QUANTITATIVE ANALYSIS OF LARYNGEAL MECHANOSENSITIVITY IN THE CAT AND RABBIT

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SUMMARY

1. Single afferent fibres in the internal branch of the superior laryngeal nerve which responded to light touch or gentle probing of discrete areas of the exposed epithelium of the opened larynx were identified in anaesthetized, paralysed cats (148 fibres) and rabbits (58 fibres).

2. A quantitative examination of the sensitivity of these laryngeal mechano-receptors to both static (step indentations) and dynamic (vibratory) forms of mechanical stimulation was undertaken using a servo-controlled mechanical stimulator.

3. In both species two predominant classes of mechanoreceptors were observed (Boushey, Richardson, Widdicombe & Wise, 1974). One class was distinguished by a regular and continuous pattern of activity at a frequency of 10–70 Hz (tonic fibres, sixty-six in cat, thirty-five in rabbit). The other class was silent or (more rarely) irregularly active at a very low frequency (silent fibres, eighty-two in cat, twenty-three in rabbit).

4. The location of the receptive fields was determined by manual probing. Inter-species and regional variations in receptive field location were observed for the two fibre groups.

5. Conduction velocity was measured for twenty-one tonic and seven silent fibres in the rabbit by a pre-triggered averaging technique. The results obtained (tonic: range 10·8–30·0, mean ± S.E. of mean 21·4 ± 1·2 m/s; silent: 14·8–28·6, 20·4 ± 1·8 m/s) were characteristic of group III afferent fibres but were not significantly different for the two classes.

6. Both classes of receptor showed a response at the onset of a step indentation of the region of the mucosa that corresponded to their receptive field. Subsequent to this brief initial response the behaviour of the two classes diverged markedly. Tonic fibres were invariably slowly adapting whereas most (forty-four out of fifty-five in cat; twenty-two out of twenty-three in rabbit) silent fibres were rapidly adapting, at least for smaller indentation amplitudes.

7. Receptors of both classes were readily entrained to discharge at the same frequency as the probe stimulator (1:1 entrainment) when this was made to vibrate upon the receptive area for test periods of 0·5 or 1·0 s. Tuning curves were constructed

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of the minimum amplitudes required to elicit 1:1 entrainment throughout an entire test period at various frequencies.

8. Individual fibres in the two classes could be entrained at frequencies up to 400 Hz or more at sensitive (e.g. < 100 μm) vibratory amplitudes. However, all fibres were less sensitive at these higher frequencies than at some lower point on the frequency scale.

9. Rapidly adapting, silent fibres had curves which usually exhibited identifiable minima (10–60 Hz), but the curves for these, and other silent fibres, were relatively broadly tuned. Slowly adapting, silent fibres had curves which were particularly flat in the lower range of frequencies. Tonic fibres were also more sensitive at the lower frequencies that approached their spontaneous discharge rate, but could not normally be 'stimulated' to fire at frequencies less than this.

10. It was concluded that the dynamic sensitivity of fibres from the various locations would ensure a high probability of the movement of a foreign particle into the larynx being signalled at most velocities and on most laryngeal surfaces.

INTRODUCTION

The larynx is richly innervated by mechanosensitive nerve fibres, the majority of which are contained in the internal branch of the superior laryngeal nerve. Single-fibre studies have described nerve endings sensitive to light touch, applied pressure indentations, transmural pressure or to the displacement of laryngeal structures and contraction of the laryngeal muscles (Andrew, 1954; Sampson & Eyzaguirre, 1964; Martensson, 1964; Storey, 1968; Boushey, Richardson, Widdicombe & Wise, 1974; Harding, Johnson & McClelland, 1978; Sant'Ambrogio, Mathew, Fisher & Sant'Ambrogio, 1983; Hwang, St. John & Bartlett, 1984; Mathew, Sant'Ambrogio, Fisher & Sant'Ambrogio, 1984).

Boushey *et al.* (1974) distinguished two major classes of superficially located laryngeal mechanoreceptors according to the presence or absence of spontaneous discharge activity, and the dynamics of the response to gentle mechanical stimulation of the exposed laryngeal mucosa. The fibres of one group were generally silent and displayed a wide range of adaptation rates to mechanical stimulation. The fibres of the other group were characterized by a regular and continuous pattern of spontaneous activity and were slowly adapting when mechanically stimulated. The sensitivity that some of these fibres displayed to mechanical and chemical stimulation implicates them in defensive reflexes. A quantitative determination of the static and dynamic sensitivity of these receptors would clearly be a useful extension of the above studies.

Although the mechanosensitivity of cutaneous sensory receptors has now been well documented and quantified (for example, Werner & Mountcastle, 1965; Talbot, Darian-Smith, Kornhuber & Mountcastle, 1968), the methods employed by these authors have not been applied to a study of laryngeal mechanosensitivity. The present study represents a continuation of the approach adopted in this laboratory of adapting such methods for the study of receptors in the upper airways (Nail & Rowe, 1976; Nail, 1980). The paper provides a quantitative description of the sensitivity of laryngeal mechanoreceptors in the cat and rabbit to both static (step

indentation) and dynamic (vibration) stimuli applied by a mechanical stimulator of the type employed in the above cutaneous receptor studies.

<center>METHODS</center>

Surgical procedures

Experiments were performed on fifty-five adult cats and twenty-seven adult rabbits. The cats were anaesthetized with intraperitoneal injections of sodium pentobarbitone (Nembutal, Abbott, 30 mg/kg) and the rabbits with intravenous injections of a mixture of sodium pentobarbitone (Nembutal, Abbott, 30 mg/kg) and urethane (Sigma, 0·75 g/kg). The trachea was cannulated, the femoral artery and vein catheterized and the animal positioned supine in a stereotaxic frame. Blood pressure and rectal temperature were monitored continuously. Throughout the recording period the animals were immobilized with gallamine triethiodide (Flaxedil, May & Baker, 20 mg/kg) and artificially ventilated; intravenous supplements of anaesthetic were given regularly throughout the experiment.

The larynx was isolated from its attachments to the trachea, the sternothyroid and sternohyoid muscles and the oesophagus in order to minimize transmission of passive movements during ventilation. The laryngeal mucosa was exposed by a ventral mid-line incision which was continued through the epiglottis and hyoid bone. Traction was applied to the left (non-experimental) cut edge of the larynx so as to expose the mucosa covering the right arytenoid cartilage, vocal cord and right half of the epiglottis. When necessary, a surgical thread was carefully inserted into the right cut edge of the tip of the epiglottis and gentle traction applied to prevent infolding of the tip of the epiglottis over the glottis. No further traction was applied to the right (experimental) half of the larynx. The exposed laryngeal mucosa was kept moist by irrigation with isotonic saline between tests.

The right internal laryngeal nerve was exposed and cut 2 cm from the larynx; the surrounding fibrous tissue was dissected away from the nerve which was covered with mineral oil contained by skin flaps. The peripheral end of the nerve was then placed on a small platform within the oil pool and dissected into fine filaments from which action potentials were recorded and analysed using conventional techniques.

