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THE LOCALIZATION, BY MEANS OF ELECTRICAL STIMULATION, OF THE ORIGIN AND PATH IN THE MEDULLA OBLONGATA OF THE MOTOR NERVE FIBRES OF THE RABBIT OESOPHAGUS

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The origin of the motor nerve fibres to the striated muscle of the mammalian oesophagus has not previously been clearly established. It has been suggested, on the basis of degeneration after section of the vagus at various levels, that the oesophagus is innervated from cells in the rostral part of the nucleus ambiguus (Kosaka, 1909; Molhant, 1912). However, Getz & Sirnes (1949), in interpreting the degeneration that they observed after vagal sections, located these neurones in the dorsal motor nucleus of the vagus.

The experiments of Bell & Lawn (1955) on the sheep demonstrated that, in this species, oesophageal contraction could be produced by stimulation in the path of the vagal fibres leaving the medulla, but could also be produced by stimulation at more ventral positions in the medulla. They could not be certain whether the responses from the ventral points resulted from stimulation of: (*a*) the nucleus ambiguus and the nerve fibres leaving it; (*b*) the fibres emerging from the dorsal motor nucleus of the vagus taking a more ventral course than is usual; or (*c*) some motor centre for the oesophagus situated in the reticular formation.

This paper describes experiments localizing the regions in the rabbit medulla whose stimulation gives rise to oesophageal contraction. The use of near-threshold stimulation and electrolytic marking of low threshold points allowed relatively concise identification of these regions. For both the cervical and the cardiac portions of the oesophagus (which in the rabbit, as in the sheep, has almost entirely striated muscle) these regions have been found to correspond with the position of the nucleus ambiguus and the pathway taken by axons from its cells leaving the medulla.

Some observations have also been made on the localization of regions which, when stimulated, give rise to pharyngeal or laryngeal contractions; these are also associated with neurones of the nucleus ambiguus.

There is evidence that the peristaltic wave in the striated muscle portion of the oesophagus, unlike that in the intestines, is not co-ordinated by the local intramural nerve plexus but by neurones in the brain. But, whatever the method of co-ordination, the problems of interconnexion between neurones would be simplified by an arrangement of the neurones in the medulla whereby those controlling successively more caudal segments of the oesophagus were aligned in a manner consonant with the relation of their peripheral fields. A wave of activity progressing through the neurones would then produce a progressing peristaltic wave in the oesophagus.

In an effort to detect such a distribution of the neurones, responses were recorded from two places in the oesophagus, one in the cervical region and the other in the abdominal region. A careful examination was made of the anatomical relation between the points in the brain from which cervical responses could be elicited and the points from which abdominal responses could be elicited.

A preliminary account of the results of these experiments has already been presented (Lawn, 1960).

METHODS

The results reported here were obtained from twelve young rabbits.

Operative procedure. After induction of anaesthesia with urethane, the skin and pretracheal muscles were divided in the ventral mid line from the mandible to the sternum and the pretracheal muscles removed on the left side. The animal was then placed upright in a support, the head being held by means of clamps on each zygomatic arch and a bar which was grooved to receive the incisor teeth. The skin over the occiput was incised and the muscles between the nuchal crest and the atlas were removed. After an opening had been made in the bone, the dura was opened and part of the cerebellum was removed so that the 4th ventricle was exposed. A pool of mineral oil was used to prevent desiccation.

The abdominal cavity was next opened on the left side by an incision immediately caudal to the last rib. The liver was deflected caudally and a special Perspex retractor was used to hold the abdominal part of the oesophagus so that a recording electrode could conveniently be applied to it.

Recording methods. Miniature bipolar suction electrodes were used to record muscle activity. The electrodes were connected to balanced a.c. amplifiers and the amplified muscle potentials were displayed on a four-channel oscilloscope. Activity at one of the electrode sites was also monitored aurally by means of a loudspeaker.

