RESPIRATORY NEURONAL ACTIVITIES IN SPINAL AFFERENTS OF CAT

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The integrative function of the bulbar reticular formation for respiratory movements has long been established by experiments of denervation, ablation or gross stimulation. Many works are in agreement with the view of the pneumotaxic mechanism of the upper pons and the vagal lung afferents, both influencing the bulbar respiratory rhythm\(^8,9,10,12,13\). Spontaneous neuronal discharges, synchronous with respiratory movements, were first recorded by GESELL, BRICKER and MAGEE\(^5\) in the dog medulla. They described that the bulbar reticular gray matter was influenced by all the types of sensory signal, but most profoundly by those coming through the fifth, ninth, tenth, eleventh and twelfth cranial nerves and the sensory roots of the spinal cord.

Using microelectrode techniques, YAMAMOTO and his coworkers\(^14,15\) could record impulse discharges occurring synchronously with respiratory movements in the dorsal funiculus and in the spinocerebellar tract of the cat. These impulse discharges are proprioceptive in nature in the sense that adaptation is very slow, and considered to originate in the chest wall. The present work was carried out to explore the spinal cord of the cat with a microelectrode for the purpose of determining the nature and distribution of respiratory afferent neurons.

METHODS

The experiments were carried out on 70 cats. The trachea was cannulated and both the common carotid arteries were ligated under ether anesthesia. After intercollicular decerebration, the anesthesia was discontinued. The first to the third segments of the cervical cord were exposed by laminectomy, and the second cervical segment was prepared for exploration. The microelectrodes were silver needles insulated with glass, as designed by YAMAMOTO et al.\(^15\). The tip of the electrode measured from 5 to 10 \(\mu\) in diameter. This electrode was inserted free-hand into the spinal cord. In order to minimize the disturbances induced by respiratory movements or arterial pulsation, the electrode was suspended by a
thin copper wire, which was connected to a CR-coupled amplifier. The indifferent electrode was placed on the surface of the spinal cord, or adjacent bone. Neuronal discharges were recorded with a twin-beam cathode ray oscilloscope, and its second beam was used to record chest movements through a mechano-electrical transducer.

The procedure of experiment is as follows: When unitary discharges were found to occur in association of the respiratory movement, it was tried to determine to what phase of respiration they were related. If the encountered discharges showed no relation to the respiratory movement, a mechanical stimulus was applied to the thorax. In the present experiments, the mechanical stimulation to the thorax was such that the thorax, held with one hand, was squeezed moderately as if artificial respiration were carried out manually. This method of squeezing the thorax was also applied when no respiratory discharges were encountered while inserting the microelectrode.

After satisfactory recordings had been performed, a lesion about 0.2 mm in diameter was made electrolytically at the site of recording in the spinal cord. The spinal cord was fixed in MÜLLER'S solution, and every site of recording was checked microscopically in serial sections by the WEIGERT-PAL method.

RESULTS

One hundred and forty-four recordings were made at the level of the second cervical segment. The distribution of the sites which gave rise to impulse discharges related to respiration could be grouped in two portions in the spinal cord. One is the cuneate fascicle of the dorsal funiculus and the other the superficial layer of the dorso-lateral funiculus which coincides with the dorsal spinocerebellar tract. In view of the localization of the recording sites, the discharges were considered to originate from afferent fibers in the spinal cord. This was proved in several experiments by cutting the tract just cranial to the recording sites.

The encountered spike discharges were always initially positive. The peak amplitude of the spike amounted to 1.2 mV at the best recording. When a discharge, though occurring synchronously with the respiratory movement, was proved to be the cutaneous origin, it was not taken in this series of experiments.

(I) Units discharging synchronously with respiratory cycle.