Receptive field location

Fibres were only selected for study if they were sensitive to gentle strokes with a cotton thread (touch-sensitive units). A further restriction on the selection of fibres was that the receptive field had to be accessible to a normal approach by the probe tip of a mechanical stimulator. After electrically isolating a 'single' mechanoreceptive fibre the location of its receptive field was determined by gentle manual probing and the most sensitive area was indicated on a map of the larynx constructed from a photograph. A few fibres were encountered that were insensitive to such gentle stimulation but which could be excited by more forceful probing, for example with a fine plastic catheter, provided this was sufficient to cause a small indentation of the mucosa. Such high-threshold tactile units which exhibited a reasonably well-defined receptive field were also examined and their properties are described separately in this study.

Mechanical stimulation

Successive trains of mechanical stimuli delivered by a servo-controlled mechanical stimulator (Darian-Smith, Rowe & Sessle, 1968; Bystrzycka, Nail & Rowe, 1977) were used to quantify fibre mechanosensitivity. The stimulator was based on a moving-coil vibrator (Alpha-M Corp., Model AV-6) to which was fixed a threaded output shaft containing the stimulating probe. The axial displacement of the probe tip was signalled by a linear displacement transducer (Schaevitz 100DC-D) permanently attached to the probe which was monitored continuously throughout these experiments on a storage oscilloscope (Tektronix 5113). Signals from the displacement transducer were used by a feed-back control circuit in the stimulator's power amplifier to ensure that differences between the desired displacement and the transducer output signal were minimal. In this manner the axial displacement of the probe tip (Lucite, 1·58 mm diameter machined to a hemisphere) was independent of the relatively slight mechanical resistance offered by the tissue being probed over the range of displacements employed in these experiments. Two input voltages were supplied to

the stimulator's control circuitry. One controlled the amplitude of the step indentation which was switch selectable in fifteen steps; the maximum indentation was set at 1050 μm and the rate of indentation, which actually followed a ramp form, was usually set at a high (5 ms/mm) value as this function was not employed in the present study. The second input accepted sinusoidal wave-forms from a function generator (Interstate Electronics F43). An adjustable d.c. voltage from a ten-turn potentiometer ensured fine control of the wave-form amplitude. These control signals were passed through a wave-shaping network, which incorporated the feed-back circuitry and a damping control, before going to the vibrator. Timing signals controlling the length of time for the indentation and the vibratory wave forms were taken from a Digitimer (Model D4030) master timing unit set to give a stimulus repetition rate of one every 10 s.

Calibration of the control settings used to preset step indentation and sine-wave amplitudes was achieved by measuring the displacement of the probe tip with a graduated micromanipulator (resolution 1 μm) at each frequency and indentation used. This procedure also validated the calibration of the linear displacement transducer. The tip of the stimulating probe was placed opposite a micromanipulator and an electrical circuit arranged to signal contact between these two surfaces. Once contact was established, the micromanipulator was withdrawn from the probe by half the peak-to-peak amplitude of the required sinusoidal vibration and the potentiometer setting then determined for achieving this probe displacement. This was done by incrementing the vibration amplitude until contact was achieved and the setting on the control potentiometer noted. Peak displacements for both indentation and withdrawal phases were determined to ensure the symmetry of the wave form and to guard against frequency-dependent drift. The procedure was carried out at frequency intervals of 50 Hz and the calibration curves so obtained were used to determine peak-to-peak probe displacement for any combination of potentiometer setting and vibration frequency. It was noted that while the relation was essentially flat for low to moderate frequencies, the potentiometer setting needed to produce a given amplitude was clearly frequency dependent for the higher frequencies where the amplitude available became progressively limited. For example at 400 Hz only 850 μm could be achieved.

Stimulation protocol

Static sensitivity. The sensitivity of single fibres to static stimuli was tested by recording the fibre's response to step indentations of 1·8 s duration over fifteen steps from 70 to 1050 μm. A 400 ms settling period was allowed immediately after each indentation was applied, following which the number of action potentials evoked during a further 1 s was recorded. The indentations were delivered during the same phase of the ventilatory pump stroke ('pump triggered'), and repeated at intervals of 10 s. The sequence of delivery of the different amplitudes of the step indentations was randomized and the mean response to three tests at each indentation amplitude was plotted (indentation curves). For some fibres, the number of action potentials in the first 500 ms (F_1), i.e. not preceded by a settling period, and those in the second 500 ms (F_2), were recorded independently for construction of an adaptation index (A.I.), where

$$\text{A.I.} = (F_1 - F_2/F_1)\,100.$$

This index is similar to that used by previous investigators (Knowlton & Larrabee, 1946; Widdicombe, 1954; Bartlett & St. John, 1979; Sant'Ambrogio, Fisher & Sant'Ambrogio, 1983).

Dynamic sensitivity. Fibres were tested for their dynamic sensitivity by superimposing a sinusoidal vibratory movement upon the step indentation of the probe. To maintain stable mechanical coupling between the probe and the surface of the mucosa during vibration, the sinusoids were imposed upon a relatively firm step indentation, for example 140 μm greater than the step indentation required to produce a threshold response. That the probe tip was withdrawn from the mucosal surface during the 8·2 s rest periods was determined by visual inspection with a dissecting microscope. A 1 s period of probe vibration was superimposed on a 1·8 s step indentation and was programmed to commence 400 ms after the onset of the step to isolate the dynamic response of the fibre to the application of the step. The minimum sinusoidal amplitude necessary to achieve 1:1 entrainment was routinely determined for each of a number of different frequencies selected from as wide a range as possible. The duration of vibration was usually 1 s; for a few fibres vibratory durations of 500 ms, or less, were used (see Results).

Conduction velocity measurement

Conduction velocity was measured 'on-line' using a pre-triggered averaging technique (Hilaire, Gauthier & Monteau, 1983). Single-fibre action potentials were used to 'pre-trigger' a fast (2·0 μs/point sampling rate) digital storage device ('Wavesaver', Epic Instruments Inc.) which was connected to record whole-nerve activity from the internal laryngeal nerve at a site as close as possible to the larynx. A laboratory computer (Apple IIe) interfaced to the 'Wavesaver' accumulated an average wave form from a preset number (usually 150) of successive pre-triggered 'Wavesaver' records. A fine thread was used to measure the inter-electrode distance at the conclusion of each conduction velocity determination.

RESULTS

Two predominant classes of mechanoreceptive fibres were observed in the internal laryngeal nerve of both species as previous reports have indicated (Boushey *et al.* 1974; Davies & Vizek, 1982). One group of fibres was distinguished by a regular and continuous pattern of tonic activity at a frequency of 10–70 Hz. The other group were silent or exhibited a low-frequency (< 10 Hz) irregular discharge. Recordings were obtained from 148 fibres in the cat (eighty-two silent and sixty-six tonic), and from fifty-eight in the rabbit (twenty-three silent and thirty-five tonic). Conduction velocity measurements for seven silent fibres in the rabbit ranged from 14·8 to 28·6 m/s (20·4 ± 1·8 m/s, mean ± S.E. of mean) and from 10·8 to 30·0 m/s (21·4 ± 1·2 m/s) for twenty-one tonic fibres.

