

An autoradiographic study of the brainstem projections of vagal visceral afferent fibres in the domestic hen

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INTRODUCTION

There have been nearly forty studies of the central projections of vagal visceral afferent fibres in many different species, employing, over the years, most of the known neuroanatomical and neurophysiological techniques. It was quickly established that the majority of these afferent fibres project to the ipsilateral nucleus tractus solitarius (Cajal, 1911). This has been subsequently shown for, amongst others, the monkey (Beckstead & Norgren, 1979), the mallard (Dubbeldam, Brus, Menken & Zeilstra, 1979) and the cat (Gwyn, Leslie & Hopkins, 1979). However, there is no general agreement about the other possible sites of central termination of vagal visceral afferent fibres. Most authorities agree that there is a smaller projection to the contralateral nucleus of tractus solitarius via the commissural nucleus of Cajal (Beckstead & Norgren, 1979; Dubbeldam *et al.* 1979; Gwyn *et al.* 1979; Kalia & Wells, 1980; Kerr, 1962). This projection was not found in the frog (Rubinson & Friedman, 1977) or in the carp (Morita, Ito & Masai, 1980), although a projection to the commissural nucleus itself was identified. Some authors have reported projections to the dorsal motor nucleus of the vagus in mammals (Harding & Leek, 1973; Kalia & Wells, 1980; Mitchell & Warwick, 1955) though many studies have failed to confirm this (Cottle, 1964; Dubbeldam *et al.* 1979; Jordan & Spyer, 1978). Projections to the reticular formation on the ipsilateral side (Anderson & Berry, 1956; Beckstead & Norgren, 1979; Kumada & Nakajuna, 1972) and on the contralateral side (Harrison & Bruesch, 1945; Kimmel, 1965) have been described. Bilateral projections to the area postrema (Beckstead & Norgren, 1979; Gwyn *et al.* 1979; Kalia & Wells, 1980) and area subpostrema (Gwyn *et al.* 1979) have been reported although other authors have not been able to confirm this (Cottle, 1964; Dubbeldam *et al.* 1979; Katz & Karten, 1978; Rubinson & Friedman, 1977). Other less frequently reported projections are to the nucleus intercalatus (Jordan & Spyer, 1978), nucleus ambiguus (Kumada & Nakajuna, 1972), upper spinal cord (Kerr, 1962; Kimmel, Kimmel & Zarkin, 1961) and the cerebellum (Sobusiak, Zinney & Matlosy, 1971). Projections to the insular cerebral cortex have been described (Seigfried, 1961) but it is not clear whether these are primary or secondary afferent fibres.

As part of a continuing study of the afferent innervation of the respiratory tract of the domestic hen, the central projections of the vagal afferent fibres in this animal were studied. The results reported here are the brainstem projections of the vagal visceral fibres with their cell bodies in the distal vagal ganglion.

MATERIALS AND METHODS

Six adult hens were used. They were anaesthetized with 70 % urethane administered intravenously. A tracheostomy was performed and the hens allowed to breathe spontaneously. The distal vagal ganglion was exposed via a dorsal approach using a method previously described (Bower & Parry, 1978). The tritiated leucine was freeze-dried, reconstituted in normal saline and injected into the inferior vagal ganglion using a micropipette. The hens were kept anaesthetized for 12 hours and then given a lethal dose of urethane and perfused transcardially with 10 % formol saline administered at a pressure of 100 mm Hg. The brains and the distal vagal ganglia were removed and stored in formol saline for 4 days. After embedding in paraffin, 10 μm sections were cut off the brainstem and the ganglia. The sections were coated with Ilford K2 nuclear emulsion diluted 1:1 with 1 % glycerol solution and at the same time sections for use as positive and negative chemography controls were prepared. All brainstem sections were left for 3–4 weeks at 4 °C in light-tight boxes containing a dessicant and those of the ganglia for 3–4 days in similar conditions. At the end of this period, all autoradiographs were developed in Kodak D19 developer, diluted 1:1, rinsed in distilled water and fixed in Amfix at 18 °C with intermittent agitation. The sections were counterstained with cresyl fast violet and the slides were viewed using a Vickers M17 microscope with both incident and transmitted light.

Of the six experimental hens, two died before sufficient labelled protein had been transported along the axons to reach the brainstem. The following results are based on the remaining four hens and any dimensions quoted are averages of these four hens.

