*J. Physiol.* (1987), **393**, *pp.* 147–155
*With 4 text-figures*
*Printed in Great Britain*



# A CONVERGENT INPUT FROM NASAL RECEPTORS AND THE LARYNX TO THE ROSTRAL SENSORY TRIGEMINAL NUCLEI OF THE CAT

BY DAVID JORDAN AND L. MICHAEL WOOD*

*From the Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF*

(*Received 6 February 1987*)

SUMMARY

1. Extracellular recordings were made from ninety-one neurones in the vicinity of the rostral trigeminal nucleus in chloralose-anaesthetized cats.

2. Sixty-two neurones within this area were activated by electrical stimulation of the ipsilateral superior laryngeal nerve (s.l.n.). Only two of the twenty-one neurones tested had an additional input from the contralateral s.l.n.

3. Fifty of these sixty-two neurones were also activated synaptically by light mechanical stimulation of the ipsilateral nasal cavity and in the eight neurones tested electrical stimulation of the ipsilateral nostril evoked activity. All these neurones exhibited characteristics of postsynaptic responses to s.l.n. and nasal stimulation, showing a variable latency to onset to either stimulus, summation and facilitation of more than one stimulus.

4. None of those neurones receiving an s.l.n. input, or those with convergent inputs from the s.l.n. and nose, could be affected by mechanical stimulation of any part of the face.

5. The activity of a further twenty-nine neurones was also recorded within this same general region. Sixteen responded to movement of the whiskers, five to touching the skin of the lower jaw, two to touching the skin of the upper jaw, three to touch around the eyebrows and three to touching other parts of the face. None of these neurones were activated by s.l.n. stimulation.

6. The location of seventeen of these neurones showing a convergent s.l.n. and nasal input was determined histologically. They were closely grouped together in a region 3·5–4·5 mm rostral to obex in and around the main trigeminal sensory nucleus, dorsolateral to the retrofacial nucleus corresponding to the parvocellular division of the alaminar spinal trigeminal nucleus.

7. The lack of somatosensory input to those neurones receiving a convergent input from the nose and s.l.n. is discussed in relation to previous studies describing somatosensory–visceral convergence to neurones within trigeminal nuclei.

* Present address: Department of Pharmacology, Smith, Kline & French Research Ltd, The Frythe, Welwyn, Herts. AL6 9AR.

## INTRODUCTION

A convergence of inputs to neurones within the medullary trigeminal nuclei has been widely reported. Darian-Smith (1960) described the spatial summation of two subthreshold stimuli to different areas of the upper lip onto units in the main sensory nucleus of the trigeminal complex of the cat. Gottschaldt & Young (1977) reported that cells in the rostral sensory trigeminal nuclei responded both to facial sinus hair stimulation and to stimulation of surrounding parts of the face. A convergence of different visceral inputs, from stimulation of the superior larygeal (s.l.n.), glossopharyngeal (IX), aortic and carotid sinus nerves to neurones in a similar area has been described (Biscoe & Sampson, 1970). Additionally, Sessle & Greenwood (1976) have reported neurones receiving both visceral and somatic inputs from stimulation of the s.l.n., glossopharyngeal nerve, tooth pulp and cutaneous facial mechanoreceptors. These neurones, located in the rostral trigeminal sensory nucleus and its vicinity, included neurones projecting directly to the thalamus, interneurones and reticular formation neurones. Neurones with a similar diverse convergent input, some of which also had axonal projections to the rostral trigeminal nucleus, were subsequently described by Hu, Dostrovsky & Sessle (1981). These were located in the caudal sensory trigeminal nucleus and may form at least part of the input to the rostral nucleus. In the present report we describe the responses of a localized group of neurones within the vicinity of the rostral trigeminal nuclei which receive a more restricted convergent input from the s.l.n. and the nose but with no demonstrable input from tactile stimulation of any other part of the face. Some of these results have been reported previously in abstract form (Jordan & Wood, 1986).