Receptive field location

In the cat the majority of fibres tested were sensitive to light stroking with a cotton thread. Significant directional sensitivity was not evident for any field. Some fibres from both the silent and tonic receptor groups which were particularly sensitive to stroking had small receptive fields, often less than 1 mm^2, although much more forceful stimulation was likely to be effective over a wider area. The receptive fields for other fibres, such as some of those on the ary-epiglottic fold or epiglottis, were oval or circular in shape and exhibited a uniform rather than punctate sensitivity over 2–3 mm. Receptive fields for the *silent* fibres were frequently located on the apex of the arytenoid cartilage as well as on the vocal process, the ary-epiglottic fold and the base of the epiglottis (Fig. 1). A few units were located below the glottis while the receptive field for one fibre was located on the contralateral side, although close to the mid line. A small number of silent fibres ($n = 4$) only responded to forces sufficient to cause mucosal indentations. The receptive fields of these silent, high-threshold touch (indentation) fibres were more difficult to define; their location is indicated in the legend to Fig. 1 by the number in parentheses. Recordings were obtained from fifty-five *tonic* fibres in the cat which were sensitive to light stroking with a cotton thread, and from eleven tonic fibres which were only sensitive to probing that resulted in obvious indentation of the mucosa (i.e. tonic high-threshold touch–indentation fibres). Receptive fields of tonic fibres were frequently located on the vocal process of the arytenoid cartilage, the ary-epiglottic fold and the epiglottis (Fig. 1). Tonic fibres were more numerous on the body of the epiglottis than were silent fibres, and some of these were exquisitely sensitive to touch.
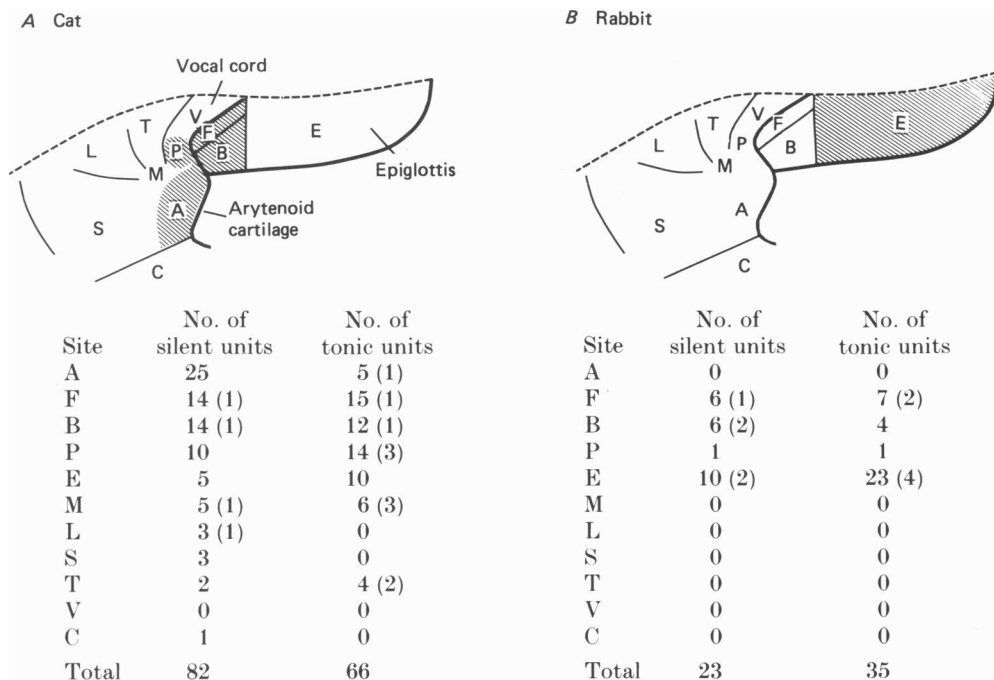
Site	No. of silent units	No. of tonic units	Site	No. of silent units	No. of tonic units
A	25	5 (1)	A	0	0
F	14 (1)	15 (1)	F	6 (1)	7 (2)
B	14 (1)	12 (1)	B	6 (2)	4
P	10	14 (3)	P	1	1
E	5	10	E	10 (2)	23 (4)
M	5 (1)	6 (3)	M	0	0
L	3 (1)	0	L	0	0
S	3	0	S	0	0
T	2	4 (2)	T	0	0
V	0	0	V	0	0
C	1	0	C	0	0
Total	82	66	Total	23	35

Fig. 1. Distribution of the receptive fields for internal laryngeal nerve mechanoreceptors identified in the cat (A) and rabbit (B) by light touch or gentle probing of the mucosa. The receptive fields for these fibres were located after the larynx had been opened by a ventral mid-line section (dashed line) of the cricoid, thyroid and epiglottic cartilages. The number of silent fibres and regular-firing tonic fibres localized to the various laryngeal structures are given separately for the cat and rabbit in the table. The parentheses indicate the number of fibres located on that structure which were sensitive only to probing which was sufficiently forceful to cause obvious indentation of the mucosa. For each species those structures associated with the greatest representation of mechanoreceptive fibres are shaded. A: apex and ventral surface of the arytenoid cartilage. F: ary-epiglottic fold. B: base of the epiglottis. P: vocal process of the arytenoid cartilage. E: body of the epiglottis. M: attachment of the thyro-arytenoid and lateral crico-arytenoid muscles to the arytenoid cartilage. L: mucosa overlying the lateral crico-arytenoid muscle. S: mucosa overlying the cricoid cartilage (sub-glottic). T: mucosa overlying the thyro-arytenoid muscle. V: inter-membranous part of the vocal cord. C: contralateral laryngeal mucosa.

In the rabbit the proportion of tonic to silent fibres was greater. Relatively few fields were located on the arytenoid cartilage or its attachments and the majority of fibres were located on the body of the epiglottis, which contained the most sensitive fibres, the ary-epiglottic fold and the base of the epiglottis. Both classes included those less-sensitive fibres which only responded to forces sufficient to cause indentations ($n = 11$, Fig. 1). There was an apparent absence of fibres from mechanoreceptors below the glottis in the internal laryngeal nerve of the rabbit. For both species, no fields for either type were isolated on the inter-membranous portion of the vocal cord (anterior two-thirds) although in the cat the vocal process (posterior one-third of the vocal cord) appeared to be quite richly innervated.