RESULTS

All the distal vagal ganglia had been successfully and completely injected with tritiated leucine and presented an appearance similar to that already described in a previous publication (Bower, Molony & Brown, 1975). The vagus nerve rootlets entering the brainstem contained labelled fibres indicating that the radioactive protein had been transported as far as the brainstem. The labelled fibres were seen entering the dorsomedial aspect of the medulla and then passing medially towards the ipsilateral tractus solitarius and its nucleus. The rostrocaudal extent of the labelled fibres was from 500 μm rostral to the obex to 2500 μm rostral to the obex. The ipsilateral tractus solitarius contained labelled fibres which presented the appearance of having been cut transversely, indicating that the vagal fibres had entered the tract and were then travelling rostral or caudal in it. The extent of this labelling was from 400 μm caudal to the obex to 2500 μm rostral to the obex. Medial to the ipsilateral tractus solitarius, its associated nucleus contained labelled fibres. The appearance of this labelling was consistent with that seen in an area where fibres terminate, i.e. scattered silver grains in the tissue around the neurons. This labelling extended from 400 μm caudal to the obex to 2000 μm rostral to the obex. The rostral pole of the ipsilateral nucleus of the tractus solitarius was not labelled. Most of the labelling in the ipsilateral nucleus of the tractus solitarius was medial to the tractus (Fig. 1A).

Just caudal to the obex, labelled fibres were seen crossing from the ipsilateral to the contralateral side (Fig. 1B). This is the region of the commissural nucleus of Cajal. The contralateral nucleus of the tractus solitarius was labelled in its medial

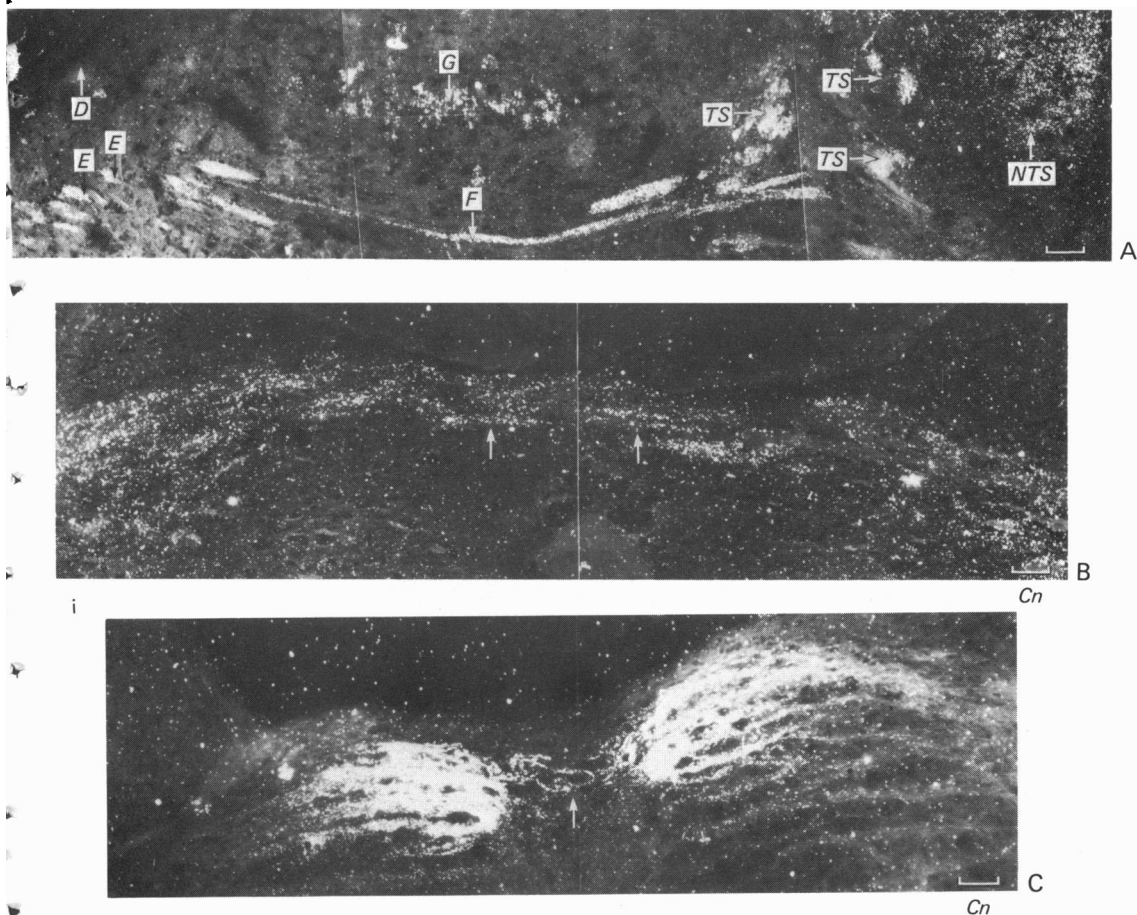


Fig. 1. The bar in each case represents $50\ \mu\text{m}$. In B and C, *i* is ipsilateral and *Cn* is contralateral. (A) This shows the ipsilateral side of the brainstem. *D* is the dorsal aspect. Labelled vagal rootlets enter at *E* and cross the brainstem as traversing fibres (*F*). They turn caudally at the tractus solitarius (*TS*). Labelling is seen in the medial part of the ipsilateral nucleus of the tractus solitarius (*NTS*). *G* is artefact. (B) The decussation via the commissural nucleus of Cajal is arrowed. (C) Showing the rostral commissure. The mid-line is arrowed.

