ACTION POTENTIALS IN THE NORMAL RESPIRATORY CENTERS AND ITS CENTRIFUGAL PATHWAYS IN THE MEDULLA OBLONGATA AND SPINAL CORD

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In 1936 Gesell et al. (3, 4) recorded the respiratory potentials from various parts including the diencephalon, mesencephalon, pons, medulla and upper cervical cord of dogs using bipolar needle electrodes separated by 1.5-2.0 mm. The respiratory potentials were found in the greatest abundance in the region about the obex, the region in which, according to them, the respiratory centers are assumed to localize. The respiratory potentials consisted of inspiratory and expiratory potentials which did not show any localized arrangement. In 1951 the experiments were repeated on rabbits by Dirken and Woldring (2, 8) using bipolar microelectrodes composed of enameled platinum wire of 50 micron gauge. According to their observations the center consists of active spots chiefly situated in the reticular mass of the medulla between the obex and the acoustic tubercles and two separate areas, against the results of Gesell et al., could be discriminated, a ventromedial inspiratory and a dorsolateral expiratory part. In 1951 Amoroso et al. (1) who explored the fastpotential changes from the medulla oblongata of dogs, cats and rats by means of a unipolar steel needle electrode with diameters from 10 to 40 micron, observed, in agreement with the results of Gesell et al., no definite separation into inspiratory and expiratory regions.

As regards the localization of the normal respiratory centers there is a wide discrepancy between our opinion (5, 6) and those of the previous authors. From the results obtained by a series of experiments in which the transection as well as the localized lesion of the brain stem was performed we concluded that the normal centers are situated at the level of the striae acusticae bilaterally in the lateral reticular formations and the centrifugal pathways pass through the lateral reticular formations of the medulla oblongata. To establish our opinion more firmly and at the same time to obtain more precise informations as to the activity of the centers we attempted to record the action potentials with a respiratory rhythm from the centers and the centrifugal pathways.

METHOD

The unipolar microelectrode devised by Tomita (7) was used to lead the action potentials. It was composed of a glass capillary with a diameter of 10-20

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micron into which a silver wire reduced electrolytically to about 5 micron in diameter at its tip was inserted. The electrode was connected to the grid of a capacity-coupled amplifier which was connected to a cathode-ray oscillograph for registration of potentials as shown in fig. 1. As a vibrator A-type was used. For auditory detection a power amplifier and loud speaker were connected to the amplifier described above.

Cats (2-3 kg.) and dogs (2-3 kg.) were used in the experiment. For anesthetic 1 gm. of urethane per kg. body weight was administered subcutaneously. The animal was then fixed in a prone position and decerebrated. The procedure of the operation was as follows: The carotid arteries were ligatured and the vertebral arteries clamped to block the blood circulation in the brain except that through the anterior vertebral artery. An artificial respiration was then carried out through a tracheal cannula. The scalp was trephined, the mid-brain was transected between the anterior and posterior colliculi and the brain situated rostral to the level of transection was completely removed. The vertebral arteries were again opened usually 7-8 minutes after the decerebration. A part of the occipital bone and of the cerebellum was removed to expose the dorsal surface of the pons and medulla oblongata.

The respiration of the animal was recorded by means of Bert's pneumograph connected with Marey's tambour provided with a small mirror. The electrode-holder was connected to the outer tube of the microscope. With one turn of the micrometer screw the tip of the electrode moved by 100 micron upwards or downwards.

To ascertain the trace of the electrode the brain stem was fixed with formalin, embedded in paraffin, sectioned serially in thickness of 30 micron and stained with Nissl's method.

RESULTS

A. Spike potentials with a respiratory rhythm and localization of points from which the spikes are detected

The electrode is inserted into the region of the brain stem situated rostrally or caudally to the under border of striae acusticae, where, according to our opinion, the normal respiratory centers lie. In the experiments carried out successfully, the following course of events is observed: As the electrode is gradu-
ally penetrated ventrally there appear at first continuous spike discharges, then at the depth of 3–4 mm., they give place to volleys of spike discharges with a respiratory rhythm and when the electrode proceeds a little deeper the amplitude of spikes reaches the maximum. The amplitude of each spike is always the same in the volleys produced successively. We may then conclude from a series of uniform spikes that the same unit was repeatedly active. The potentials may reach the magnitude up to 100 μV.