Sensitivity to maintained indentation

Silent fibres. In the cat a total of fifty-five silent fibres were tested with step indentations ranging from 70 to 1050 μm. Twenty-three fibres adapted rapidly, discharging only one or two spikes at the indentation and retraction phases of the step ('on' and 'off' responses, Fig. 2); many of these (twelve of twenty-three) were located on the arytenoid cartilage or its attachments. The receptive fields for rapidly adapting fibres appeared to be less well defined than those for the more slowly adapting silent fibres described below.
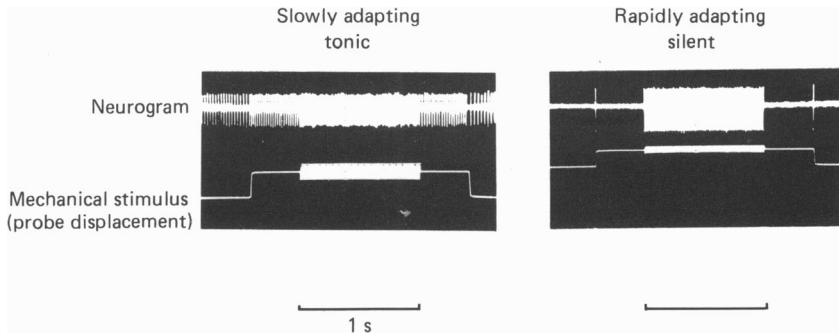
Fig. 2. Neurograms of fibre discharge evoked by a mechanical probe; the lower traces show the probe displacement transducer output. A 1·8 s step indentation of the mucosa is depicted, upon which a 1 s period of sinusoidal vibration is superimposed. Note that the regular tonic discharge (40 Hz) for the fibre on the left (rabbit) is increased by the application of the step (indentation 210 μm) and is subsequently entrained by the sinusoidal vibration (120 Hz, 131 μm). The fibre on the right (cat) is silent at rest but displays a rapidly adapting response at the indentation and retraction phases of the step indentation (140 μm) and is entrained by the probe vibration (120 Hz, 56 μm).

For the remainder of the silent fibres tested with step indentations, twenty-one displayed high (50–100 %) adaptation indices at low indentation amplitudes, for example less than 350 μm above threshold, but this index became smaller (30–60 %) as the indentation amplitude was increased. More than half (twelve of twenty-one) of these fibres were located on the apex of the arytenoid cartilage. The response of two such silent fibres to a large step indentation is depicted in an instantaneous frequency plot in Fig. 3 *A* and *B*. The adaptation index for these fibres at a 840 μm indentation was 54 % (fibre in *A*) and 33 % (fibre in *B*) although at smaller indentations (70–210 μm for fibre in *A*; 70–560 μm for fibre in *B*) both of these fibres displayed only 'on' and 'off' responses. Silent fibres of this type appeared to form a large proportion of the silent receptor class in the cat although only twenty-one were tested with a variety of step indentations.

Other silent fibres ($n = 11$) adapted more slowly to the step indentations at all amplitudes tested (adaptation index less than 40 %). Some had an irregular, low rate of tonic discharge (for example less than 10 Hz), or were silent and became tonically active with a regular type of discharge for periods of a few minutes or longer after having been stimulated by probing. These fibres had receptive fields which were widely distributed over the glottic and supra-glottic areas depicted in Fig. 1.
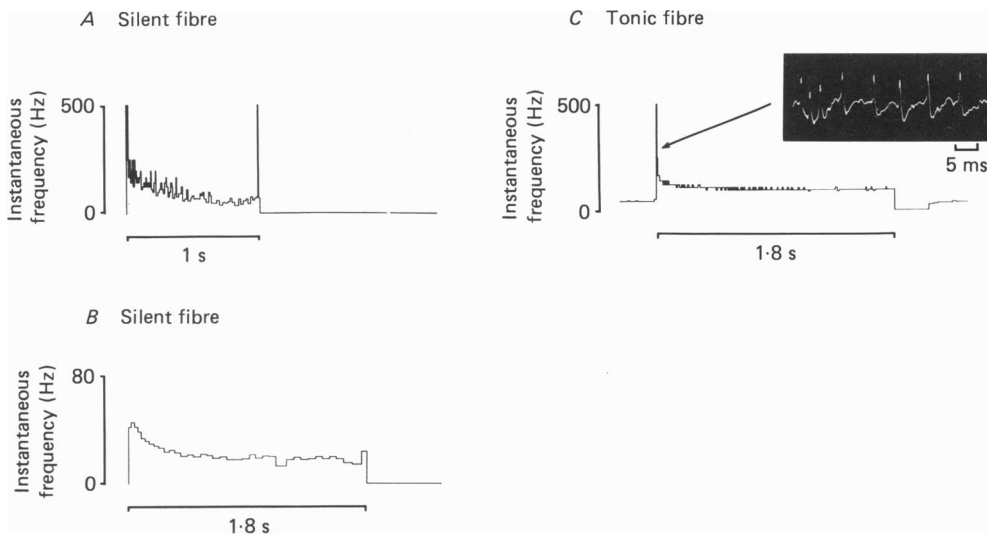
Fig. 3. Instantaneous frequency plots of the discharge evoked in two silent fibres (*A* and *B*) and one tonic fibre (*C*) in the cat by step indentations (840 μm) of the fibre's receptive field. (The frequency resolution of these plots is limited by the time base (1 ms) used for interspike interval measurement.) Note the different calibration for instantaneous frequency in *B* and *A*, *C*. The duration of the indentation is indicated by the bar beneath each plot. Note the dynamic response during the indentation and retraction movements of the mechanical probe for the silent fibre depicted in *A* and at the indentation phase for the tonic fibre depicted in *C*; the inset shows a neurogram of the initial discharge of this fibre at the commencement of the indentation for which the instantaneous frequency plot was derived. Each of the silent fibres (*A* and *B*) displayed only 'on' and 'off' responses to smaller step indentations (see text). Other silent fibres (not depicted in this Figure) showed only 'on' and 'off' responses at all step indentation amplitudes tested.

Fig. 4*A* illustrates the sensitivity to 1 s step indentations (following a 400 ms settling period) for some of the silent fibres which displayed a high adaptation index at low indentation amplitude (continuous line) and some of the more slowly adapting silent fibres (dashed line).

In the rabbit the majority (seventeen of twenty-three) of silent fibres were rapidly adapting to identation amplitudes up to 1050 μm and displayed only 'on' and 'off' responses at the indentation and retraction phases of mucosal indentation. Most of these were sensitive to light stroking, but four were classified as high-threshold touch (indentation) fibres. Each of the remaining six more slowly adapting silent fibres discharged at an increasing frequency as the indentation amplitude was increased (see results for two fibres in Fig. 4*B*) although, as seen in the cat, the adaptation index was considerably larger (60–100 %) for small indentation amplitudes than for larger amplitudes (adaptation index 30–60 %).

