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ANATOMICAL AND FUNCTIONAL HETEROGENEITY OF THE MEDULLARY RESPIRATORY NEURONS

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Abstract. In a systematic study we attempted to activate the respiratory neurons antidromically by stimulating the spinal cord and the vagus. This has led to an anatomical and functional classification of the neurons: bulbo-spinal neurons whose axons enter the spinal cord, vagal motoneurons, propriobulbar neurons which cannot be activated antidromically. We used simultaneously other tests (localization, response to Hering–Breuer reflexes or to cortical stimulation) which gave positive results. Two groups of respiratory neurons were apparent: a dorsal respiratory nucleus with only inspiratory bulbo-spinal neurons and a ventral respiratory nucleus with inspiratory and expiratory bulbo-spinal neurons, vagal motoneurons and propriobulbar neurons. The bulbo-spinal neurons and some propriobulbar neurons are normally related to Hering-Breuer reflexes evoked by lung inflation or deflation, but the recurrent laryngeal motoneurons or fibres and some propriobulbar neurons can give a paradoxical response to lung inflation. By cortical stimulation the propriobulbar neurons show only an arrest of their discharge, whereas the bulbo-spinal neurons give a burst of spikes followed by an inhibition. These results led to the following conclusions: (i) the propriobulbar neurons do exist; (ii) some neurons classified as medullary respiratory neurons have not really a ventilatory function: they belong to the laryngeal system; (iii) it is fundamental to use systematically the antidromic test and some other ones to know exactly the hodological situation of the neurons in the respiratory complex.

INTRODUCTION

Neurons in the medulla which give bursts of discharge having the same rhythm as those of the phrenic fibres are called "respiratory neurons". In fact, it is known from different studies, that they do not constitute a homogeneous population and that it is possible to classify them in many types if we consider different features: anatomical localization and pattern of discharge (most of the workers), response to Hering–Breuer reflexes (Baumgarten and Kanzow 1958, Cohen 1969) and effect of end-tidal carbon dioxide tension (Cohen 1968).

The purpose of our experiments is to obtain a classification of the respiratory neurons based predominantly upon the possibility of activating them antidromically by stimulation of the spinal cord or vagus nerve. We believe that this test, used for the first time by Nakayama and Baumgarten (1964), is fundamental, because it gives an accurate idea of the hodological localization of the neurons into the "respiratory centres". Other tests used simultaneously gave complementary results.

More details can be found in our previous publications (Planche et al. 1970, Bianchi 1971, Bianchi and Barillot 1971).

METHODS

Our studies were made in cats either anaesthetised (chloralose urethane or Dial) or conscious (section of the spinal cord at the thoracic level). All the animals were paralysed by gallamine and artificially ventilated in conditions giving an end-tidal CO_2 between 4 and 5%. Both vagus nerves were generally cut in the neck, but of course they were kept intact when we secured a Hering–Breuer reflex. The electrical activity of single medullary neurons was recorded extracellularly with floating tungsten microelectrodes (Koepchen and Langhorst 1967). We recorded also the global neurogram from the central end of a cut phrenic nerve root.

It is well known that, when an electrical stimulus is applied to an axon, the generated action potential runs antidromically towards the cell body and can invade it. Four criteria were used to prove the antidromic origin of the activation (Fig. 1):

1. Identity of shape of antidromic and spontaneous (orthodromic) potentials;

2. Shortness and approximative constancy of the antidromic spike latency;

3. Ability for a neuron to respond to each stimulus at rates of 100/sec and more;

4. Collision between antidromic and spontaneous potentials; thus a potential is an antidromic one if it does not appear when a spontaneous potential precedes the stimulus within a brief interval; this last test is the most important one.

