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DISTRIBUTION OF RESPIRATORY MODULATED UNITS IN THE PONS

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The existence of pontine respiratory units, which would support the localization of part of the respiratory centre in the pons (Cohen and Wang 1959, Hukuhara et al. 1969, Cohen 1970), has given rise to much controversy (Salmoiraghi and Burns 1960, Salmoiraghi 1963, Batsel 1964, Carregal et al. 1967). When we previously designated the pneumotaxic system as located in the dorso-lateral upper pons (Bertrand and Hugelin 1971), one of our main arguments was based on our ability to record from closely packed respiratory units in this area. Since then, we have started a series of recording experiments, with the purpose to make a systematic survey of the upper and middle pons, looking for pontine structures containing cells with a respiratory modulation.

This kind of experiments required the maintenance of the animals in the steadiest possible state, so as to compare one animal to another; it required also recordings from different areas in the same animal. To meet the first condition, we resorted to a special type of "encéphale isolé" preparation, which is very suitable for respiratory neurophysiology. It is the cat, whose spinal cord is cut at the C_7 level, vagotomized, curarized with gallamine and maintained in normocapnia with appropriated artificial ventilation. The phrenic nerves are cut, and the phrenic discharge recorded as a monitor of the spontaneous breathing rhythm. Such preparations enabled us to extend the recording sessions up to 36, and sometimes 50 hr, during which arterial PCO_2, PO_2, pH and fluid balance were checked at regular intervals. The fluid intake consisted of a perfusion, 50% saline, 50% glucose, with gallamine, at the speed of 4 ml per hour.

To meet the second condition, we tried to improve our recording methods: first we increased the signal to noise ratio by using glass insulated tungsten microelectrodes; then we resorted to a computer to filter the noise and sort out the spikes, thus increasing the number of cells we were able to record in a given area. In addition, two microelectrodes were inserted simultaneously, thus increasing the number of the explored areas in an individual cat.

At the present time, we have results in 19 cats, in which about 3,000 cells were recorded. The regions that were explored are shown on the histological sections in Fig. 1. In order to avoid the bony tentorium, the electrodes were tilted to a 45° posterior angle to the Horsley–Clarke vertical plane; the histological sections were made with the same angle. In the middle section is seen the pneumotaxic system, in which we include the nucleus parabrachialis medialis, the adjacent part of the brachium conjunctivum, and the Kölliker–Fuse nucleus, situated laterally and more caudally. In the lower section is seen the trigeminal system whose motor nucleus and main sensory nucleus were explored; the mesencephalic root of the fifth nerve is seen on all the sections.

Some punctures were made in auditory nuclei: inferior colliculus and lateral lemniscus. The mesencephalic and pontine reticular formations were investigated and also a few other areas, such as the locus caeruleus, once believed to be the site of the pneumotaxic centre (Johnson and Russel 1952).

In order to achieve a greater accuracy in the location of the recorded cells, each puncture was marked by two very restricted lesions (100 to 200 μ in diameter); one at the bottom of the electrode track, the other 3 to 5 mm above; examples can be seen in Fig. 1. These two reference marks enabled us to calculate the shrinking due to formalin fixation, and to localize any given cell with a ± 75 μ precision.

The existence of a respiratory discharge pattern of a given unit was ascertained by establishing cycle-triggered time histograms giving the average time distribution of spikes in relation to the start of inspiration, most often for 30 cycles of preselected duration. Some of the histograms representing the main types of respiratory discharge pattern have been grouped in Fig. 2. Two histograms (Fig. 2, upper right) come from respiratory units recorded at the bulbar level, their discharge begins and ends abruptly during one of the two phases of the cycle. All the other histograms come from units recorded in various pontine structures. It can be seen that most of the pontine cells have a continuous firing, the firing rate increasing sharply during a given part of the cycle. According to the position of the peak frequency, the patterns fall into the categories already described by several authors (Cohen and Wang 1959, Cohen 1970),