Tonic fibres. Step indentations of 70–1050 μm were tested on twelve tonic fibres in the cat and five in the rabbit. Each fibre so tested responded with an approximately linear increase in discharge frequency as the indentation amplitude was increased (Fig. 4*C* and *D*). The discharge pattern was slowly adapting with adaptation indices generally less than 10 % except for the larger indentation amplitudes where this index
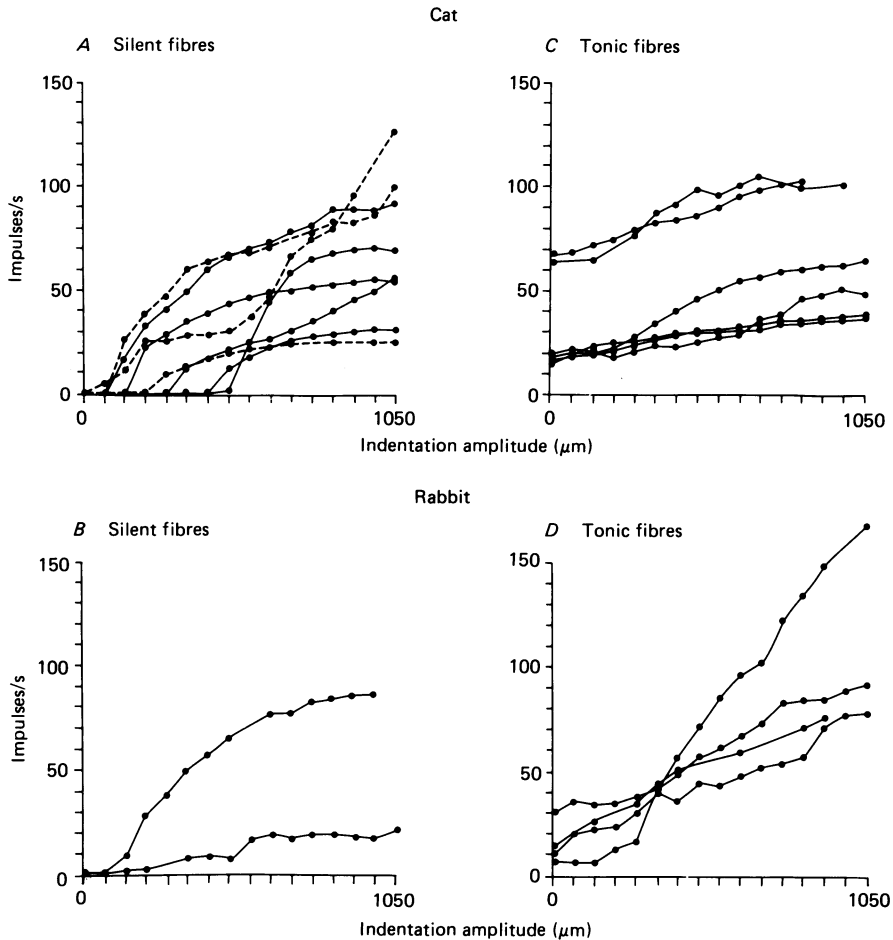
Fig. 4. The number of impulses evoked in individual fibres by different amplitudes of mucosal indentation. Impulses were counted during the second which immediately followed a 400 ms settling period from the onset of indentation. For each fibre, individual points represent the mean for three separate presentations of a particular indentation amplitude. The sequence of amplitudes presented was randomized. In *A* the continuous curves identify silent fibres which displayed a high adaptation index at low indentation amplitudes and the dashed curves the slowly adapting silent fibres. Note that the silent fibres which respond to all amplitudes of step indentations with only brief 'on' and 'off' responses (see text) are not represented in this Figure.

sometimes increased to 30%. Most tonic fibres discharged briefly at a higher frequency (for example 500 Hz) during and immediately after the indentation movement of the probe, as can be seen in the neurogram in Fig. 2 and in the instantaneous frequency plot in Fig. 3*C*. In the latter Figure the inset depicts the fibre discharge in the initial 45 ms following mucosal indentation; two interspike intervals of 2 ms are apparent and the second and third spikes are reduced in amplitude indicative of the relative refractory period (Tapper, 1964). As illustrated in this Figure, the tonic fibres did not generally display 'off' responses at the moment of retraction of the probe.

Sensitivity to vibratory stimuli

This section of the Results describes the relative capacities of individual fibres to be entrained to discharge at the frequency of the vibrating probe used to stimulate them. It should be noted that entrainment in the sense used here refers to perfect (1:1) entrainment, i.e. to the pattern of response where one action potential is evoked by each individual vibratory cycle throughout the entire vibratory test period (see Methods). The form of tuning curve which results when minimum vibratory amplitude for perfect entrainment is plotted against vibratory frequency provides a useful characterization of the dynamic sensitivity of the endings being so examined (Talbot *et al.* 1968).

Silent fibres. In the cat most silent fibres tested (forty out of fifty-nine) were sensitive to vibration at frequencies between 10 and 100 Hz and their discharge could be readily entrained at some or all frequencies within this range at low vibratory amplitudes (10–100 μm). Some of these fibres could be entrained by these low amplitudes to considerably higher frequencies, e.g. 200 Hz ($n = 3$), 300 Hz ($n = 1$), 400 Hz ($n = 2$), 500 Hz ($n = 1$) and even 600 Hz ($n = 2$). Other fibres (twenty-three out of forty) were entrained at frequencies of 200 Hz or more, but only at vibratory amplitudes greater than 100 μm. The remaining silent fibres (nineteen out of fifty-nine) either exhibited a threshold greater than 100 μm amplitude at all frequencies tested ($n = 11$), or could not be entrained to the frequency of vibration for the full 1 s (or 500 ms) vibratory test period ($n = 8$). All fibres in this latter group had rapidly adapting responses to step indentations (as did four of the eleven fibres in the former, higher threshold group) but also showed adaptation during the vibratory train.

Tuning curves, showing the minimum vibratory amplitude required to perfectly entrain the receptor discharge to the vibratory sequence at various frequencies (see note above), are shown in Fig. 5. Results for the silent fibre class are depicted in parts *A* and *B* of this Figure. The rapidly adapting fibres, i.e. adaptation index 100 % at all indentation amplitudes, appeared to be most sensitive to frequencies between 10 and 60 Hz at vibratory amplitudes from 45 to 200 μm (Fig. 5*A*). The curves depicted in Fig. 5*B* represent results for the less rapidly adapting subset of the silent fibre group; fifteen of the fibres depicted displayed high adaptation indices only at low indentation amplitudes and five were slowly adapting (adaptation index < 40 % at all indentation amplitudes). The latter, although initially silent, were induced into periods of moderately long-lasting background tonic activity by the vibratory stimuli with the result that these fibres could not be entrained at frequencies lower than this background discharge. The Figure appears to indicate also that some apparently very sensitive fibres could not be entrained at frequencies below 5–10 Hz. This was not due to the fibre becoming insensitive at low frequencies, but rather because the minimum amplitude required to elicit entrainment at such low frequencies tended to produce more than one impulse for each vibration of the probe tip, as a consequence of which the criterion of perfect (i.e. unitary) entrainment could not be achieved.