part and again the labelling had the appearance of an area where fibres terminate. The rostrocaudal extent of this labelling was from $400\ \mu\text{m}$ caudal to the obex to $1200\ \mu\text{m}$ rostral to the obex. The contralateral tractus solitarius was also labelled for a similar extent and presented an appearance of fibres cut in cross section. This was a shorter distance than was seen on the ipsilateral side and, in addition, the intensity of label was less, indicating that fewer fibres had gone to the contralateral than to the ipsilateral side.

When the brainstem below the floor of the fourth ventricle was examined $900\ \mu\text{m}$ rostral to the obex, a second discrete decussation of fibres from the ipsi- to contralateral sides was seen (Fig. 1 C). This second decussation had a rostrocaudal extent of $900\ \mu\text{m}$ rostral to the obex to $1300\ \mu\text{m}$ rostral to the obex. Direct continuity of fibres between the vagus and the decussation was not seen but bundles of labelled fibres were seen passing medially beyond the ipsilateral nucleus of the tractus solitarius in this region. Associated with the rostral decussation was an area of diffuse labelling

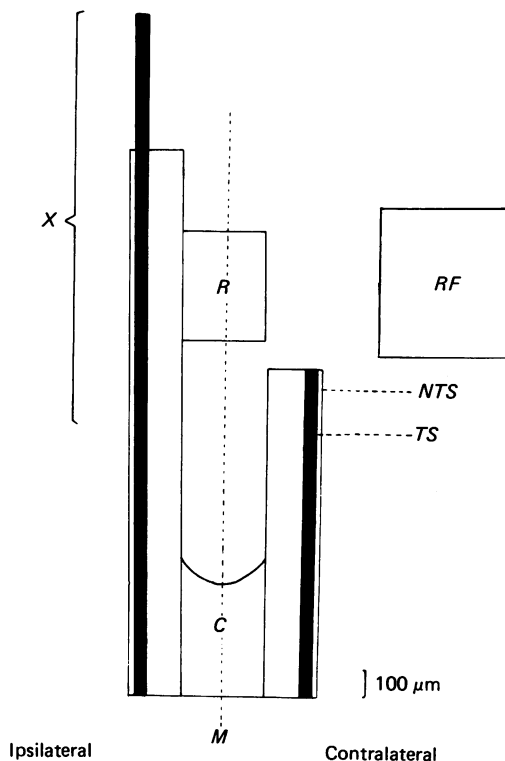


Fig. 2. Schematic diagram of the extent of labelled afferent vagal fibres. *M* is the mid-line; *R*, the rostral commissure; *C*, the caudal commissure; *RF*, the reticular formation; *NTS*, nucleus tractus solitarius; *TS*, tractus solitarius; *X*, extent of labelled vagal fibres entering the brainstem. The lateral dimensions are not to scale.

in the contralateral ventromedial reticular formation. The precise boundaries of this labelling were difficult to determine because of the variation between hens. However, the area of labelling shown in the summary Figure (Fig. 2) was seen in all the animals.

Significant negative results obtained were that no labelling was seen in the dorsal motor nucleus of the vagus, the area postrema or subpostrema, the nucleus ambiguus or any other brainstem nuclei. The slides for chemography controls provided no evidence of either positive or negative chemography. All the results are summarised in schematic form in Figure 2.

DISCUSSION

Because the inferior vagal ganglion contains only afferent neurons (Wakley & Bower, 1981), and because fibres of passage do not transport tritiated amino acids (Cowan *et al.* 1972), it can be concluded that the only labelling seen was in afferent vagal fibres. The survival time was chosen to be too short for transneuronal transport of the tritiated amino acid so the only labelling was in primary afferent fibres. The technique employed labels all vagal visceral afferents including important groups of taste, respiratory, cardiovascular and gastrointestinal (which individually may have different projection sites) within the gross terminal areas described in the

results. The fact that the control autoradiographs showed no evidence of positive chemography means that the results are due to the presence of vagal afferent fibres and not to the spurious interactions between brain tissue and the nuclear emulsion.