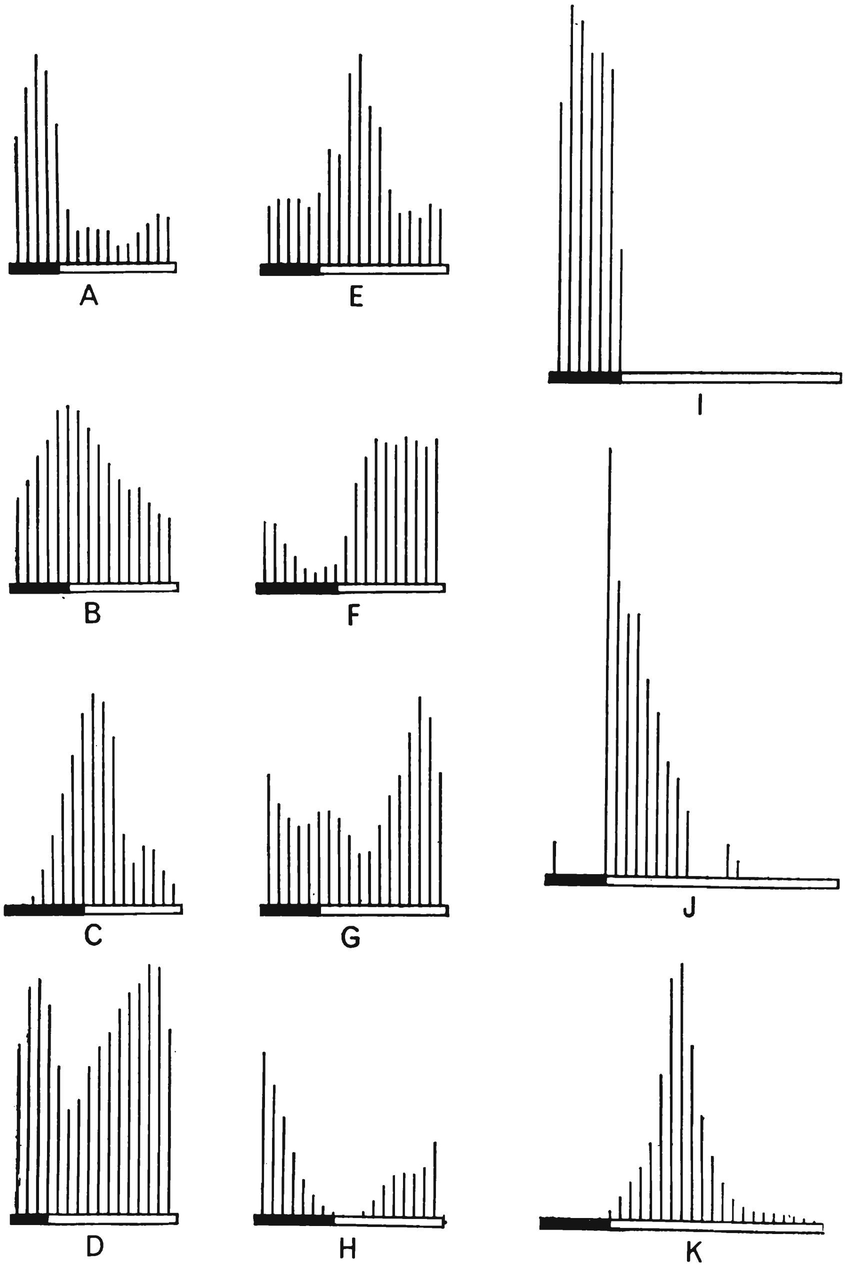

Fig. 2. Cycle triggered histograms of respiratory cells. *A–H,* recordings made in various pontine structures; *I, J,* recordings at the bulbar level; *K,* recording in the Vth motor nucleus. Explanations in text.

inspiratory, expiratory, and phase-spanning, either inspiratory-expiratory, or expiratory-inspiratory. One of these firing patterns (Fig. 2, bottom right) was to be found almost exclusively within the Vth motor nucleus area; it consists of a discontinuous discharge, with an early expiratory peak frequency.

We tried at first to classify the various pontine formations according to their abundance in respiration related units, as well as the type of firing pattern. As can be seen in Fig. 3, the distribution in each structure has been represented in a semicircle whose radius varies in direct ratio to the mean cellular density, i.e. the mean number of cells to be met in 1000 μ. The very high density (up to 17 cells in 1000 μ) that we found repeatedly in a few structures is probably due to the improvement of the signal to noise ratio, which allowed us to distinguish cells which otherwise would have been lost in the noise.