The entrainment thresholds of most silent fibres located on the epiglottis (93 %) and apex of the arytenoid cartilage (89 %) were less than 100 μm (Fig. 6*A*). The frequencies at which fibres were the most sensitive, according to the criterion adopted

A Silent fibres

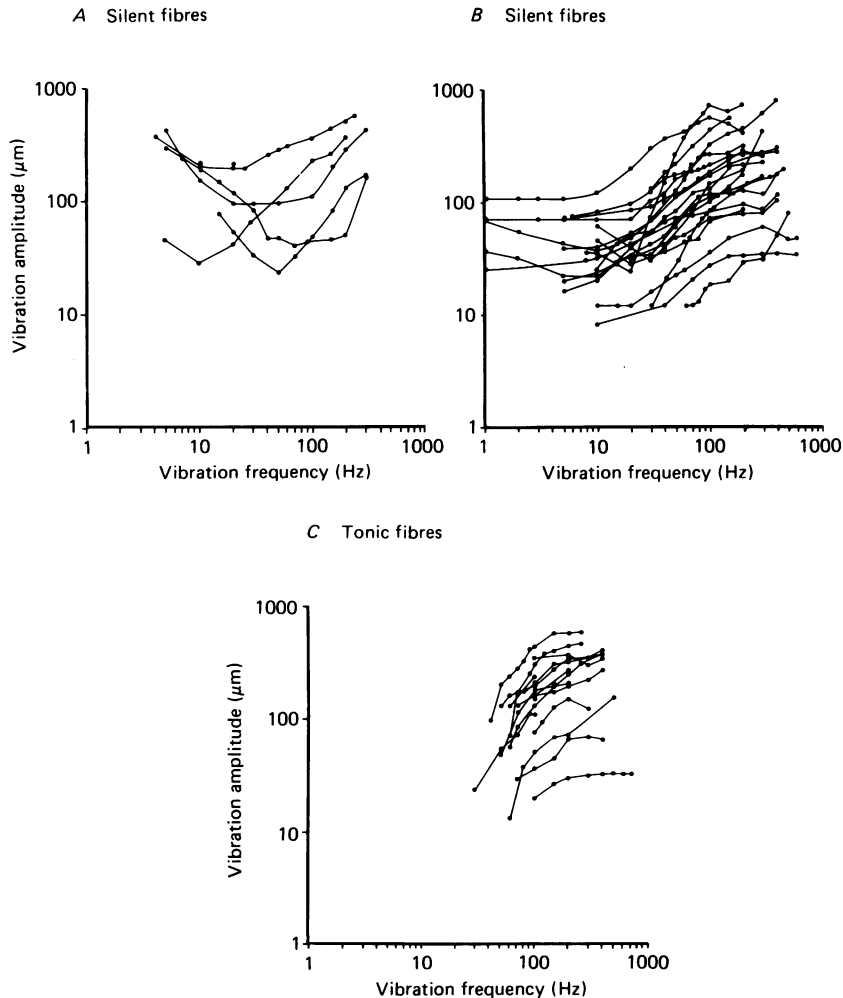B Silent fibres

C Tonic fibres

Fig. 5. Tuning curves (see Methods) of internal laryngeal nerve mechanoreceptors in the cat. The minimum amplitude (μm) required to entrain perfectly fibre discharge to the stimulating sinusoidal vibration for durations of 1 s is depicted (for the highest threshold fibre in *A* the duration of the sinusoidal vibration was shortened to 500 ms). *A*, silent, rapidly adapting fibres (adaptation index 100 % at all step indentation amplitudes tested). *B*, silent fibres which displayed a high adaptation index only at low indentation amplitudes ($n = 15$) or which were slowly adapting (adaptation index < 40%, $n = 5$) at all indentation amplitudes; the latter, initially silent fibres were induced into tonic activity by the vibratory stimuli and for these, entrainment could not be produced at frequencies lower than this background discharge. *C*, tonic fibres. Note logarithmic scale on both axes.

for this study, ranged from 5 to 40 Hz (mean $15 \pm 2 \cdot 8$ Hz) for those fibres on the epiglottis and from 5 to 200 Hz ($35 \cdot 2 \pm 11 \cdot 1$ Hz) for those on the arytenoid cartilage. A smaller proportion (60 %) of silent fibres with fields on other structures (Fig. 1 areas P, F, M, T, S) displayed entrainment thresholds less than 100 μm at their most sensitive frequency ($20 \cdot 1 \pm 3 \cdot 5$ Hz).

In the rabbit thirteen silent fibres were tested for their dynamic sensitivity. Three

fibres could not be entrained and others were only entrained by vibratory amplitudes greater than 100 μm (six fibres) and/or vibratory test periods of 500 ms or less (five fibres). While some of these curves were more peaked, and in this respect like the curves for the most rapidly adapting fibres in the cat (Fig. 5A), the tuning curves for other silent fibres in the rabbit were relatively flat from 10 to 100 Hz, like those of the cat depicted in Fig. 5B.

Tonic fibres. The tonically active, slowly adapting fibres were also very sensitive to vibratory stimuli. In the neurogram depicted in Fig. 2 the background discharge can be seen to be both increased by the step indentation and entrained by vibration. For ten out of nineteen tonic fibres tested in the cat, entrainment to frequencies in the range 30–150 Hz was achieved at vibration amplitudes between 10 and 100 μm. Six of these fibres could also be entrained at frequencies up to 400 Hz or higher (for example 700 Hz, see Fig. 5C) and of these, three achieved such levels of activity at amplitudes less than 100 μm. The remaining nine tonic fibres showed similar responses (including entrainment to 400 Hz for four out of nine fibres) although only at larger (i.e. > 100 μm) vibration amplitudes. In the rabbit two tonic fibres exhibited low-threshold (< 100 μm) entrainment in the range of 60–500 Hz and two were entrained in the range 40–200 Hz at amplitudes greater than 100 μm.

Tuning curves obtained from sixteen tonic fibres in the cat are depicted in Fig. 5C. Normally entrainment could not be induced at frequencies close to, or less than, the frequency of the spontaneous discharge, which explains the abrupt termination of the curves in their sensitive low-frequency range. Note that when entrained, i.e. at frequencies greater than that of the spontaneous firing rate, the tonic fibres are as sensitive to dynamic stimuli as the silent fibres. The entrainment threshold for 85 % of tonic fibres located on the epiglottis or arytenoid cartilage was less than 100 μm (cf. Fig. 6A and B). The three fibres with fields located on the belly of the thyro-arytenoid muscle each displayed high (> 200 μm) entrainment thresholds; two of these were high-threshold touch (indentation) fibres. For the tonically active fibres the most sensitive frequency ranged from 30 to 150 Hz and was generally the lowest frequency at which 1:1 entrainment could be achieved above the level of tonic background discharge.