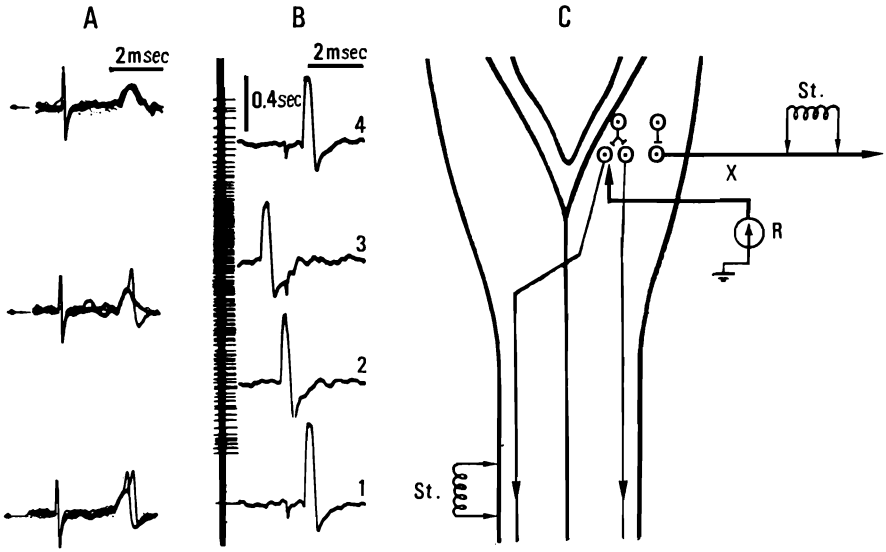

Fig. 1. Antidromic activation of medullary respiratory neurons. *A*, antidromic responses of bulbo-spinal neurons after cord stimulation at 100/sec showing blockage of potential at axo-somatic junction. *B*, inspiratory discharge of a bulbo-spinal neuron (vertical sweep) and interaction between antidromic (*1* and *4*) and spontaneous (*2* and *3*) potentials (horizontal sweeps). *C*, diagram of experimental procedure. (*see* Bianchi 1971).

RESULTS SUPPLIED BY THE TESTS

1. Antidromic activation

The antidromic test permits us to distinguish three types of respiratory neurons:

Bulbo-spinal neurons which are antidromically activated by stimulation of the spinal cord; that is to say that their axons have a spinal destination;

Vagal motoneurons which are antidromically activated by stimulation of the vagus or of the recurrent laryngeal nerve: they innervate the laryngeal muscles;

Propriobulbar neurons which cannot be antidromically activated by either stimulation and have perhaps short axons which lie wholly within the bulb.

2. Localization (Fig. 2)

The active sites have been accurately marked by electrocoagulation. Two groups of respiratory neurons are apparent. The first, a *dorsal respiratory nucleus,* lies just rostral to the obex and ventrolateral to the

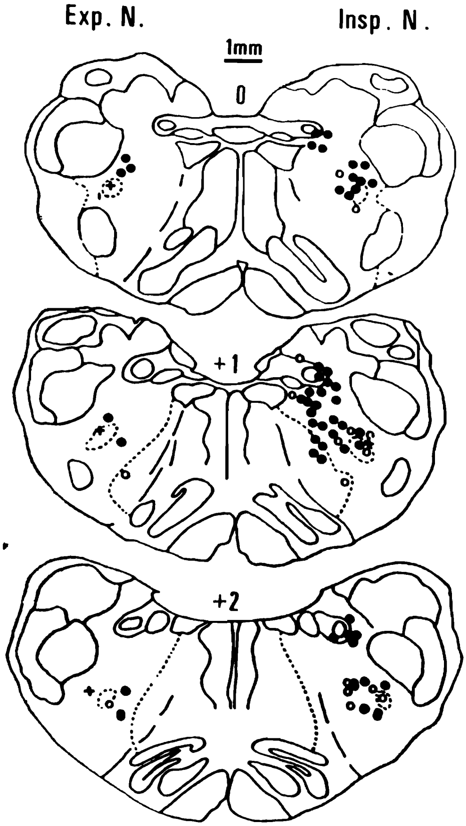

Fig. 2. Frontal sections of the medulla of the cat showing localization of the respiratory units at level of obex (*0*), 1 mm and 2 mm rostral to the obex (+1 and +2). All the inspiratory units are plotted on the right and all the expiratory units are plotted on the left. ●, bulbo spinal neurons; o, propriobulbar neurons; +, vagal motoneurons (*see* Bianchi 1971).

solitarius complex. It was first studied by Baumgarten, Baumgarten and Schaeffer (1957) and includes only inspiratory neurons. The second, a *ventral respiratory nucleus,* quantitatively more important, lies in the ventrolateral reticular formation, close to the nucleus ambiguus. This ventral nucleus includes inspiratory and expiratory neurons.