In the majority of experiments four recording electrodes were employed. These were attached on the left side of the body to (1) the surface of the pharynx at the level of the thyroid cartilage; (2) the surface of the cricothyroid muscle; (3) the surface of the cervical oesophagus in the mid-cervical region and (4) the surface of the abdominal oesophagus (after removal of its peritoneal covering). Only three main amplifiers were available, but as the laryngeal response was brief and the abdominal oesophageal response had a long latency, these two responses could be displayed on one beam of the oscilloscope without the one obscuring the other. The corresponding electrodes were each connected to a pre-amplifier; the outputs from the two pre-amplifiers were mixed and then further amplified by a single main amplifier.

Stimulation method. The medulla was stimulated through an electrode which was made from a straight length of Diamel insulated, stainless-steel wire, 0·005 in. diameter, cut obliquely at one end. The opposite end was held in a stereotaxic instrument employing rectangular co-ordinates (Rosenberg & Tindley, 1949).

Stimuli from an electronic stimulator were applied, through an isolating transformer, between the monopolar wire electrode and an indifferent electrode attached to the margin of the skin incision. The stimulus was a brief pulse approximately 200 μsec in duration.

Each photograph taken by the oscilloscope camera contains the responses, at the four electrode sites, to five stimuli of increasing strengths. A single horizontal sweep synchronized with the stimulus was imposed on three beams at each strength of stimulus. In order

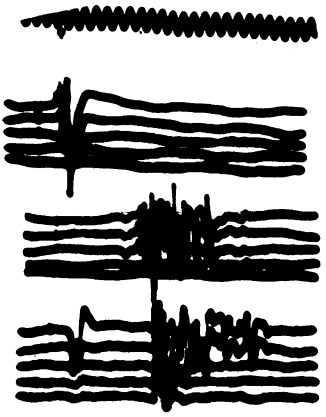

Fig. 1. A typical response to stimulation of the medulla. The upper trace is of a 500 c/sec sinusoidal wave. The five traces in the group below this are the responses at the pharyngeal electrode to stimulus strengths of, from above downwards, 16, 8, 4, 2 and 1 units. The five traces in the middle group are the responses at the cervical oesophageal electrode and the five in the lowest group the responses at the laryngeal electrode (early response) and at the abdominal oesophageal electrode (late response) to the same five stimuli. In this record the threshold for the pharyngeal response is between 1 and 2 units, for the laryngeal response between 4 and 8 units, for the cervical oesophageal responses between 1 and 2 units and for the abdominal oesophageal response less than 1 unit.

that the records should not overlap, each beam was displaced vertically between successive sweeps (see Fig. 1). The five stimuli were separated from each other by an interval of 2 sec. The stimulus strengths were in the ratios 1:2:4:8:16. In different experiments the maximum strength delivered by the stimulator was either 8 V or 16 V which were reduced to approximately 4·7 V and 9·4 V respectively by the isolating transformer (ratio 1·7:1). Another beam displayed a sinusoidal wave generated by an audio-oscillator. All four beams were swept at the same velocity. An apparatus was designed and constructed to perform the above operations automatically (Lawn, 1962).

The medulla was mapped in the following manner. The electrode was inserted vertically and lowered in steps of 0·5 mm, applying the five stimuli of ascending strengths and recording the response at each step. When the ventral surface of the medulla was reached repetitive stimulation (1 to 2 stimuli/sec) was commenced and the electrode track searched for the most sensitive regions, progressively decreasing the stimulus strength. When such

points were found to have a low threshold they were marked by passing a small current (from a battery) between the stimulating and indifferent electrodes (stimulation electrode positive). The threshold strength at each point was noted. The needle was then withdrawn and inserted at a new point, 0·5 mm from the previous insertion. In some experiments the insertions were made in saggital rows, while in others they were made in transverse rows. Successive rows of insertion points were separated by 1·0 or 1·5 mm.