Since the discharge recorded in the dorsal funiculus were quite similar in pattern to those in the dorso-lateral funiculus, activities in both funiculi were discussed altogether in one category. It was observed in most of the recordings that the respiratory unit in the spinal afferents continuously showed some background of discharge upon which the change in discharge frequency occurred in association with respiratory movements. Such a pattern of the unitary activity is exemplified by the record in Fig. 1. On the other hand, the units, activated in one phase of respiration and completely silent in the other phase, were very rare and were encountered in only five cases of 91 recordings. Examples of this type of
The patterns of respiratory discharges could be classified according to the phases of respiration during which activation occurred. Two types were found, an inspiratory discharge and an expiratory one. Each of them was divided into three subgroups (Fig. 4). Among the inspiratory discharges of the afferents, the first subgroup is the slow increment type which gradually increases the frequency of impulse discharge upon the beginning of inspiration and maintains the maximum frequency during inspiration, with the abrupt return to the previous level of activity at the onset of the expiration. This type was observed in 61 per cent of the cases. The second subgroup is the steady type in which activity increases abruptly to the maximum at the onset of the inspiration, and subsides abruptly to the previous level at the onset of expiration. This type was seen in 26 per cent. The last one is the slow increment-decrement type in which activity increases and decreases gradually. This was seen in 13 per cent of the cases. Among the afferents which were activated in the expiratory phase, there are the following subgroups: i) the rapid increment type in which activity increases abruptly at the onset of the expiration and, maintaining its maximum during the pause, subsides gradually to their previous level as the inspiration is started; ii) the steady type which shows a sudden increase in activity and subsides abruptly to the previous level at the onset of the inspiratory phase; iii) the slow increment-decrement type in which activity increases and decreases with gentle gradients. The frequencies of occurrence of three expiratory subgroups were 56%, 31% and 13% in the rapid increment type, the steady type and the slow increment-decrement type, respectively.
Fig. 4. (Left) Types of activity patterns of respiratory afferents. Thin line indicates respiratory phase, inspiration upwards. Thick line indicates frequency diagram of impulses. A, inspiratory discharge. B, expiratory discharge.

Fig. 5. (Right) Discharge evoked by pressure on thoracic wall recorded in second cervical segment. Onset of pressure on thoracic wall is indicated by arrow. Sweep interval, 1 sec.; time, 0.1 sec.

(II) Units which respond to passive distortion of the thoracic wall.

As seen in Table 1, 53 cases of total 144 recordings (37%) were found to respond only to distortion of the thorax. Discharges in this category were slowly

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adapting in nature and not modified by the normal respiratory movements (Fig. 5). It is to be noted that the respiratory discharges as discussed in the previous item also responded to passive distortion of the thoracic wall with discharges adapting slowly. Statistical representation of all the picked-up discharges will be found in Table 1.

FIG. 6.

FIG. 7

FIGS. 6 and 7. Distribution of points showing respiratory discharge in second cervical segment. Solid circle, inspiratory discharge. Open circle, expiratory discharge.
(III) Topographical arrangement of respiratory afferents in the spinal cord.

In the second cervical segment, the respiratory afferents encountered in the dorsal funiculus were observed mostly near the medial border of the cuneate fascicle (Fig. 6). Far less numbers of afferents were encountered in the lateral area of the fascicle, and some of them were found to be activated by stimulation of pectoral muscles or muscles surrounding the axillar cavity. The distribution of inspiratory and expiratory afferents intermingled with each other, and any difference in localization could not be observed between them.

Since the distribution of afferents in the dorso-lateral funiculus appeared to be the same between the left and right sides, the results were combined and shown in a single diagram of one lateral funiculus (Fig. 7). This distribution of the sites of recording coincides with the area of the dorsal spinocerebellar tract\(^1\).

The sites of afferents responding only to passive distortion of the thoracic wall were shown in Fig. 8. Their distribution appeared to be quite similar to that of the respiratory afferents as indicated in Figs. 6 and 7.

![Fig. 8. Distribution of points in second cervical segment responding only to passive distortion of thoracic wall.](image)

DISCUSSION

Concerning the afferent impulses essential for maintaining the normal rhythmic respiration of animals decerebrated at the midcollicular level, many works have pointed to the vagal afferents arising from stretch receptors of the lung. COOMBS\(^3\) was the first who described the role of dorsal root afferents in control of respiratory movements. She found that section of the dorsal roots of the thoracic and cervical spinal nerves resulted in a diminution or cessation of active costal...
respiration. Thus the spinal sensory inflow seems to be indispensable for maintaining the normal respiratory movements. Such spinal sensory inflow will find its electrophysiological correlate in the spike discharge which we recorded from upper spinal segments in association with the respiratory movement.