3. Hering–Breuer reflexes

The Hering–Breuer reflexes were induced by airway occlusion and lung inflation or deflation. Low "encephale isolé" or dial anaesthetised cats were used. The Hering–Breuer reflex produced by lung inflation gives two types of responses on respiratory neurons: responses in the same direction as the Hering–Breuer reflex (inspiratory neurons inhibited and expiratory neurons facilitated) and responses in the opposite direction to the Hering–Breuer reflex (inspiratory neurons facilitated and expiratory neurons inhibited). These effects have been previously described by Cohen (1969). For the sake of simplification, they will be named respectively normal and paradoxical effects.

4. Stimulation of cerebral cortex

By stimulation of different regions of the cerebral cortex with single electric shocks, we obtain in some cases the occurrence of a burst of discharge, often followed by an arrest of the spontaneous discharge. In other cases, we observe only the arrest of the discharge.

BULBO-SPINAL NEURONS

Probably, bulbo-spinal neurons control, directly or by the way of interneurons, the motoneurons driving the thoracic respiratory muscles. Thus, it is not surprising that in our experiments, all the bulbo-spinal neurons, either inspiratory or expiratory, give normal responses to the Hering–Breuer reflex.

These neurons are distributed in two groups. The first group is in the dorsal respiratory nucleus which seems to include only inspiratory neurons of this type. The neurons of the second group are found in the ventral respiratory nucleus; they are either inspiratory or expiratory, and are mixed with other types (motoneurons, propriobulbar neurons).

The distribution of the inspiratory bulbo-spinal neurons into two groups is not only a matter of anatomical localization. It seems to correspond also to a functional differentiation: (i) neurons of the dorsal group have a pattern of discharge very similar to that of the phrenic nerve, and their axons usually end at the 8th cervical level or above. This suggests that they are concerned mainly with the command of phrenic motoneurons; (ii) neurons of the ventral group, in contrast, show various types of discharge and their firing is not necessarily parallel to that of the phrenic nerve. Often their axons end in the spinal cord below the cervical level, i.e. caudal to the phrenic nucleus. So they are probably more important that those of the dorsal group for the command of the motoneurons driving the intercostal muscles.

Some differences between the two groups of inspiratory neurons appear also when we stimulate the cerebral cortex by single shocks. The latency of the burst of discharge is 10 msec for neurons in the ventral nucleus. In the dorsal nucleus, the response latency is more variable, with 4–10 msec for some neurons, and 25–30 msec for others.

The expiratory bulbo-spinal neurons show a pattern of discharge of the same type as that of the expiratory intercostal fibres studied by Sears (1964).

VAGAL MOTONEURONS

These motoneurons belong certainly to the nucleus ambiguus. Besides the antidromic test, the *expiratory* vagal motoneurons can be easily distinguished from the bulbo-spinal neurons if one considers their pattern of discharge: into the burst, a decreasing frequency is observed, instead of increasing. The pattern of discharge of *inspiratory* motoneurons is not typical.

Since these neurons send their axons into the recurrent laryngeal nerve, we can study their response to the Hering-Breuer reflex by recording the electrical activity of either single fibres of the recurrent laryngeal nerve or medullary neurons. In both experimental conditions we observe that all the expiratory vagal motoneurons give a paradoxical response. For the inspiratory neurons we have only at this time results dealing with single laryngeal fibres in cats under Dial anaesthesia: 60% of them gave a paradoxical, and 40% a normal response (Fig. 3).