<center>DISCUSSION</center>

Quantitative methods which have been successfully used to characterize the different classes of cutaneous mechanoreceptors (Werner & Mountcastle, 1965; Talbot *et al.* 1968) were employed in an attempt to define the mechanoreceptor properties of laryngeal nerve endings more precisely. The justification for applying this particular form of artificial stimulation to the larynx is that it permits the separation and quantification of the dynamic, as well as static, sensitivity for each ending, and that both these particular properties are likely to be of functional importance. The chief function of the larynx is to guard the airways against the entrance of particulate and other matter and it may be supposed that the most sensitive endings would serve that role. The endings studied here are the most sensitive to mechanical stimulation that may be detected from the laryngeal surfaces. Moreover, it is unlikely that successful defence of the airways depends solely on
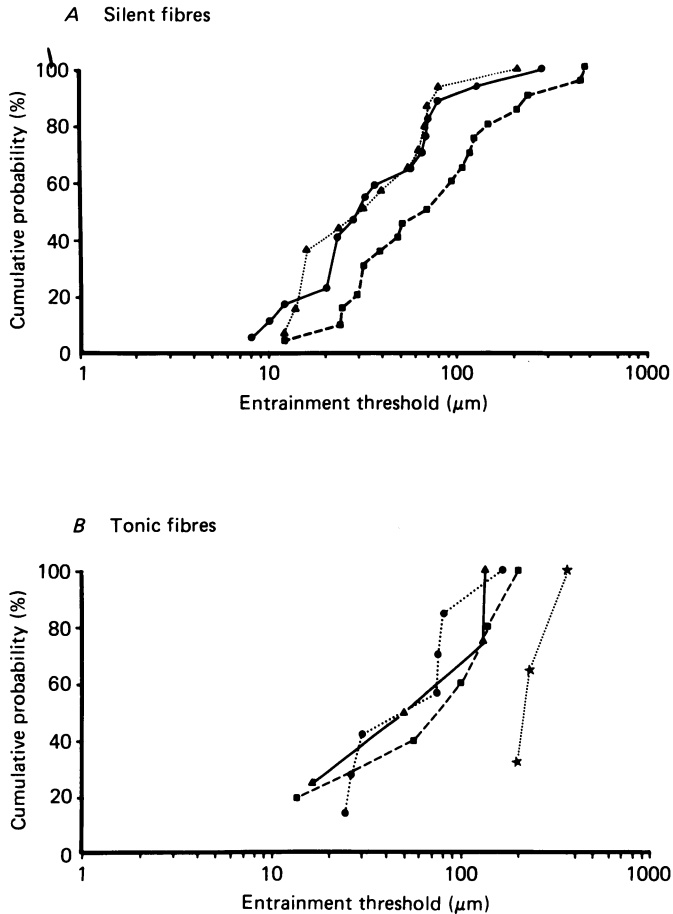
Fig. 6. The distribution of entrainment thresholds in the cat (expressed as the minimum amplitude of sinusoidal vibration capable of perfectly entraining each fibre's discharge) at different areas of the larynx. Note that these are minimum thresholds, i.e. thresholds determined at the most sensitive frequency of each fibre (see Fig. 5). *A* represents the cumulative percentages of the thresholds for silent fibre populations with fields on the arytenoid cartilage (●, $n = 17$), the epiglottis (▲, $n = 14$) and pooled for fibres on the vocal process, ary-epiglottic fold, thyro-arytenoid attachment to the arytenoid cartilage, overlying the thyro-arytenoid muscle or the subglottic mucosa (■, $n = 20$). Results for tonically active fibres with fields on the arytenoid cartilage and epiglottis (●, $n = 7$), the ary-epiglottic fold (▲, $n = 4$), the vocal process and thyro-arytenoid attachment to the arytenoid cartilage (■, $n = 5$) and overlying the thyro-arytenoid muscle (★, $n = 3$) are depicted in *B*.

sudden closure and violent expulsive reflexes. Note for example that even in the cat, a most sensitive species in this respect, coughs cannot be elicited from all of the surfaces here shown to contain sensitive mechanoreceptors (Korpas & Tomori, 1979). Rather, it is suggested that this function would also be well served by a distributed set of afferent neurones if these were able to precisely signal the location and trajectories of food and other particles which must approach the larynx; such signals would provide for continuous monitoring and anticipation, and perhaps adjustment,

of the paths of particulate matter. Vocalization represents another important laryngeal function for which a more detailed knowledge of the dynamic sensitivity of the mechanoreceptors contained in the larynx is of considerable interest. Accordingly the discussion which follows attempts to evaluate the relevance of the results obtained with sinusoidal probe stimulation for these two aspects of laryngeal function. However, it should be noted that other modes of stimulation are capable of affecting these endings. In particular their ability to respond to events associated with the flow of air over them, as described by Boushey et al. (1974) and others, implicates them in additional functions.

The sensitivity of most of the laryngeal mechanoreceptors to vibratory stimuli was striking. No fibres were as sensitive as Pacinian corpuscles, for which 1:1 entrainment to considerably higher frequencies of cutaneous vibration at amplitudes less than 10 μm have been described (Talbot et al. 1968; Janig, Schmidt & Zimmermann, 1968). However, many fibres, including the slowly adapting, tonically active fibres, were capable of signalling to 400 Hz or more, even when stimulated with quite small vibratory amplitudes, such as 10–100 μm. None of the tuning curves was narrow. On the contrary, the majority of fibres displayed a relatively flat tuning curve, usually with the most sensitive frequency below 100 Hz. These results confirm and extend the semi-quantitative report by Sampson & Eyzaguirre (1964) that the dynamic sensitivity of the internal laryngeal nerve fibres was greater in the low-frequency range.

For the tonically active, slowly adapting receptor group the dynamic sensitivity appears noteworthy. Although some cutaneous slowly adapting mechanoreceptors display dynamic responses at the indentation phase of the probe displacement (Tapper, 1964; Janig et al. 1968), it appears that slowly adapting, tonically active cutaneous mechanoreceptors cannot be entrained to 1 s of vibration by the frequencies and amplitudes here shown to entrain laryngeal endings with similar adaptation characteristics. For example, Iggo & Ogawa (1977) reported that only a few of the slowly adapting receptors in the cat's glabrous skin could be entrained to 100 Hz. On the other hand, the sensitivity of muscle spindle primary endings to vibration has been documented by Brown, Engberg & Matthews (1967) who showed these nerve endings could be driven for 1 s by a vibrating probe attached to the muscle tendon at frequencies up to 500 Hz and at amplitudes as small as 20 μm. However, on the basis of the distribution of the diameters of myelinated fibres in the laryngeal nerves (Evans & Murray, 1954; Murray, 1957) and the lack of any convincing histological or physiological evidence for the presence of muscle spindles in the laryngeal musculature of the species studied here (Martensson, 1964; Rudomin, 1966), it would appear unlikely that the dynamically sensitive, slowly adapting fibres are muscle spindle afferents. Moreover, the inappropriate conduction velocities, the discrete receptive areas most of these endings displayed and the apparent lack of directional sensitivity within such areas all argue against encounters with spindle endings in the present study.

Conduction velocity measurements were consistent with the myelinated nature of internal laryngeal nerve fibres (fibre diameter < 12·8 μm with the majority < 4 μm) in these species (Miller & Loizzi, 1974). For both the silent and the tonic fibre groups the conduction velocities were broadly distributed over a range from 10·8 to 30·0 m/s

or predominantly in the group III range (< 40 m/s) as previously reported (Sessle, 1973; Boushey *et al.* 1974; Shingai, 1977; Harding *et al.* 1978). The values for conduction velocity conform with the distribution of compound action potential peaks for the internal laryngeal nerve reported by Miller & Loizzi (1974) except that we found no fibres with conduction velocities in the range of their fast peak (63·6–70·6 m/s). Possibly their fast-conducting fibres are not touch sensitive and thus not sampled by us.