With a few exceptions, only points giving oesophageal responses at low threshold were marked. Only the left side of the medulla was explored. At intervals, the constancy of location of the recording electrodes was checked by connecting the stimulator to a pair of electrodes applied to the vagus in the mid-cervical region.

Histological identification of the electrode positions. The method used to mark the position of the most sensitive regions in each electrode track was the Prussian blue method of Adrian & Moruzzi (1939). A current of 10 μA was passed through the electrode from a battery via a resistance of 10 MΩ for a period of 15 sec. After the experiment had been completed the animal was killed and perfused through the ascending aorta, first, with 5 % potassium ferrocyanide solution, then with 10 % formol-saline. The head was cut off and immersed in formol-saline overnight. On the following day the medulla was removed from the skull. Fixation was continued in 10 % formol-saline for a further 3–5 days. The appropriate part of the medulla was then serially sectioned at a thickness of 100 μ, either horizontally or transversely, using a freezing microtome (Marshall, 1940). The sections were stained with a modification of the Luxol fast blue technique of Kluver & Barrera (1953) substituting pyronin G for cresyl violet as the cell stain.

RESULTS

Maps of the distribution of reactive sites

In Fig. 2 are shown diagrammatic cross-sections of the rabbit medulla upon which are plotted the positions of points giving low threshold responses as identified from Prussian blue marks in the brain sections. All the most sensitive points (threshold below 1 V applied to the isolating transformer) from eleven rabbits have been plotted, a total of 126 points. The various symbols show sites at which the relevant electrode (pharynx, larynx, cervical oesophagus or abdominal oesophagus) recorded a response to a strength of 1 V or less. In Fig. 2 to avoid confusion, the points from which responses from the cervical and abdominal oesophagus could be aroused are plotted on the side on which they lay, but those for the larynx and pharynx have been plotted on the opposite side (all stimuli were applied to the left side of the medulla). The numbers above each section indicate the distance, in millimetres, of that particular section from the transverse level of the rostral extremity of the nucleus ambiguus, negative figures indicating sections caudal to this level and positive figures sections rostral to this level. Each section shows all the points localized between the level of that section and the level of the section preceding it (caudal sections) or succeeding it (rostral sections). All these measurements are those made on the mounted sections without correction for shrinkage. The vertical and lateral position of the points was determined, not by direct

measurements, but by determining the relation of the marks to easily recognizable medullary structures.

In Fig. 3 the same points are plotted on diagrammatic plan views of the medulla. Symbols are used to distinguish responsive points which were situated dorsally in the medulla from points which were situated more ventrally.