## METHODS

The present experiments were carried out on twenty cats (2·5–2·9 kg body weight). The animals were anaesthetized initially with sodium pentobarbitone (Sagatal, May & Baker, 40 mg $kg^{-1}$ I.P.) but following the surgical procedures described below. $\alpha$-Chloralose (BDH Chemicals Ltd, 70 mg $kg^{-1}$ I.V.) was given and supplemented as necessary with 20 mg bolus doses. The right femoral artery and vein were cannulated for monitoring systemic blood pressure and the administration of drugs respectively. The trachea was cannulated below the larynx and initially the animals breathed spontaneously. Following pneumothorax they were artificially ventilated with air enriched with $O_2$ (Harvard Apparatus Small Animal Ventilator) delivering a tidal volume of 20 ml at a rate of 20–40 cycles $min^{-1}$. Arterial blood gas composition was monitored at frequent intervals throughout the experiment (Corning Blood Gas Analyzer Model 158). The arterial blood $P_{O_2}$ was maintained between 100 and 150 mmHg by adjusting the rate of $O_2$ insufflation and the arterial blood $P_{CO_2}$ between 35 and 40 mmHg by adjusting the rate of the ventilator, bolus infusions of molar sodium bicarbonate being used to maintain the pH between 7·35 and 7·40. Rectal temperature was maintained between 36·5 and 38 °C by a heating coil placed under the animal through which warm water circulated. The animals were placed in a stereotaxic head holder (David Kopf Instruments) and the rostral medulla was exposed by removing the occipital bone and reflecting the overlying dura mater. In some animals the caudal part of the cerebellum was either retracted or removed by suction.

Both superior larygeal nerves (s.l.n.) were dissected clear of connective tissue, cut close to the larynx, and the central ends placed on bipolar silver stimulating electrodes. The right phrenic nerve was also isolated in the neck, the cut central end placed on bipolar silver wire recording electrodes and its activity differentially amplified (Neurolog; NL 104) to monitor central respiratory drive. Mechanical stimulation of the ipsilateral nasal cavity was achieved by lightly probing the inside of the nose with a cotton bud and electrical stimulation by placing a bipolar silver stimulating

electrode into the ipsilateral nostril. The animals were paralysed with gallamine triethiodide (Flaxedil, May & Baker Ltd, 4 mg $kg^{-1}$) and supplemented with 1–3 mg $h^{-1}$ after assessing the level of anaesthesia.

Extracellular recordings were made (Dagan 2400 Extracellular Preamplifier) from the right medulla, 3–5 mm rostral to obex, using filament glass microelectrodes whilst stimulating the ipsilateral s.l.n. at 1 Hz, 0·1 ms, up to 10 V. All neurones exhibiting an input from the s.l.n. were then tested for an input from the nose as described above. The electrodes were filled either with 4 M-sodium chloride or a 2% solution of pontamine sky blue in 0·5 M-sodium acetate which could then be used to mark the site of recording with an ionophoretic current of 5 $\mu$A for 10 min (Hellon, 1971).

Arterial blood pressure and tracheal pressure were measured using Statham P23Db pressure transducers (Statham Ltd, Puerto Rico) and conditioning amplifiers (Gould Instrument Division). All variables were recorded on magnetic tape (Racal Store 7 tape-recorder) and displayed on an electrostatic recorder (ES1000, Gould Instrument Division). Analysis of the data was carried out off-line using a spike processor (Digitimer 130), a digital storage oscilloscope (Nicolet Explorer 204A) and a minicomputer (Cambridge Electronic Design Slam 2 System). All anatomical locations are named in accordance with those of Berman (1968).

TABLE 1. Summary of the inputs to the ninety-one neurones studied

| Receptive field | | s.l.n. input | | |
|---|---|---|---|---|
| Nasal | Facial | Number of cells | Ipsilateral | Contralateral |
| 50 | 0 | 29 | + | — |
| | | 19 | + | 0 |
| | | 2 | + | + |
| 0 | 0 | 12 | + | — |
| 0 | 29 | 0 | 0 | — |

+, input present; 0, no input; —, input not tested.

## RESULTS

### *Responses of neurones receiving a convergent input from the superior laryngeal nerve and the ipsilateral nasal cavity*

The activity of a total of ninety-one neurones was recorded in the vicinity of the trigeminal nucleus between 3 and 5 mm rostral to obex. These were either silent or fired irregularly with a low rate of ongoing activity. Table 1 summarizes the data obtained.