The dynamic sensitivity of both the silent and the tonic fibres is presumably at least partly determined by the visco-elastic properties of the tissue environment which surrounds their nerve endings. The sensory innervation to the larynx consists of non-encapsulated myelinated nerve fibres which ramify and terminate between and beneath the epithelial cells and fine, beaded non-myelinated fibres (Feindel, 1956; Ironada, Yokoyama, Nemoto & Yamaguchi, 1957; Hatakeyama, 1960). For both fibre groups the receptive fields, particularly those on the arytenoid cartilage, were generally small and well defined. This may relate to the relatively superficial location of these nerve endings, at least on the basis of their sensitivity to light touch, as Janig (1971) found that after removing the corneal layer the receptive fields of cutaneous mechanoreceptors were smaller and the receptors easier to locate.

The functional significance of the dynamic sensitivity of these endings may be related to defensive reflexes. The broadly tuned frequency response displayed by most fibres in a variety of locations means that they would be sensitive to the movement of a foreign particle into the larynx over a broad range of velocities and over most laryngeal surfaces, except perhaps the anterior part of the vocal cord. Similarly, the ability of these fibres to signal at the frequency at which the vocal cords vibrate during phonation in these species suggests they could provide a significant source of afferent input during vocalization, but more direct evidence for such a role is needed. In general, the lowest thresholds for vibratory stimuli were seen on the apex of the arytenoid cartilage and the laryngeal surface of the epiglottis. A relatively large number of silent, rapidly adapting fibres were isolated on the former site in the cat, although none were seen in the rabbit. It is possible that these nerve endings are important in provoking appropriate defensive reflexes such as coughing, and less important in the rabbit for which species the ary-epiglottic folds and epiglottis are well developed (in contrast to the cat, Pressman & Keleman, 1955) and may afford better protection of the glottis. Supporting this hypothesis is the observation by Korpas & Tomori (1979) that the cough reflex cannot be provoked in the rabbit by mechanical stimulation of the larynx although this is a sensitive site for eliciting this reflex in the cat. The findings of a paucity of internal laryngeal nerve mechanoreceptors sensitive to light touch below the glottis in the cat, and an absence of such receptors below the glottis in the rabbit, or on the inter-membranous portion of the vocal folds in both species, is in agreement with histological reports in these species (Ironada *et al.* 1957; Hatakeyama, 1960; Jeffery, Korpas & Widdicombe, 1978).

A rationale for attempting to differentiate the receptor groups on the basis of adaptation rates is provided by consideration of the likely effects of different properties of the spike-generating mechanism (Nakajima & Onodera, 1969). The inability to sustain a regular and continuous discharge when stimulated is in striking contrast to the behaviour of all of the slowly adapting fibres in the tonic firing group

and has been used as a criterion for classifying laryngeal afferent fibres in previous studies (Mei & Nourigat, 1968, see below). However, this criterion fails with some silent fibres. Boushey *et al.* (1974) observed a wide range of adaptation rates (measured as the time within 20 s to complete adaptation in response to a clamped probe indentation of the receptive field) for the silent class of receptor. This study has quantitatively extended these results by using precise control of the indentation amplitude and confirms that the silent fibres do not form a homogeneous group on the basis of adaptation to sustained indentation.

Most fibres tested with step indentations, including the tonic, slowly adapting fibres, signalled the indentation movement of the probe with a discharge burst, the frequency of which was related to indentation amplitude. During the subsequent maintained indentation, all fibres which discharged with a regular firing pattern (including those normally silent but intermittently active fibres which produced a period of steady tonic discharge activity only after being probed) displayed little or no further adaptation. However, the majority of the silent fibres were rapidly adapting and responded with only short bursts of impulses to the indentation and retraction movements of the probe at low indentation amplitudes. Many of these, particularly in the cat, sustained an adapting discharge throughout the maintained indentation if stimulated at higher indentation amplitudes. Storey (1968) observed in the cat that fibres frequently adapted rapidly to weak mechanical stimuli (such as puffs of air) and more slowly to pressure indentation (forces 0·01–0·32 g) and it is possible that he was describing similar fibres to these. A differential dynamic response to indentation amplitude may be related to the visco-elastic properties of the receptor environment since fibres of this type appeared to be preferentially located on the arytenoid cartilage; on this site the mucous membrane is very thin and the propria beneath is in direct contact with the perichondrium of the cartilage (Hatakeyama, 1960). Fibres which only displayed 'on' and 'off' responses at all indentation amplitudes tended to be less sensitive and their fields more difficult to define; some adapted during applied vibration even if the stimulating period was reduced to 500 ms or less.

Boushey *et al.* (1974) considered the slowly adapting, regular-firing tonic fibres as a separate group. Possibly the slowly adapting silent fibres observed in the present study (adaptation index less than 40 %, see Results) could be also considered as part of this group, as generally the 'spontaneous' discharge induced by probing was also very regular for as long as it was maintained, usually several minutes or more. Such a group of slowly adapting fibres might represent the high end of a threshold continuum for receptors with different thresholds. The regularity of the discharge pattern may reflect discharge from fibres with a single, or dominant, spike-generating zone (Chambers, Andres, von During & Iggo, 1972).

The tonically active fibres were conspicuous by the regularity of the discharge which was frequently maintained for hours (Andrew, 1954). The source of the regular tonic discharge is unknown. Stimulation by respiratory muscular activity or passive movement can be excluded as a neuromuscular block was maintained and the larynx was isolated as much as possible from its caudal attachments. Although a few fibres were studied under conditions where the tip of the epiglottis was gently retracted, the ipsilateral laryngeal mucosa was not obviously under stretch from its rostral

attachments. Andrew (1954) reported a similar pattern of tonic discharge for internal laryngeal nerve fibres in the isolated larynx, a preparation for which passive stretch of the larynx from its attachments to rostral or caudal structures would not be present. In support of a hypothesis that the source of this tonic activity is mechanically induced is the observation that the tonic discharge could be inhibited by pressure applied at some locations adjacent to the receptive field. Although internal laryngeal nerve fibres displaying regular tonic activity patterns have been observed in the rabbit in preparations involving an intact larynx (Davies & Vizek, 1982), the possibility cannot be excluded that the tonic activity patterns observed in the present investigation may have been associated with mechanical stresses imposed on the receptor environment as a result of our open-larynx preparation. The relative ease with which tonic discharge could be induced in some of the more slowly adapting silent fibres by mechanical probing implicates these fibres in particular.

The precise location of the endings of the tonic fibres described in this study is not known but if they are connected to those non-encapsulated terminals which ramify beneath and between the epithelial cells (Feindel, 1956; Ironada *et al.* 1957; Hatakeyama, 1960), then the possibility exists that at some locations the junctional forces between the cells might result in sufficient compression or local distortion of the receptor membrane to produce and maintain a tonic pattern of discharge. Local application of tobacco smoke and other chemicals can inhibit such tonic activity (Boushey *et al.* 1974) but it is conceivable that these agents also alter the junctional forces between cells. Alternatively, inhibition of the tonic activity pattern after local applications of tobacco smoke may be due to a direct action of these drugs at the regenerative region of the nerve ending, or to some other direct or indirect action on the generator potential.

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