Respiratory afferents encountered in the dorsal funiculus distributed mostly near the border of the cuneate fascicle. This portion consists of afferent fibers from thoracic dorsal roots. A smaller number of respiratory afferents were found in the lateral part of the cuneate fascicle which consists of afferent fibers from cervical dorsal roots. This may suggest that not only thoracic afferents, but cervical ones also contribute to respiratory mechanism as shown by Coombs\(^3\). It is classically known that the cuneate fasciculus has a relay station at the dorsal funiculus nucleus which lies in the vicinity of the bulbar respiratory center. It is reasonable to consider that the arcuate fibers and the lemniscal system send some branches to the respiratory center. This idea can be supported by the recent neurophysiological findings that neurons in the brain stem reticular formation receive convergence of various sensory afferents\(^1,11\).

The recordings in the lateral funiculus in the present experiments were less in number as compared with those in the dorsal funiculus. They were, however, found exclusively included in the region of the funiculus occupied by the dorsal spinocerebellar tract. One may argue that the dorsal spinocerebellar tract may be a throughway connecting the Clarke’s column and the cerebellum, having no relation to the bulbar reticular formation. This is not true. Since Coombs\(^3\) showed a spinocerebellar relay in the bulbar reticular formation, we can safely suppose that the afferent impulses conducted through the dorsal spinocerebellar tract may preserve a possibility for affecting activity of the respiratory center lying in the bulbar reticular formation. Actually, Gesell, Bricker and Magee\(^5\) found respiratory discharges in the brachium conjunctivum and suggested a contribution of the cerebellum to the respiratory act. We suppose that the respiratory sensory discharge climbing up the dorsal spinocerebellar tract may also serve for the integrative action of the cerebellum in respiration.

Respiratory discharges encountered in spinal afferents were classified according to the phases of the respiratory cycle, inspiratory discharge and expiratory one. With respect to the change in the discharging frequency associated with the respiratory movement, each type of discharge could be divided into three subgroups. The most frequently encountered subgroup in the inspiratory discharge was the slow increment type in which activity builds up slowly and subsides abruptly. The one in the expiratory discharge was the rapid increment type in which activity builds up suddenly and subsides slowly. These two kinds of pattern of discharges appear to be the types encountered most frequently not only in respiratory muscle\(^6\), but also in the bulbar reticular formation\(^4,6,7\).

It is worth noting that most of picked-up discharges were found to be continuous without any appreciable “silent period”. The only discernible change
in discharging frequency was that associated with respiration. These respiratory afferents were also activated by passive distortion of the thorax, suggesting that the respiratory afferents in the spinal cord originate from proprioceptive receptors of mostly the thoracic wall. The afferents whose discharges could be induced only by passive distortion of the thorax are considered to originate in the same proprioceptive receptors of the thoracic wall as in the case of respiratory afferents. If the respiratory movements is big, this type of afferents is supposed to be recruited in the regulation of respiratory cycle.

SUMMARY

1. In a series of intercollicular decerebrated cats, the spinal cord at the level of the second cervical segment was explored with a microelectrode in search for neuronal afferent discharges synchronous with the respiratory movement.
2. Respiratory discharges could be encountered in the cuneate fascicle and in the portion corresponding to the dorsal spinocerebellar tract.
3. Most of the respiratory discharges were found continuous without appreciable silent period, but the frequency of discharge varied in different phases of the cycle. Discharges were classified according to the phase of the cycle during which they occurred. Two types were found, inspiratory and expiratory. Each of them was again divided into three subgroups. Arranged in the order of frequent occurrence, the following types were obtained. A) Inspiratory discharge: i) the slow increment type in which activity builds up slowly and subsides abruptly, ii) the steady type in which constant level of high activity appears suddenly with inspiration, iii) the slow increment-decrement type in which activity builds up and subsides slowly. B) Expiratory discharge: i) the rapid increment type in which activity builds up suddenly and subsides slowly, ii) the steady type in which activity is periodically maintained at a constant high level, iii) the slow increment-decrement type in which activity slowly builds up and subsides.
4. Numerous units were found whose discharges were elicited only by passive distortion of the thorax. This type of discharges was considered to be of the same origin as the respiratory discharges.

We are deeply indebted to Dr. Kitsuya IWAMA, whose advice and helpful criticism are acknowledged with gratitude and appreciation.

REFERENCES