Sixty-two of these neurones were activated by electrical stimulation of the ipsilateral s.l.n. (0·5–10 V, 0·1 ms, 1 Hz) with a minimum onset latency of $7{\cdot}3 \pm 0{\cdot}4$ ms (mean + S.E.M., range 1·5–15·8 ms) and duration of $11{\cdot}2 \pm 0{\cdot}9$ ms (range 2·1–28·1) (Figs 1–3). Only two of the twenty-one neurones tested were also activated by stimulation of the contralateral s.l.n. (Fig. 3). Fifty of these neurones activated by stimulation of the ipsilateral s.l.n. could also be activated by light mechanical stimulation of the ipsilateral nasal cavity. Eight of these were subsequently activated by electrical stimulation of the nasal cavity (10–20 V, 0·1 ms, 1 Hz) with a minimum onset latency of $6{\cdot}0 \pm 1{\cdot}0$ ms (range 3·5–9·6 ms) and duration of $15{\cdot}7 \pm 3{\cdot}2$ ms (range 0·5–26 ms). Finally, two of the neurones which had an input from both the ipsilateral s.l.n. and the nose were excited also by blowing ammonia vapour into the ipsilateral nostril.

Examples of activity recorded in neurones shown to receive convergent input from

the nose and s.l.n. is shown in Figs 1–3. The response of a neurone to stimulation of the ipsilateral s.l.n. at 4 V (Fig. 1, left) is enhanced when the s.l.n. stimuli are applied during continual light mechanical stimulation of the nasal cavity (Fig. 1, right). Post-stimulus time histograms (p.s.t.h.) of the responses of the neurone shown in Fig. 1 to stimulation of the ipsilateral s.l.n. are illustrated in Fig. 2 (top). The response evoked by s.l.n. stimulation (latency range 6–20 ms) is enhanced when stimulation of the ipsilateral s.l.n. is applied during a background of continual light mechanical stimulation of the nose as shown in the middle trace. The bottom p.s.t.h. shows the response of the neurone to continuous light mechanical stimulation of the nose alone. Less temporal dispersion of the nasal input was observed during electrical stimulation

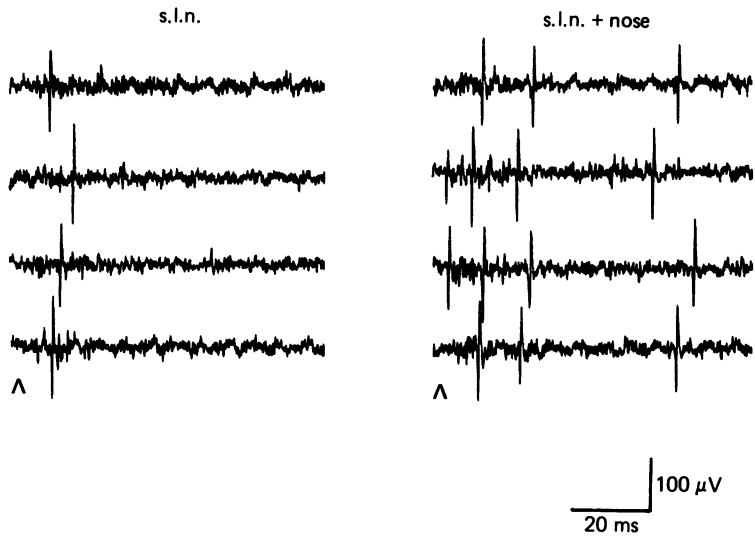


Fig. 1. Four consecutive single oscilloscope sweeps showing on the left the response of a neurone to electrical stimulation of the s.l.n. (4 V, 0·1 ms, 1 Hz) given at ∧. On the right is shown the same s.l.n. stimuli given on a backround of light mechanical stimulation of the nasal cavity.

within the nostril (Fig. 2, right, bottom). This produced an even greater facilitation of the s.l.n.-evoked response than mechanical nasal stimulation (Fig. 2 right, middle).

The responses reported above exhibited characteristics typical of postsynaptic responses. The variable latency to the onset of the evoked response is shown in Figs 1 and 2. Summation and facilitation of both threshold and subthreshold stimuli to the s.l.n. and nose are illustrated in Fig. 3. This facilitation was evident from the shortening of the minimum onset latency, increased duration of activation or an increase in the neuronal discharge rate. None of the fifty neurones which received this convergent input from the s.l.n. and nose could be shown to be affected by touching or prodding any part of the face.

The locations of seventeen of these neurones possessing convergent nasal and laryngeal inputs were determined histologically, ten were directly marked with pontamine sky blue and seven were interpolated from marked points by plotting the

depth of the microelectrode in the brain stem. Figure 4 shows a composite transverse section of the brain stem made up from ten sections taken between 3·5 and 4·5 mm rostral to obex.