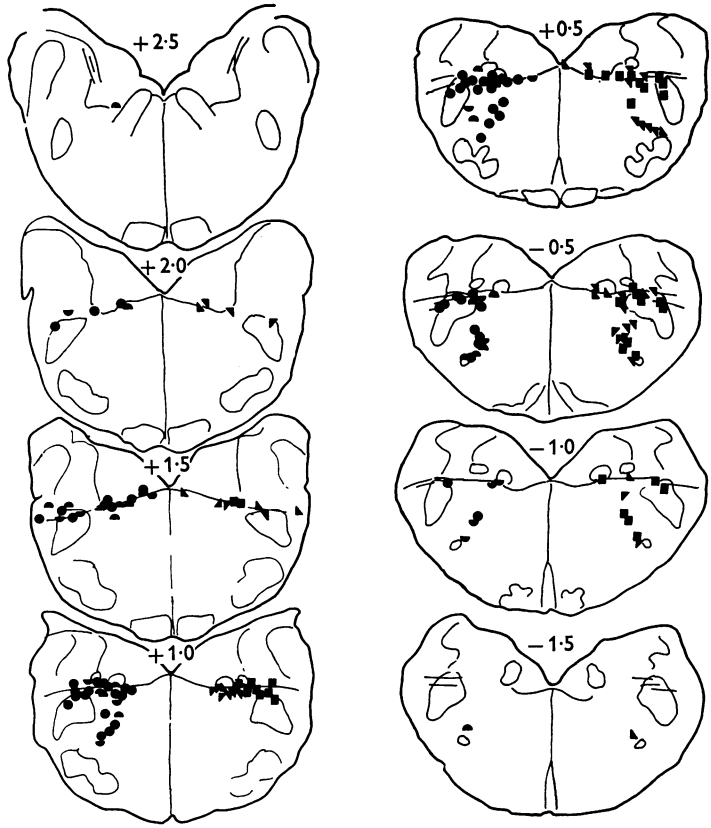

Fig. 2. Diagrammatic transverse sections of the medulla illustrating the distribution of low threshold stimulation points for oesophageal, laryngeal and pharyngeal responses. The figures above each section indicate the distance from the rostral tip of the nucleus ambiguus in mm. For details of the methods of plotting see text. Except in a few cases, points giving laryngeal and pharyngeal responses without any response of the oesophagus were not marked. For this reason this map does not represent the full extent of the area from which low threshold laryngeal and pharyngeal responses can be elicited. All responses were produced by stimulation of the left side of the medulla, but laryngeal and pharyngeal points have been transposed to the right side of the diagrams. The symbols used are as follows: pharynx, ◥; larynx, ◣; pharynx and larynx, ■; anterior oesophagus, ◓; posterior oesophagus, ◒; anterior and posterior oesophagus, ●.

Additional evidence for the correspondence of marked points with medullary structures can be gained by examining the records from single experiments. A series of records comprising one transverse row of needle tracks were analysed and the threshold for each plotted approximately in its correct position on an appropriate diagrammatic cross-section of the medulla, with only the sterotaxic co-ordinates of each point as a guide. In this way a threshold 'contour' map was constructed by drawing lines of approximately equal threshold, freehand, between the plotted points. Two maps based on data from one animal are presented in Fig. 4.

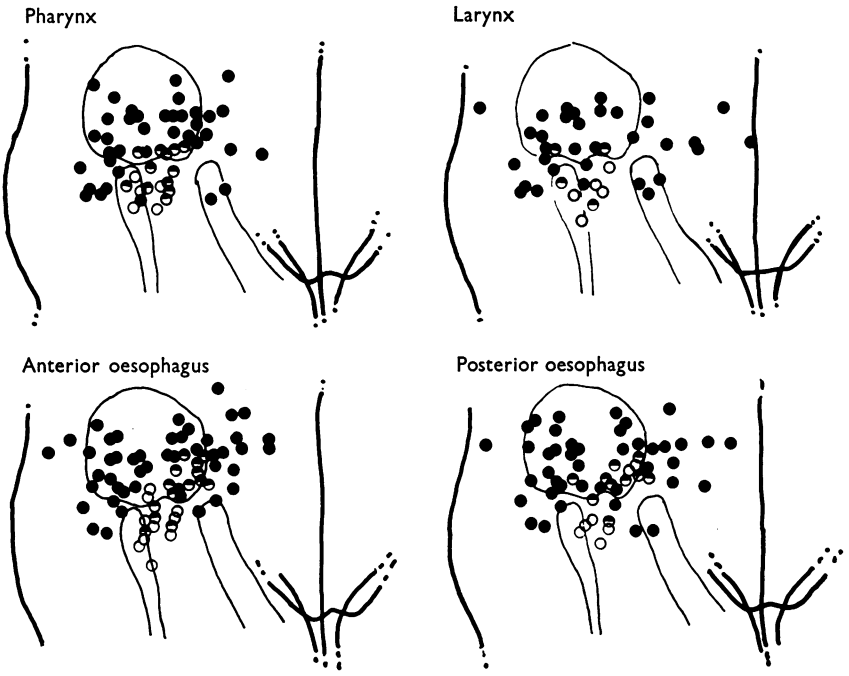

Fig. 3. Diagrammatic plan views illustrating the distribution of low threshold points for oesophageal, laryngeal and pharyngeal responses. The full area for the pharyngeal and laryngeal responses is not represented (see legend to Fig. 2). Superficial (dorsal) responses, ●; deep (more ventral) responses, ○; deep and superficial responses, ◓.