The results confirm some findings of other workers, but there are also some new findings and negative results which may represent species differences. The hen, like other animals, has a major projection of vagal afferent fibres to the ipsilateral nucleus of the tractus solitarius with a smaller, sparser projection to the opposite nucleus via the commissural nucleus of Cajal. Confirmation of the projection of vagal afferent fibres to the caudal part of the ipsilateral nucleus of the tractus solitarius, as described by other workers (Beckstead & Norgren, 1979; Cajal, 1911; Dumbledam *et al.* 1979), was obtained. It has been reported (Beckstead & Norgren, 1979; Cottle, 1964; Dumbledam *et al.* 1979) that vagal afferents project mainly to the medial part of the ipsilateral nucleus of the tractus solitarius, which is in agreement with the findings reported here. However, it was noticed that the rostral part of the ipsilateral tractus solitarius was labelled with fibres which were probably passing more caudally, but the rostral part of the associated nucleus was not labelled. Similar findings have not been reported in previous studies and may represent a species difference. The nucleus of the tractus solitarius has been divided in several ways, with no clear agreement between the authors (Beckstead & Norgren, 1979; Cajal, 1911; Dumbledam *et al.* 1979) but there is agreement that specific vagal afferent fibre types project to specific subnuclei of the nucleus, e.g. aortic arch afferents project to the nucleus sulcalis dorsalis (Katz & Karten, 1978). The results presented here confirm that the hen is similar in that it has extensive bilateral projections to the medial part of the nucleus of the tractus solitarius, but because all fibre types were labelled, no specific projections could be identified. An area of termination can be identified using light microscopic autoradiography, but it is impossible to determine the details of synaptic configuration without using electron microscopic autoradiography.

The usual site of decussation of vagal afferent fibres at the commissural nucleus of Cajal has been confirmed, but in addition our results show that there is an extra decussation of vagal afferent fibres rostral to the obex (Fig. 1 C). This second decussation is discrete and is situated some 900 μm rostral to the commissural nucleus of Cajal. An unidentified rostral commissure has been alluded to in earlier studies (Cajal, 1911; Harding & Leek, 1973) but it has never been fully demonstrated. Associated with this rostral commissure was an area of labelling in the contralateral, ventromedial reticular formation which would suggest that some vagal afferent fibres terminate in this region. Because there was no evidence of labelling rostral or caudal to this region of the reticular formation, it is probably an area of termination of vagal afferent fibres rather than an area which fibres were just passing through. Again, synaptic details will have to await electron microscopic study. With the use of physiological techniques there have been previous reports of vagal afferent termination in the reticular formation in cats (Harrison & Breusch, 1945) and similar reports derive from the use of a Nauta technique in the cat, rat and guinea-pig (Kimmel, 1965). There have been no previous reports of a vagal termination in the reticular formation in birds using any sort of technique. There is as yet no further clue as to what modality such a termination might be serving. In mammals, a projection of vagal afferents to the area postrema and area subpostrema has been found (Beckstead & Norgren, 1979; Gwyn *et al.* 1979; Kalia & Wells, 1980). However, no such projection has been found in the mallard (Dumbledam *et al.*

1979; Katz & Karten, 1978), the frog (Rubinson & Friedman, 1977) or the carp (Morita *et al.* 1980). The results presented here are also negative for a projection to the area postrema and subpostrema in hens, and this may be further evidence that such a projection is a phylogenetic development that occurs only in mammals.

There is more controversy over a possible projection of vagal afferent fibres directly to the dorsal motor nucleus of the vagus. Most reports (Dubbledam *et al.* 1979; Gwyn *et al.* 1979; Jordan & Spyer, 1978; Robinson & Friedman, 1977) deny the existence of such a projection. There are a few reports, however, of a direct projection (Allen, 1923; Mitchell & Warwick, 1955; Beckstead & Norgren, 1979). Of these, only that of Beckstead & Norgren was similar to the present report in so much as it used an autoradiographic technique. However, the authors admit that the sparse labelling that they saw could equally well have been due to fibres passing through as to fibres terminating in that region. The present results did not confirm any sort of labelling in the dorsal motor nucleus of the vagus. If there is a direct projection to this nucleus, it is clearly a very sparse one and it may be that it is another projection like those to the areas postrema and subpostrema and of recent phylogenetic origin.

SUMMARY

Using an autoradiographic technique, the central brainstem connections of vagal visceral afferent fibres were studied. The results show that there is a large projection of vagal afferent fibres to the ipsilateral nucleus of the tractus solitarius and a smaller, sparser projection to the contralateral nucleus of the tractus solitarius. That vagal afferent fibres cross the mid-line via the commissural nucleus of Cajal is confirmed. In addition, a second, more rostrally placed decussation of vagal afferent fibres is shown. Associated with this more rostral decussation is an area of termination of vagal afferent fibres in the contralateral ventromedial reticular formation. No direct projection of vagal afferent fibres to the dorsal motor nucleus of the vagus was seen.

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