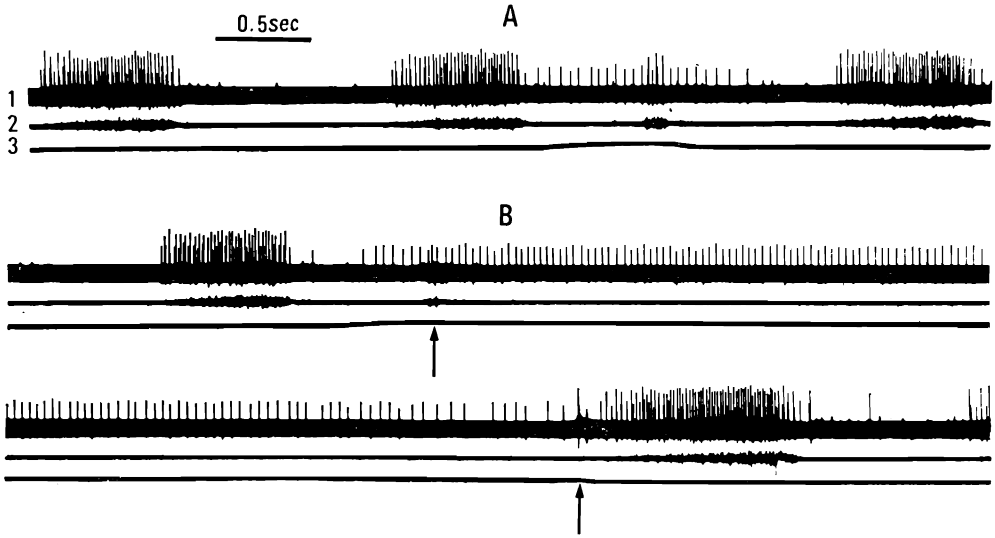

Fig. 3. Two inspiratory activities recorded simultaneously from a small bundle of recurrent laryngeal fibres. *A*, control; *1*, fibre activity; *2*, phrenic discharge; *3*, tracheal pressure (inflation upward). *B*, continuous tracing, tracheal occlusion at peak of inflation (indicated by arrow): the small spike activity remains firing (paradoxical reflex) whereas the largest one is depressed (normal reflex).

PROPRIOBULBAR NEURONS

Propriobulbar neurons are located in the ventral respiratory nucleus. They are either inspiratory or expiratory. Two main problems have to be examined: the reality of their existence, and their function.

1. Proofs of their existence

These neurons are identified on the basis of a negative result: the lack of possibility of antidromic activation. We could think that it is due only to technical difficulties. This is not our opinion, for the following reasons:

1. In our experimental conditions, propriobulbar neurons do not exist in the dorsal respiratory nucleus, but constitute nearly half of the cells of the ventral nucleus. It is difficult to think that technical difficulties for an antidromic activation exist only for this nucleus.

2. The ratio of bulbo-spinal to propriobulbar neurons varies through different transversal sections of the medulla: the propriobulbar neurons are more numerous in the oral region. This feature suggests a differential localization.

3. The proportion of the different types of neurons classified on the basis of their discharge pattern (early, late, phase spanning, tonic dis-