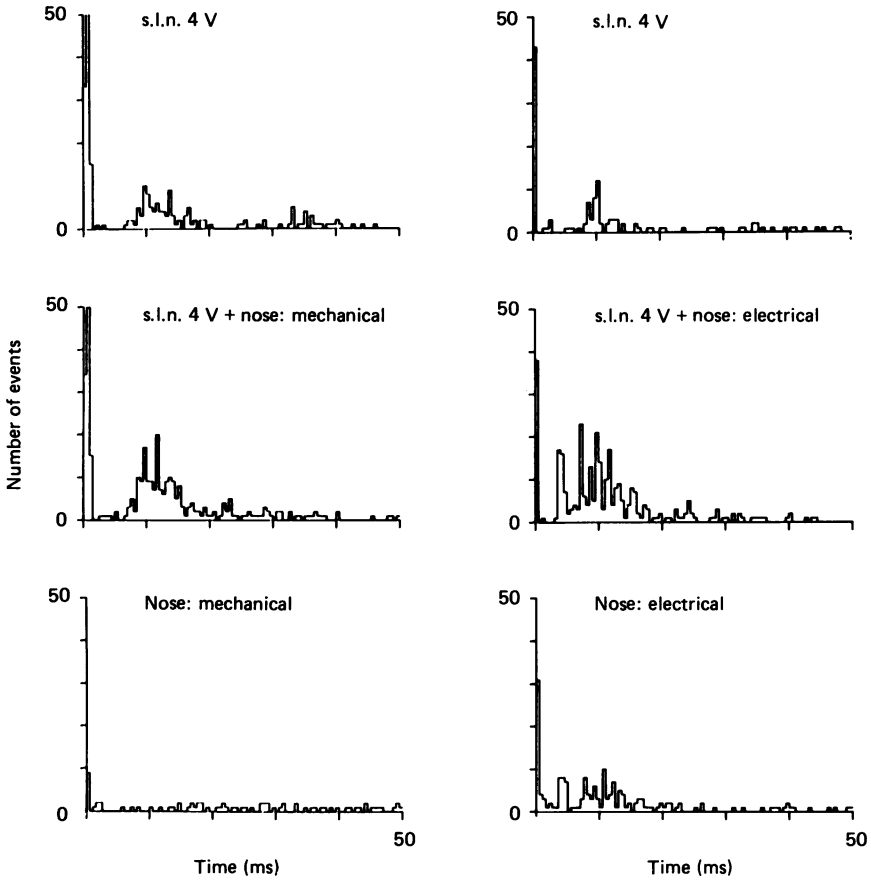


Fig. 2. Post-stimulus time histograms (fifty sweeps, 0·5 ms bins). Top: the response evoked in a neurone by stimulation of the s.l.n. (4 V, 0·1 ms, 1 Hz) given at 0 ms (stimulus artifact shown in the first three bins). Bottom left: the effect of continuous mechanical stimulation of the nasal cavity. Bottom right: the effect of electrical stimulation of the nasal cavity (10 V, 0·1 ms, 1 Hz) given at 0 ms (stimulus artifact shown in the first bin). Middle: combined stimulation of the nasal and s.l.n. stimuli.

These neurones were closely grouped together and located in the vicinity of the main trigeminal nucleus dorsolateral to the retrofacial nucleus. The area corresponds to the parvocellular division of the alaminar spinal trigeminal nucleus (Berman, 1968).

*Responses of other neurones located in the same area*

Located amongst the neurones receiving s.l.n. inputs we have also recorded activity from twenty-nine other neurones. None of these received input from the nose or s.l.n. but were clearly activated by light touch or prodding various areas of the