In order to compare more accurately the rostro-caudal distribution of points giving responses from the different recording sites, similar threshold contour maps have been constructed from the results of an experiment in which the rows of electrode tracks were in a saggital plane. Figure 5 shows a map from an experiment in which a complete rostro-caudal exploration was made.

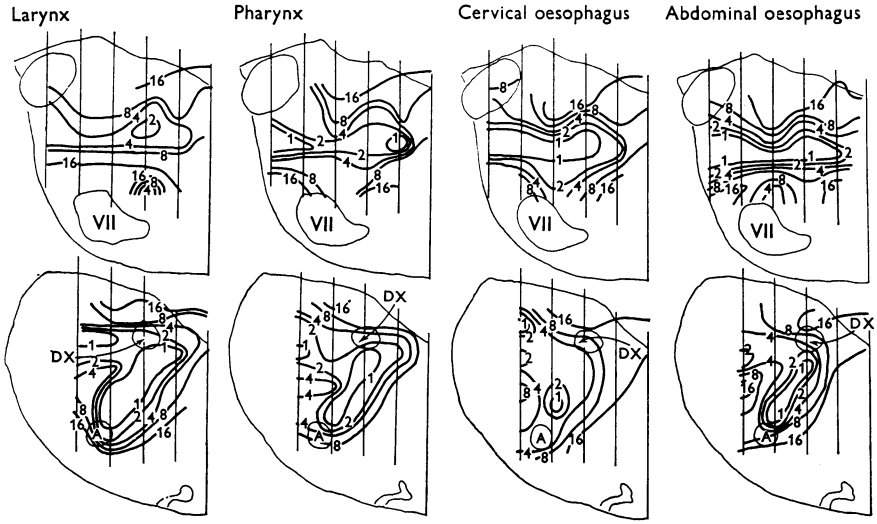

Fig. 4. Transverse threshold contour maps for oesophageal, laryngeal and pharyngeal responses. For details of construction see text. Each line outlines the area within which responses at the appropriate recording electrode could be elicited by stimulation at the strength marked on the line. The upper diagrams represent a transverse plane 1·1 mm rostral to the tip of the nucleus ambiguus, the lower diagrams represent a plane 0·6 mm posterior to this point. VII, Facial nucleus; DX, dorsal motor nucleus of the vagus; A, nucleus ambiguus.

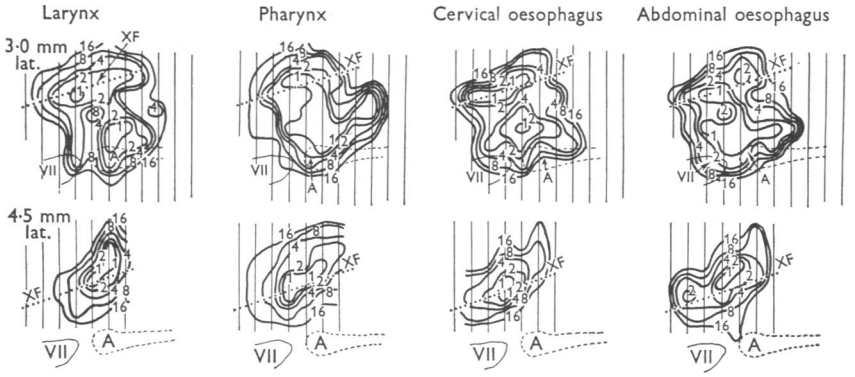

Fig. 5. Saggital threshold contour maps for oesophageal, laryngeal and pharyngeal responses. For methods of construction see text. For significance of the lines see legend to Fig. 4. The upper diagrams represent a saggital plane 3·0 mm lateral to the mid line, the lower diagrams a plane 4·5 mm lateral to the mid line. The nucleus ambiguus lay approximately 3·3 mm from the mid line in this animal. XF, level of fibres of the vagus and glossopharyngeal nerves entering and leaving the medulla; VII, facial nucleus; A, projected position of nucleus ambiguus.