The spike discharges are classified in inspiratory and expiratory discharges according to their appearance during the inspiratory or expiratory phase. The inspiratory and expiratory neurons are intermingled together in the respiratory centers. On gradually penetrating the electrode, inspiratory, expiratory and inspiratory discharges are successively detected in some cases; in other cases, expiratory, inspiratory and expiratory discharges are successively detected. In short we cannot localize any region of inspiratory and expiratory neurons. The appearance of inspiratory potentials have predominance over the expiratory ones on the animal decerebrated, while the latter appears more and more abundantly when the animal is asphyxiated. From these facts we are inclined to think that the relatively few expiratory neurons work in the normal condition of the animal, but further studies must be carried out to solve this problem conclusively.

Respiratory discharges are also recorded from the lateral reticular formations in the medulla oblongata and from the spinal reticular formations in which all nerve cells are undoubtedly not endowed with automaticity. According to the results of our previously reported experiments these regions must be the centrifugal pathways of respiratory impulses. As regards the frequency and variation of the periods of the discharges no essential difference is perceivable between those from the centrifugal pathways and those from the centers. From these facts it can be concluded as follows: When the spike potentials with a respiratory rhythm have been detected from some region, the authors have no reason to believe that the centers are localized in that region, unless the results of another corroborating experiments have been taken into account. From this reason we believe that the concepts of previous authors are not reliable as far as the localization of the centers is concerned.

Fig. 2 shows the dorsal surface of the brain stem and the succeeding portion of the spinal cord. The points show the positions of insertion of the electrode. The inspiratory and expiratory spike discharges detected from the level of A, B, C, . . . , F are illustrated in fig. 4. The precise localization of points from which the spike discharges are detected are shown in fig. 3.

As shown in fig. 3, in the region of the striae, i.e., the centers, discharges are detected from the part which is situated ventrolaterally in the lateral reticular formation and adjacent to the dorsolateral surface of the facial nucleus. The more caudally we explore along the centrifugal respiratory pathways, the more dorsolaterally move the points of detection of the respiratory potentials and at the level of the decussatio pyramidum and the succeeding spinal cord these points of detection locate in the reticular formations situated laterally to the lateral horn. In the section of the spinal cord shown in fig. 5 the point for detecting the respiratory spike potentials is clearly seen.
FIG. 2. Dorsal surface of the brain stem and the spinal cord illustrating the positions of insertion of electrode.


FIG. 3. Localization of points from which the action potentials with a respiratory rhythm are detected.

Pictures *A, B, C,..., F* show the microscopic sections at the level of *A, B, C,..., F* in fig. 2. On the left side of each section white as well as black points show respectively the points from which the inspiratory as well as the expiratory potentials are detected. On the right side of each section the centrifugal pathways are schematically with the bundle of lines illustrated.
B. Spike discharges from the inspiratory neurons

As to the frequency and variations of the periods of spike discharges no remarkable differences are perceived between the discharges detected from the centers and those from the centrifugal pathways, so that it is permissible to describe en bloc (fig. 4).

1) Number of spike discharges. In cats the spike of the inspiratory neurons are generally produced successively during the whole inspiratory phase which is about one second. The number of spikes dose not differ greatly in the successive volleys of one neuron, but differs considerably from one neuron to another varying from 5 to 57 and in most cases from 17 to 30.

In dogs the results are similar to those in cats. In the volley which lasts about one second, the number of spikes discharged varies from 15 to 63 and in most cases from 15 to 35 according to the characters of neurons.

2) Various patterns of spike discharges. To study the patterns for the variations of the periods of spike discharges, frequency diagrams have been constructed from records in which only one unit is discharging. The inverse value of the interval between two spikes is placed on the ordinate against the time elapsed since the appearance of the first spike of the volley on the abscissa. The former quantity is drawn on a logarithmic scale. The diagrams can be classified in 5 patterns as shown schematically in fig. 6.

In pattern A which is most frequently encountered three stages can be observed; a) at the stage of crescent frequency, which is in the beginning, a number of periods shorten themselves successively from one to another, then follows b) the stage of constant frequency in which each period shows only slight variation from each other, and lastly follows c) the stage of decrescent frequency, in which several periods prolong successively till the spike disappears. The volleys shown in fig. 4, I, C and D belong to this pattern. From the form of this pattern it can be referred to as the trapezoidal pattern. Among other patterns it can be remarked that the left or right side of the trapezoid prolongs (B and A in fig. 4, I). The periods are sometimes constant during the whole inspiratory phase (F in fig. 4, I). In rare cases these constant periods are followed by prolonged periods at the end stage of inspiratory phase. It is also in rare cases that spikes continue to appear yet during the expiratory phase and the pause, although their time intervals are relatively great, i.e., more than 0.2 seconds. It may be thought that pattern A is the basic and other patterns are its variants.