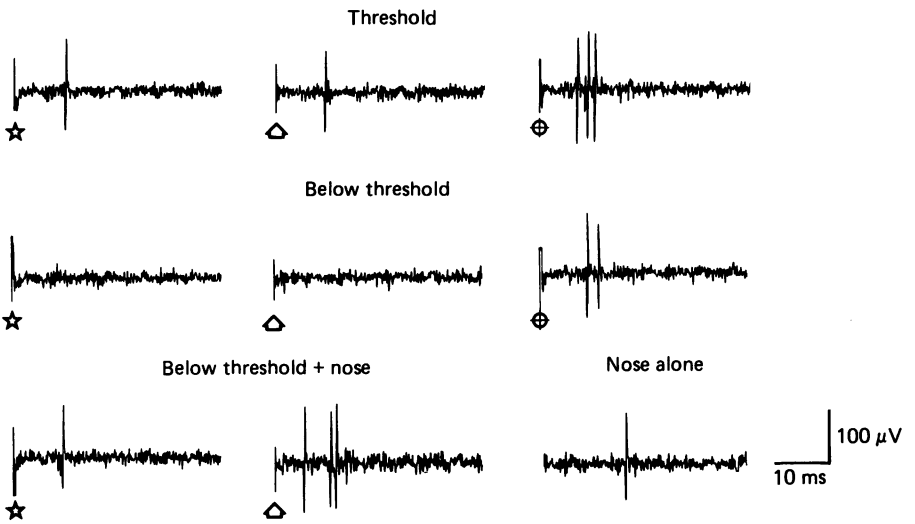


Fig. 3. Single oscilloscope sweeps showing the responses evoked in a neurone by electrical stimulation of either/or both ipsilateral and contralateral s.l.n.s (0·1 ms, 1 Hz), and light mechanical stimulation of the nose. Top: stimulation of s.l.n.s individually (left and middle) and together (right) at 1 V which was just suprathreshold when given singly. Middle: stimulation of s.l.n.s individually (left and middle) and together (right) at 0·9 V which was just subthreshold when given singly. Bottom: stimulation of the s.l.n.s individually at subthreshold intensity (0·9 V) during a background of light mechanical stimulation of the nose (left and middle); the effect of nasal stimulation alone is illustrated (right). ☆, ipsilateral s.l.n.; ⌂, contralateral s.l.n.; ⊕, ipsilateral and contralateral s.l.n.

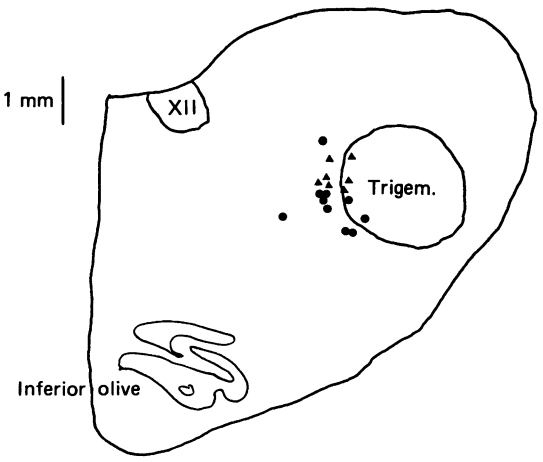


Fig. 4. A composite transverse section of the brain stem between 3·5 and 4·5 mm rostral to obex showing the location of neurones which received convergent input from both the nose and s.l.n. These were plotted directly from pontamine sky blue spots (●) or extrapolated from other marked sites (▲). Abbreviations: XII, hypoglossal nucleus; Trigem., alaminar spinal trigeminal nucleus.

face and mouth. Sixteen responded to movement of the whiskers, five to touching the skin of the lower jaw, two to touching the skin of the upper jaw, three to touching around the eyebrows and three had inputs from other parts of the face.

## DISCUSSION

In the present study we have recorded from neurones in the rostral medulla in and close to the sensory trigeminal nuclei which could be activated both by mechanical stimulation of the ipsilateral nasal cavity and electrical stimulation of the s.l.n.s. We have shown that these two inputs are indeed acting postsynaptically on the same neurone since the two stimuli when given together evoked a greater response than each individually. In addition, subthreshold s.l.n. stimuli given on a background of nasal stimulation were able to activate the neurones. None of these neurones were affected by mechanosensory input from other parts of the face. However, in this same region, sometimes at the same electrode position, we have also discriminated neurones which were activated by light touch of areas of the face. These were completely unaffected by the s.l.n. stimuli. In addition, we have reported a group of cells which were only shown to have an input from the s.l.n. Whilst these may be a separate population of cells, it is possible that they too belong to the group of convergent neurones but whose nasal input we were unable to activate. We have reported no cells receiving only a nasal input in this study. However, this is not so surprising since our experimental protocol was to search for cells with an s.l.n. input and then test for nasal stimulation rather than vice versa.