Injury discharges

On rare occasions when the electrode was lowered into the medulla a barrage of muscle potentials was heard from the loudspeaker, or observed on the oscilloscope screen, in the absence of stimuli. Wherever such points could be subsequently accurately located, they were found to be in the immediate vicinity of the nucleus ambiguus.

Short path motor fibres to the cervical oesophagus

In one experiment two bipolar recording electrodes were applied to the cervical oesophagus. On examination of Fig. 6*a*, which is the response at these electrodes to stimulation of the cervical vagus, it can be seen that

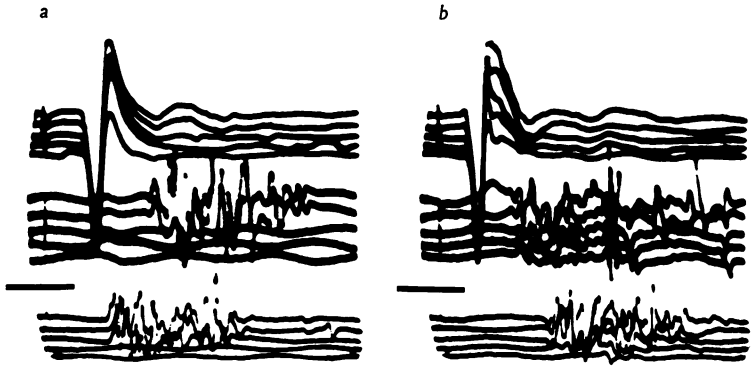

Fig. 6. Records illustrating evidence for the presence of short path motor nerve fibres to the cervical oesophagus. The traces in the three groups represent the responses at the larynx (upper group), the rostral end of the cervical oesophagus (middle group) and the caudal end of the cervical oesophagus (lower group). *a*, The stimuli were applied to the vagus; *b*, the stimuli were applied to the medulla. Stimulus strengths are as for Fig. 1. Note that although the latency of the caudal cervical oesophagus is longer with stimulation of the medulla (consistent with increased conduction distance) the latency of the rostral cervical oesophageal response is shorter (calibration: 10 msec).

the latency of the more caudal cervical oesophageal response (lowest trace) is less than that of the more cranial oesophageal record (middle trace). In Fig. 6*b*, when the stimulus was applied to the medulla, the latency relation was reversed, the cranial response having the shorter latency. These results may be explained as follows. When the vagus is stimulated, nerve impulses reach the cranial portion of the cervical oesophagus by ascending rostrally in the recurrent laryngeal nerve. The decrease in the latency of the response of the cranial oesophagus to stimulation of the medulla must mean that impulses are reaching this portion of the oesophagus by a shorter route than that through the

recurrent laryngeal nerve. At this point of stimulation in the medulla, in addition to the short latency cranial response, some potentials with an unusually long latency can be seen in the record from the cranial portion of the oesophagus. These are most probably the result of activating motor fibres reaching this portion of the cervical oesophagus through the recurrent laryngeal nerve. The two sets of motor fibres must, therefore, have been close to each other in the medulla in this case. A similar pattern of response could be elicited from other medullary points in this animal.

