1948; Dickenson, 1950; Neil & Zotterman, 1950), however, they also contain afferent fibres from the lungs (Dickenson, 1950; Oberg & Thoren, 1973; S. Donoghue, R. E. Fox & C. Kidd, unpublished observations); furthermore, it must be assumed that oesophageal afferent fibres may pass into these branches. The quantitative distribution of afferent fibres from the heart and lungs between individual branches is unknown and is likely to be variable: certainly, we noted a variability in the number and diameter of the different thoracic branches which may reflect different admixtures of afferent fibres from different areas.

Excitation of atrial receptors and lung stretch receptors with vagal afferent fibres evokes a tachycardia (de Burgh Daly & Scott, 1958; Linden & Kappagoda, 1982). However, the efferent limb of the reflex increase in heart rate evoked by atrial receptors is exclusively sympathetic (Linden & Kappagoda, 1982) whereas the tachycardia evoked by lung inflation is vagally mediated (Daly & Scott, 1958). Atrial receptors selectively inhibit renal sympathetic nerve activity but have no effect on lumbar and splenic sympathetic nerves (Karim, Kidd, Malpas & Penna, 1972) or on

peripheral vascular resistance (Carswell, Hainsworth & Ledsome, 1970). By contrast, lung inflation evokes either a sympathetically mediated reflex vasoconstriction or vasodilatation (de Burgh Daly, Hazzledine & Ungar, 1967; Hainsworth, 1974; Greenwood, Hainsworth, Karim, Morrison & Sofola, 1977). Additionally, pulmonary receptors activated by stretch or irritant substances have contrasting effects on respiration, while atrial receptors do not influence respiration (see Linden & Kappagoda, 1982). The different reflex patterns elicited by stimulation of cardiac and pulmonary receptors with myelinated vagal afferent fibres clearly are mediated by separate central pathways. However, our results, using electrical stimulation of the cardiac and pulmonary vagal branches, show excitatory convergent effects on n.t.s. neurones. The differences may be explained by the admixture of afferent fibres from different origins within different vagal branches (see above). This problem will only be resolved by the use of physiological stimuli.

Pharmacological stimulation of receptors in the heart and lungs with non-myelinated vagal afferent fibre results in a reflex vagally mediated bradycardia and hypotension (Thoren, 1979; Coleridge & Coleridge, 1983). Thus the central pathway engaged by such afferents must converge at some point. The interneurones in the n.t.s. which are excited by afferent fibres in both cardiac and pulmonary vagal branches are likely to represent the site of such convergence.

The marked differences between the reflex effects of stimulation of myelinated and non-myelinated vagal afferent fibres (see above) and the complete separation of neuronal populations between those engaged by myelinated fibres and non-myelinated vagal fibres is consistent with the hypothesis that there may be two separate systems within the central organization of the brain stem.

Neurones excited by cardiac and pulmonary vagal fibres were located almost exclusively in the n.t.s.; those influenced by myelinated fibres lay in medial and lateral regions of the nucleus while those influenced by non-myelinated afferent fibres were confined to the medial region of the nucleus. Such a distinction is consistent with our previous account (Donoghue *et al.* 1981*b*). Neurones responding to myelinated and non-myelinated afferent fibres of the aortic nerve were shown to have a similar differential distribution (Donoghue *et al.* 1981*b*). Non-myelinated afferent fibres in the carotid sinus nerve were shown to project to the medial portion of the n.t.s. (Jordan & Spyer, 1977). Such a distribution may have a functional significance for the general organization of reflexes which involve non-myelinated afferent fibres.

The restricted distribution of neurones in the ventrolateral portion of the n.t.s. which fire during lung inflation, and are presumably being influenced by fast conducting afferents attached to lung stretch receptors (von Euler *et al.* 1973; Berger, 1977; Richter, Camerer & Rohrig, 1979), can be contrasted with the wider distribution of short latency neurones responding to stimulation of the pulmonary vagal branches seen in this study. Many neurones excited by myelinated pulmonary afferent fibres lay in the medial n.t.s.; these may be part of the central reflex pathways engaged by thinly myelinated fibres of 'irritant' receptors. Such a distribution is compatible with recent histological accounts of the central distributions of pulmonary and cardiac afferent fibres (Kalia & Mesulam, 1980*b*).

We found no evidence of topographic organization in the n.t.s. although errors involved in comparing locations within the n.t.s. between different animals and the

admixture of fibres of different origins between vagal branches may have blurred such an organization. The population studied is clearly coextensive with that of the neurones influenced by the aortic and carotid sinus nerves (e.g. Lipski, McAllen & Trzebski 1976; Muira & Kitamuira, 1979; Donoghue *et al.* 1981*b*).

A small population of synaptically activated neurones lay in the dorsal motor vagal nucleus. They were not antidromically excited by stimuli to the cardiac and pulmonary branches tested and they may correspond to the 'small interneurones' of the dorsal motor vagal nucleus (McLean & Hopkins, 1981, 1982). However, the possibility that their efferent axons lay in other vagal branches cannot be excluded: nor can we exclude the possibility that the recording sites were remote dendrites or axons of n.t.s. interneurones, although the stability and depth over which recordings could be retained suggests otherwise. Cottle (1964) did not demonstrate vagal afferent fibres in the dorsal motor vagal nucleus of the cat although others (Beckstead & Norgren, 1979; Kalia & Mesulam, 1980*a*) reported autoradiographic labelling of fibres in this nucleus in the monkey and cat respectively following the injections into the nodose ganglion it is not possible to differentiate terminal areas from those of fibres '*en passage*'. Further electrophysiological studies are necessary.

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