Since we have used only electrical stimulation of the s.l.n. we cannot state with certainty the function of the afferent fibres stimulated. However, some are likely to be those stimulated by mechanical disturbances of the larynx since Angell-James & Daly (1975) showed that similar patterns of cardiovascular and respiratory response were elicited both by such mechanical laryngeal stimulation and by electrical stimulation of the s.l.n. similar to that performed here.

Similarly, the input from the nose has not been identified functionally in the present study. In most cases stimulation was performed by light touch or probing within the nasal cavity. This is most likely to activate mechanoreceptors in the nasal mucosa, but we cannot rule out the possibility than other types of afferent might also have been stimulated by such stimulation. Whilst in the two cases tested blowing ammonia vapour into the nares profoundly activated the neurones studied, this itself would activate a variety of afferent inputs.

The diversity of convergence of somatic inputs to neurones in the trigeminal main sensory nucleus has been well documented (Darian-Smith, 1960; Darian-Smith, Phillips & Ryan, 1963; Gottschaldt & Young, 1977) and in addition, such neurones may also receive inputs from a variety of visceral structures (Sessle & Greenwood, 1976). With respect of visceral inputs, there is anatomical evidence for a limited direct projection of vagal afferents to the region of the trigeminal nucleus (Kerr, 1962) and an electrophysiological study (Car, Jean & Roman, 1975) suggested that s.l.n. afferents bifurcate, one branch terminating in the nucleus tractus solitarius (n.t.s.) whilst the other terminated in a region medial to the rostral sensory trigeminal nucleus. Clearly, these projections could account for the inputs noted

previously and in the present study. However, there are also suggestions that the convergence seen in the rostral trigeminal nuclei is the result of inputs acting via the caudal brain stem. Sessle (1973) reported neurones in the n.t.s. itself and the reticular formation ventral to it, which received convergent input from the s.l.n., glossopharyngeal nerve and infraorbital nerves. Hu *et al.* (1981) subsequently described a group of neurones in the trigeminal subnucleus caudalis (caudal to obex) which received either cutaneous nociceptive or low-threshold mechanoreceptive input and a tooth pulp input. Some of each group also received visceral input from the s.l.n. and/or glossopharyngeal nerve of which between 30 and 50% projected rostrally to the trigeminal subnucleus oralis. Whilst this earlier work documents somatic–visceral convergence in some detail it clearly indicates that the neurones identified in the present study are distinct since they were never shown to receive any facial somatic input.

Whilst we have clearly defined a group of neurones showing a distinct convergent input from the nose and larynx, the physiological role of these neurones has not been determined. The larynx, particularly the epiglottis, is known to play an important role in olfaction by maintaining airflow through the nose whilst the mouth is used for other functions (Negus, 1949). It would therefore be plausible for there to be a site of specific convergence of sensory information from the larynx and nasal receptors to aid in this co-ordination. Indeed, in man, hyposmia (reduced sense of smell) has been demonstrated following laryngectomy (Henkin, Hoye, Ketcham & Gould, 1968; Hoye, Ketcham & Henkin, 1970; Henkin & Larson, 1972). However, a recent study (Moore-Gillon, 1985) has questioned the inevitability of hyposmia following laryngectomy and found little evidence to support the essential role of a neuronal interaction between afferents from the nose and larynx in olfaction.

Apnoea, bradycardia and a hindlimb vasoconstriction can be produced in dogs both by stimulation of the nasal mucosa and by either mechanical stimulation of the larynx or electrical stimulation of the s.l.n. (Angell-James & Daly, 1972, 1975). In cats, a similar pattern of response to s.l.n. stimulation (Daly, Litherland & Wood, 1983), nasal stimulation and combined stimulation of the two (Jordan, Paton & Wood, 1986, 1987) has been described. Clearly, the similarity of the patterns of response may lead to the suggestion that the same neuronal substrate may be involved in their generation. However, since in the present study we have no information regarding either other afferent inputs to these neurones, or the areas they are projecting to, then this must remain as pure speculation.

In conclusion, a distinct group of neurones in the rostral medulla which receive convergent input from nasal and laryngeal regions have been identified. Whilst they are unresponsive to facial somatosensory input, the possibility that other somatic or visceral afferents may also influence them still remains. Their function is as yet unclear.

This work was supported by a grant from the MRC.