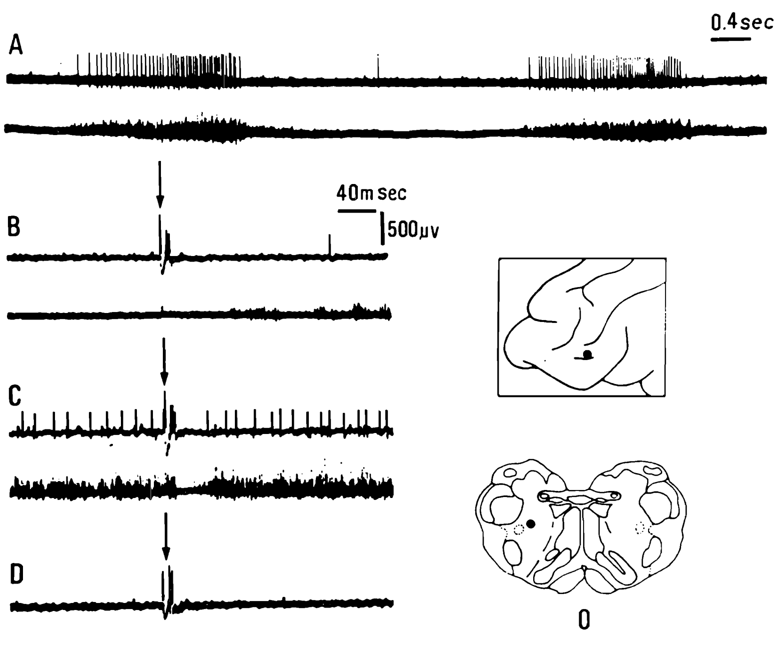

Fig. 4. Response of an inspiratory bulbo-spinal neuron after cortical stimulation. *A*, control; *B*, stimulation at the beginning of an inspiratory phase: excitatory effect; *C*, stimulation during an inspiratory phase: excitatory effect followed by an inhibitory one; *D*, stimulation during an expiratory phase. The diagrams on the right show the stimulation site on the cerebral cortex and the localization of the unit in the medulla oblongata (obex level).

charge) is not the same among bulbo-spinal and propriobulbar neurons. The probability that these two types of neurons belong to the same population is less than 0.01 (X^2 test).

4. The bulbo-spinal and propriobulbar inspiratory neurons respond in different ways when the cerebral cortex is stimulated by a single electric shock: the propriobulbar neurons show only an arrest of their discharge, whereas the bulbo-spinal give a complex response: a burst of spikes followed by an inhibition of spontaneous activity (Fig. 4).

2. Function of these neurons

At present we have not much information on this. However, it is possible to make some suggestions on the basis of the responses exhibited by the expiratory neurons during the Hering–Breuer reflex. We have two groups of propriobulbar neurons: (i) some of them give a normal response, like the bulbo-spinal neurons. They have also the same pattern of discharge, with a steady or increasing spike frequency; (ii) the others have a paradoxical behaviour during the Hering–Breuer reflex, like the vagal motoneurons, the pattern of discharge of which they exhibit, i.e. an early occurring discharge, with a decreasing spike frequency (Fig. 5).

Thus, all the propriobulbar neurons do not seem to have the same function: probably some of them are connected to the bulbo-spinal neu-

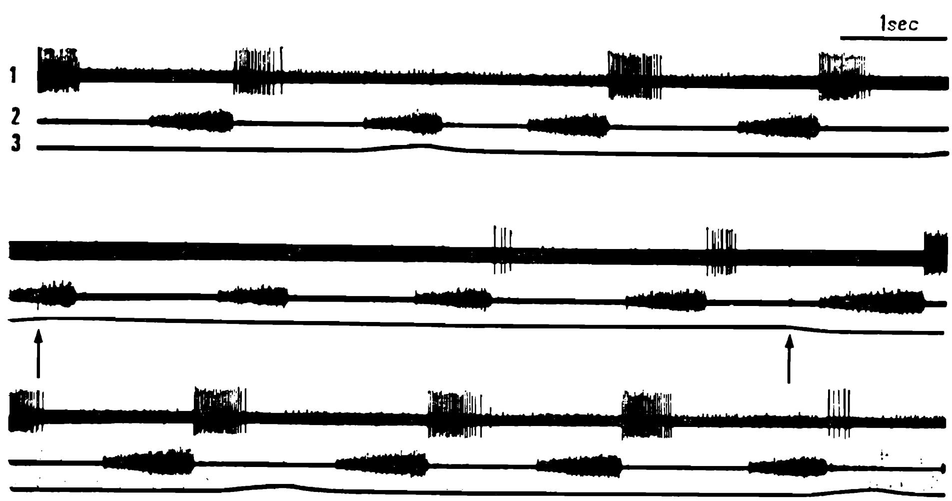

Fig. 5. Expiratory propriobulbar neuron depressed by lung inflation during tracheal occlusion at peak of inflation. Continuous tracing. *1*, medullary activity; *2*, phrenic discharge; *3*, tracheal pressure (inflation upward).

rons and are responsible for the movements of the chest, whereas others are connected to the vagal motoneurons and belong to the system of command of laryngeal muscles.