DISCUSSION

If the results of stimulation of the brain are to be used to define the position of motor neurones and their axons it must first be shown that the responses obtained are not the result of indirect activation of the motor neurones through stimulation of sensory nerve fibres. Although there is no irrefutable proof that motor pathways were stimulated directly in the experiments reported here, there is ample evidence favouring this interpretation. The animals were deeply anaesthetized with urethane during stimulation and in rabbits under these conditions it is extremely difficult to elicit reflex activity in the oesophagus by repetitive stimulation of sensory nerves, e.g. the central end of the superior laryngeal nerve or the cervical vagus. Consequently a single shock applied to a few sensory fibres in the medulla is unlikely to provoke a reflex response. In nearly every case the latency of the response to central stimulation at a particular recording site was remarkably constant in spite of varying stimulus strength. A reflex response would be expected to show a longer latency at near-threshold stimulation. In one or two cases, a response with varying latency was in fact recorded and one is illustrated in Fig. 7. As the stimulus strength was increased the latency decreased. This type of response was almost certainly reflex in nature, and its very rare occurrence, in spite of the ease with which it can be recognized, is evidence that the great majority of the responses are the result of direct stimulation of motor nerves.

For all recording sites, the position of the region in the medulla from which low threshold responses could be initiated was in every respect consistent with the identification of the responding structures with the motor neurones of the nucleus ambiguus (ventral motor nucleus of the vagus) and their axons which follow the well-known course dorsomedially then laterally to emerge from the lateral border of the medulla. This is particularly well shown in the transverse threshold 'contour' maps (Fig. 4) constructed from results from a single animal, but it is also apparent in the maps of all low threshold points (Fig. 2).

Only two out of 126 marks indicating low threshold points were situated within the dorsal motor nucleus of the vagus, although many were situated at its ventral border. In this region there is a close approximation of the neurones of the dorsal motor nucleus and the axons from cells of the nucleus ambiguus immediately below them. All the responses could have resulted from stimulation of axons of the ambiguus neurones but the presence of neurones in the dorsal nucleus innervating the striated muscle of the oesophagus cannot be completely ruled out.

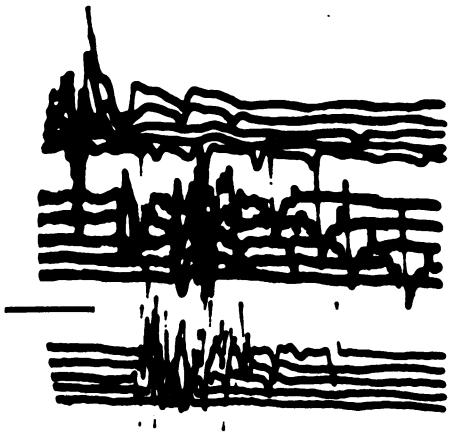

Fig. 7. An apparent reflex component observed in a response to stimulation of the medulla. The recording conditions are the same as for Fig. 6. (calibration: 10 msec). Following the direct response at the rostral oesophageal electrode is a potential which first appears at strength 2 units, and whose latency varies with stimulus strength. The varying latency suggests a reflex response. Note that the other components have a relatively constant latency at different stimulus strengths.

When injury potentials were produced as a result of moving the stimulating electrode, however, its tip was invariably found to have passed through or in the immediate neighbourhood of the nucleus ambiguus, a low threshold region. Injury potentials were never initiated by penetration of the dorsal motor nucleus of the vagus although at this position the electrodes passed through the region of axons from the nucleus ambiguus, which is another low threshold region. One interpretation of these findings is that the injury potentials are produced by damage to nerve cell bodies but not axons; no injury potentials are produced from the dorsal motor nucleus because none of the relevant motor neurones are situated in this nucleus. It can be seen from examination of the plots in Fig. 3 that the ventrally situated sensitive points have a more caudal distribution than those situated dorsally. Most of the axons from cells in the nucleus ambiguus leave the medulla rostral to their origin. In this respect the

intramedullary course of these axons of the glossopharyngeal and vagus nerves closely parallels the intramedullary course of the facial nerve.