The periods of spike discharges vary in a wide range, i.e., from 0.008 sec. to 0.233 sec. and at the constant stage of periods the frequency of discharges is in most cases 30-60/sec. in cats and dogs.

3) Relation of spike discharges to pneumogram. The first spike usually starts 0.09-0.14 seconds before the inspiratory rise of the pneumogram, the following spikes continue to appear during the entire inspiratory phase and the last spike is produced about 0.09-0.14 seconds before the end of inspiratory phase. The first spike starts sometimes, however, at the same time with or a moment later
FIG. 4. Inspiratory and expiratory potentials detected from the normal respiratory centers and their centrifugal pathways on cats.

Pictures $A, B, C, \ldots, F$ show the inspiratory (on the left (I)) as well as the expiratory potentials (on the right (II)) which are detected from the level of $A, B, C, \ldots, F$ in fig. 2. As to the localization of points of detection see fig. 3.
to the beginning of the pneumogram; the spikes are not always abolished before the end of the inspiratory phase, but continue to appear sometimes further during the expiratory phase and the pause; and discharges are abolished sometimes already in the middle of the inspiratory phase. From these facts it can be supposed that each neuron does not discharge at the same time, but always with a minute difference of start and with spike-frequency and -number characteristic of it. In view of the above consideration, the conclusion given by Dirken and Woldring that the vagal impulses begin after the beginning of the pneumogram seems to be fallacious.

FIG. 5. Point of detection of the respiratory potentials in the spinal cord (section at the level of F in fig. 2). 

× shows the trace of the micro-electrode.

C. Spike discharges from the expiratory neurons

No remarkable differences are perceived between the spike discharges detected from the centers and those from the centrifugal pathways.

1) Number of spike discharges. The spikes from the expiratory neurons are produced as usual during the expiratory phase and the pause. The sum of these two respiratory phases is about 1.3 seconds in cats and the number of spikes produced during these intervals is 20–92; in dogs the former is about 1.5 seconds and the latter is 18–95. Both the interval and number of spikes are greater than those in the cases of inspiratory neurons.

2) Various patterns of spike discharges. The frequency diagram can be classified in the patterns as shown in fig. 7. Pattern A is the basic one which is most frequently encountered. In this pattern the spikes are continually discharged with fairly constant interval and are abolished abruptly. From the form of this pattern it can be referred to as the rectangular pattern. The discharges illustrated in fig. 4, II, B and D belong to this pattern. Variants can be remarked among other patterns in which the prolongation of the periods occurs at the beginning (A in fig. 4, II) or at the end stage of the discharges (C in fig. 4, II). In rare cases the trapezoidal pattern is also observed (E in fig. 4, II).

The periods of discharges vary from 0.008 to 0.100 seconds and at the con-
stant stage of periods the frequency of discharges is in most cases 30-80/sec. in cats and dogs.

A
B
C
D
E

FIG. 6. Patterns of inspiratory discharges. Explanations in text.

A
B
C
D
E

FIG. 7. Patterns of expiratory discharges. Explanations in text.

3) Relation of spike discharges to pneumogram. The spike discharges generally start a moment before the expiratory fall of the pneumogram; sometimes, however, at the same time with or a moment later to the fall of the pneumogram. The discharges start sometimes in the latter half or in rare cases already in the former half of the inspiratory phase. The volley is abolished always a moment before the beginning of the next inspiration. The facts described above show that each expiratory neuron begins to discharge independently and with some time-lag from each other.

CONCLUSIONS

The action potentials with a respiratory rhythm were detected by means of unipolar microelectrode from the normal respiratory centers and the centrifugal respiratory pathways which have been established by our previous experiments. The results were summarized as follows.

1) No essential difference as to the frequency and variations of the periods of spike discharges exists between the spike discharges detected from the centers (reported previously by Hukuhara et al.) and those from the centrifugal pathways, i.e., from the lateral reticular formations in the medulla oblongata (already reported by Hukuhara et al.) and the spinal reticular formations found newly. The inspiratory and expiratory neurons do not show any localized arrangement in the centers. In the former the volley of spike discharges appears generally during the entire inspiratory phase, while in the latter it appears generally during the expiratory phase and the pause.

2) In the basic pattern of inspiratory discharges, after the first spike was produced, the periods of succeeding spikes shorten themselves gradually, then become constant and prolong again gradually till the discharges disappear. Besides this pattern many variants are found.

3) In the basic pattern of expiratory discharges, the spikes are produced as well as abolished abruptly, and during the stage of discharges the periods are fairly constant. Besides this pattern many variants are found.

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