GENERAL CONCLUSIONS

We wish to emphasize two main ideas:

1. Generally, the expression "medullary respiratory neurons" is used for all the neurons identical to those we previously described. This is not right because the neurons commanding the laryngeal muscles are not directly concerned with the command and regulation of the thoracic muscles. Of course, they can have an indirect action through very long loops, but they are not truly respiratory neurons.

Special care has to be taken also with the bulbo-spinal neurons: at present it is not possible to state if they have a function in the genesis of the respiratory rhythm, because we do not know if they have a recurrent axon able to secure a feedback.

2. More generally, if we wish to know how the bulbar respiratory centres work, it seems necessary to search for the exact meaning of the neurons studied, by systematic use of different tests. The antidromic test is a fundamental one, but it has to be completed by others. Unfortunately, with this type of experiment, the work becomes time consuming and it is possible to study only a few neurons in each animal. We do think that, in the future, it will be absolutely necessary to work in such a way to decrease as much as possible the number of speculations.

Note added in the proofs

Results can be found also in the following papers:

BARILLOT, J. C. and BIANCHI, A. L. 1971. Activité des motoneurones laryngés péndant les réflexes de Hering–Breuer. J. Physiol. (Paris) 63: 783–792.

PLANCHE, D. and BIANCHI, A. L. 1972. Modification de l'activité des neurones respiratoires bulbaires provoquée par stimulation corticale. J. Physiol. (Paris) 64: 69–76.

REFERENCES

BAUMGARTEN, R. von, BAUMGARTEN, A. von and SCHAEFFER, K. P. 1957. Beitrag zur Lokalisationsfrage bulboreticulärer respiratorischer Neurone der Katze. Pflüg. Arch. 264: 217–227.

BAUMGARTEN, R. von and KANZOW, E. 1958. The interaction of two types of inspiratory neurons in the region of the tractus solitarius of the cat. Arch. Ital. Biol. 96: 361–373.

BIANCHI, A. L. 1971. Localisation et étude des neurones respiratoires bulbaires.

Mise en jeu antidromique par stimulation spinale ou vagale. J. Physiol. (Paris) 63: 5–40.

BIANCHI, A. L. and BARILLOT, J. C. 1971. Modification de l'activité des neurones respiratoires bulbaires an cours des réflexes de Hering–Breuer. J. Physiol. (Paris) 63: 116*A*.

COHEN, M. I. 1968. Discharge patterns of brain stem respiratory neurons in relation to carbon dioxide tension. J. Neurophysiol. 31: 142–165.

COHEN, M. I. 1969. Discharge patterns of brain stem respiratory neurons during Hering–Breuer reflex evoked by lung inflation. J. Neurophysiol. 32: 356–374.

KOEPCHEN, H. P. and LANGHORST, P. 1967. Verbessertes Verfahren zur Einführung "reitender" Mikroelektroden ins Gewebe. Pflüg. Arch. 294: 65–67.

NAKAYAMA, S. and BAUMGARTEN, R. von 1964. Localisierung absteigender Atmungsbahnen im Ruckenmark der Katze mittels antidromer Reizung. Pflüg. Arch. 281: 231–244.

PLANCHE, D., BIANCHI, A. L. and DUSSARDIER, M. 1970. Contrôle cortical de la respiration: étude au niveau des neurones bulbaires. J. Physiol. (Paris) 62 (Suppl. 3): 430–431.

SEARS, T. A. 1964. Investigations on respiratory motoneurones of the thoracic spinal cord. Progr. Brain Res. 12: 259–273.

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