In most cases only those points were marked from which low threshold responses could be obtained at the oesophageal electrodes. The medulla was not explored systematicaly for laryngeal or phargyngeal responses, therefore the maps are not a guide to the over-all distribution of such points. It can be seen that there are no obvious differences in distribution between the points giving responses in the cervical oesophagus and those giving responses in the abdominal oesophagus. At 63 % of the low threshold points giving oesophageal responses, the larynx or the pharynx (or both of them) also responded at low threshold, and these points were not distributed differently to those giving oesophageal responses only. Even when the results from single experiments were examined, differences of distribution were small and not consistent.

There are several possible explanations for this inability to detect consistent differences between the distribution of neurones supplying the two parts of the oesophagus, the pharynx and the larynx. One possibility is that the spread of the stimulus was so great with respect to the total area occupied by the neurones concerned that differences of distribution could not be detected. The method used to determine the sites which were marked in these experiments (that of determining the points with lowest threshold) reduced the spread of current from the exposed surface of the electrodes to a minimum. It is estimated that this surface did not exceed $130 \times 200\ \mu$. Responses from the chosen muscle recording sites could be obtained by stimulation of a region 1·5 mm in rostro-caudal extent, and the nucleus ambiguus is approximately 0·5 mm in diameter. Although the relative size of the exposed surface of the stimulating electrode is rather large it would be expected that a rostro-caudal distribution of the different groups of neurones, if it were clear-cut, would be detected under these conditions of stimulation.

Nevertheless, failure to detect grouping of functionally distinct neurones does not prove its absence, for it could be masked if the axons from one group travelled in a rostro-caudal direction through neurones of another group in their path out of the medulla. However, recent experiments based on the method of retrograde cell degeneration (Lawn, 1962) also suggest that considerable rostro-caudal overlap of the neurones supplying the cricothyroid muscle, pharynx and oesophagus does occur. The results of using these two different techniques reinforce each other and it must therefore be assumed that a clear-cut rostro-caudal grouping of these neurones does not exist in the nucleus ambiguus.

The detection of motor pathways to the cervical oesophagus which are shorter than that in the recurrent laryngeal nerve is consistent with the

discovery by Hwang, Grossman & Ivy (1948) that part of the cervical oesophagus in the rabbit is supplied by the pharyngo-oesophageal nerve (pharyngeal branch of the vagus). Although these peripheral pathways are divergent, the nerve fibres concerned may be found close together in the medulla, and the cell bodies probably lie in the same region of the nucleus ambiguus.

SUMMARY

1. The medulla oblongata was stimulated electrically through a monopolar wire electrode. Activity of the striated muscle of the pharynx, larynx (cricothyroid muscle) and oesophagus (cervical and abdominal) was detected electromyographically using miniature bipolar suction electrodes applied to these muscles.

2. Points in the medulla from which oesophageal activity could be excited at a low stimulus strength were localized from serial frozen sections, using the Prussian blue marking method.

3. Low threshold points were distributed over the rostral part of the nucleus ambiguus and over the course of the nerve fibres leaving this nucleus for the periphery. Only two of 126 such points lay within the dorsal motor nucleus of the vagus. Injury discharges from the muscle concerned were obtained only when the stimulating electrode was advanced into the vicinity of the nucleus ambiguus.

It is concluded that the cells of origin of the motor nerve fibres to the striated muscle of the pharynx, larynx and oesophagus lie exclusively in the nucleus ambiguus.

4. No clear-cut differences in distribution could be detected between the low threshold points for pharyngeal, laryngeal and for cervical and abdominal oesophageal responses. Considerable rostro-caudal overlap must exist between the zones of the nucleus ambiguus occupied by these groups of neurones.

5. Further evidence is presented for a short pathway for motor nerve fibres to the cervical oesophagus in addition to the principal pathway through the recurrent laryngeal nerve. Axons entering these two different pathways can be detected close to each other in the medulla.

I wish to express my thanks to Professor E. C. Amoroso for valuable advice and encouragement and to Dr F. R. Bell for instructions in the techniques of medullary stimulation and for helpful discussions. I am indebted to the Agricultural Research Council for the oscilloscope used in this investigation.

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