In the semicircles are represented the percentages of each type of cells recorded. These percentages enabled us to distinguish two groups among the pontine structures: in the first group we include five structures where more than 55⁰/₀ of the units have a respiratory modulation (Fig. 3, left, lst five). Two of them belong to the pneumotaxic system: the NPBM, 75⁰/₀, the adjacent part of the brachium conjunctivum, 63⁰/₀. We may emphasize that all the types of respiratory modulated units are represented [1]. The last three structures belong to the trigeminal system, i.e. the fifth motor nucleus (88⁰/₀ of respiratory units), its surrounding area (73⁰/₀) and the interneurons surrounding the sensory cells of the trigeminal mesencephalic root (58⁰/₀). But in each of these structures we find a striking predominance of a given type of respiratory cells, since in the motor nucleus, 71⁰/₀ of the units have an early expiratory pattern, and since a phase-spanning inspiratory-expiratory pattern is predominant (38⁰/₀) in the interneurons of the trigeminal mesencephalic root. The presence of so many respiratory cells and their specific firing pattern could be explained by the fact that the trigeminal system supplies accessory respiratory muscles and therefore comes into action at a given time during the respiratory cycle.

The second group contains nearly all the remaining explored areas (Fig. 3, middle and right) in which the percentage of respiratory units is low, most of the time less than 20⁰/₀. Among these regions, we find the posterior part of the brachium conjunctivum, the mesencephalic reticular formation and the locus caeruleus. We can probably discard the possibility that they play an important role in the origin of respiratory rhythm,

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[1] Their topographical organization has been discussed in the paper by Hugelin and Bertrand, this Symposium.