## REFERENCES

ANGELL-JAMES, J. E. & DALY, M. DE B. (1972). Reflex respiratory and cardiovascular effects of stimulation of receptors in the nose of the dog. *Journal of Physiology* **220**, 673–696.

ANGELL-JAMES, J. E. & DALY, M. DE B. (1975). Some aspects of upper respiratory tract reflexes. *Acta otolaryngologica* **79**, 242–252.

BERMAN, A. L. (1968). *The Brain Stem of the Cat*. Madison: University of Wisconsin Press.

BISCOE, T. J. & SAMPSON, S. R. (1970). Responses of cells in the brain stem of the cat to stimulation of the sinus, glossopharyngeal, aortic and superior laryngeal nerves. *Journal of Physiology* **209**, 359–373.

CAR, A., JEAN, A. & ROMAN, C. (1975). A pontine primary relay for ascending projections of the superior laryngeal nerve. *Experimental Brain Research* **22**, 197–210.

DALY, M. DE B., LITHERLAND, A. S. & WOOD, L. M. (1983). The modification of the respiratory, cardiac and vascular responses to stimulation of the carotid body chemoreceptors by a laryngeal input in the cat. *IRCS Medical Sciences* **11**, 861–862.

DARIAN-SMITH, I. (1960). Neurone activity in the cat's trigeminal main sensory nucleus elicited by graded afferent stimulation. *Journal of Physiology* **153**, 52–73.

DARIAN-SMITH, I., PHILLIPS, G. & RYAN, R. D. (1963). Functional organization in the trigeminal main sensory and rostral spinal nuclei of the cat. *Journal of Physiology* **168**, 129–146.

GOTTSCHALDT, K.-M. & YOUNG, D. W. (1977). Quantitative aspects of responses in trigeminal relay neurones and interneurones following mechanical stimulation of sinus hairs and skin in the cat. *Journal of Physiology* **272**, 85–103.

HELLON, R. F. (1971). The marking of electrode tip position in nervous tissue. *Journal of Physiology* **241**, 12*P*.

HENKIN, R. I., HOYE, R. C., KETCHAM, A. S. & GOULD, W. J. (1968). Hyposmia following laryngectomy. *Lancet* **ii**, 479–481.

HENKIN, R. I. & LARSON, A. L. (1972). On the mechanism of hyposmia following laryngectomy in man. *Laryngoscope* **82**, 836–842.

HOYE, R. C., KETCHAM, A. S. & HENKIN, R. I. (1970). Hyposmia after paranasal sinus exenteration or laryngectomy. *American Journal of Surgery* **120**, 485–491.

HU, J. W., DOSTROVSKY, J. O. & SESSLE, B. J. (1981). Functional properties of neurons in cat trigeminal subnucleus caudalis (medullary dorsal horn). I. Responses to oral–facial noxious and nonnoxious stimuli and projections to thalamus and subnucleus oralis. *Journal of Neurophysiology* **45**, 173–192.

JORDAN, D., PATON, J. F. R. & WOOD, L. M. (1986). Hind limb vascular responses to combined stimulation of the nasal mucosa and superior laryngeal nerve in the anaesthetized cat. *Journal of Physiology* **373**, 18*P*.

JORDAN, D., PATON, J. F. R. & WOOD, L. M. (1987). Effects of stimulation of nasal and superior laryngeal inputs on the hind-limb vasculature of anaesthetized cats. *Journal of Physiology* **393**, 135–145.

JORDAN, D. & WOOD, L. M. (1986). A convergent input from the larynx and nasal mucosa of the cat. *Journal of Physiology* **372**, 23*P*.

KERR, F. W. (1962). Facial, vagal and glossopharyngeal nerves in the cat. *Archives of Neurology* **6**, 264–281.

MOORE-GILLON, V. (1985). The nose after laryngectomy. *Journal of the Royal Society of Medicine* **78**, 435–439.

NEGUS, V. E. (1949). *The Comparative Anatomy and Physiology of the Larynx*. New York: Grune and Stratton.

SESSLE, B. J. (1973). Excitatory and inhibitory inputs to single neurones in the solitary tract nucleus and adjacent reticular formation. *Brain Research* **53**, 319–331.

SESSLE, B. J. & GREENWOOD, L. F. (1976). Inputs to trigeminal brain stem neurones from facial, oral, tooth pulp and pharyngolaryngeal tissues: I. Responses to innocuous and noxious stimuli. *Brain Research* **117**, 211–226.