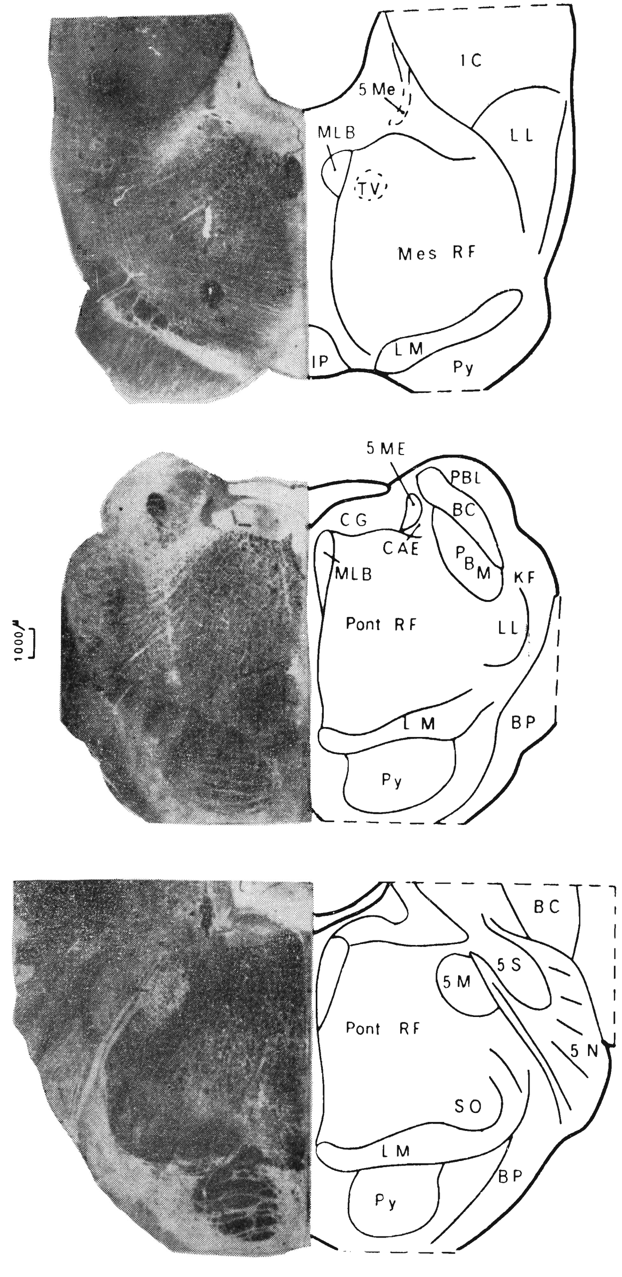


Fig. 1. Sagittal sections, inclined at a 45° angle to the Horsley–Clarke vertical plane. Abbreviations: BC, brachium conjunctivum; BP, brachium pontis; CAE, locus caeruleus; CG, central gray; IC, inferior colliculus; IP, nucleus interpeduncularis; KF, Kölliker–Fuse nucleus; LL, lemniscus lateralis; LM, lemniscus medialis; MLB, medial longitudinal bundle; MesRF, mesencephalic reticular formation; PontRF, pontine reticular formation; PBL, nucleus parabrachialis lateralis; PBM, nucleus parabrachialis medialis; Py, pyramidal tract; 5M, Vth motor nucleus; 5Me, Vth mesencephalic root; 5N, Vth nerve; 5S, Vth main sensory nucleus.

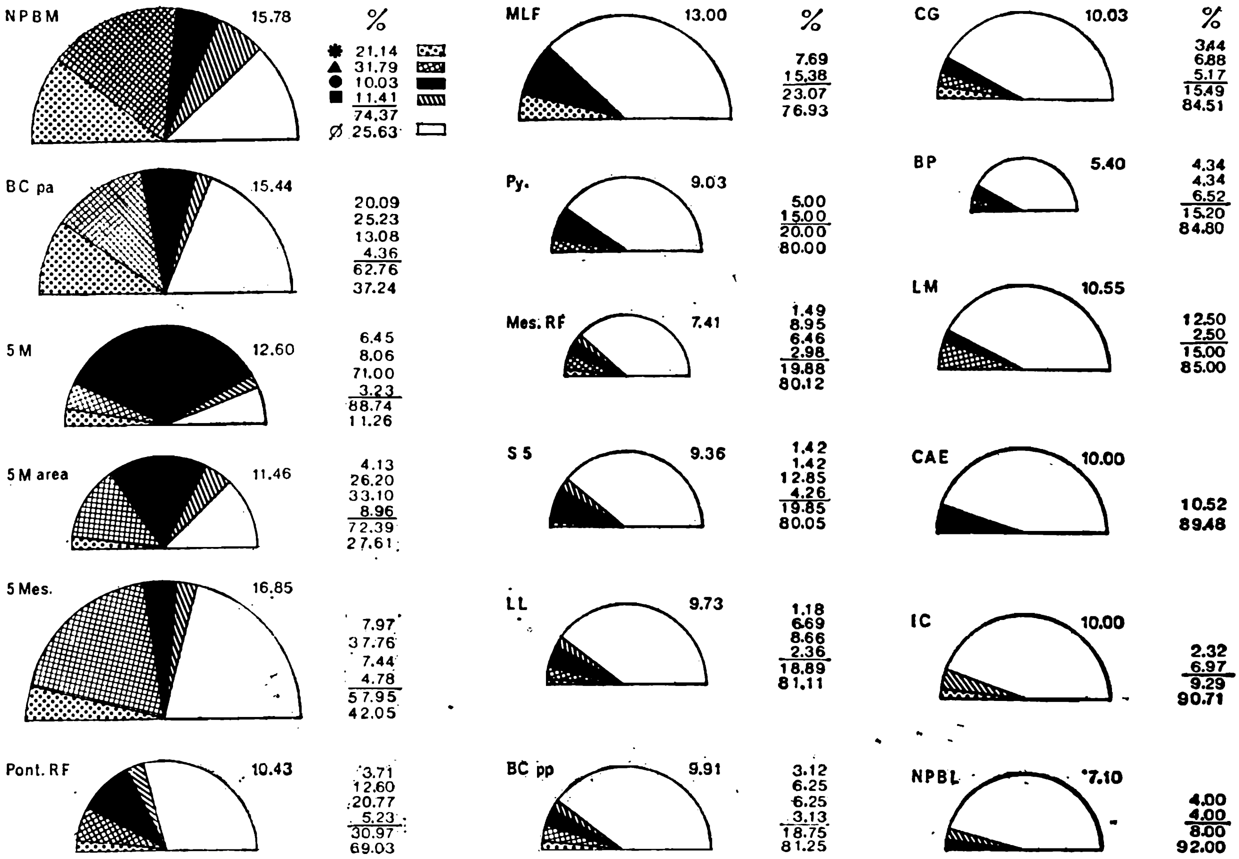


Fig. 3. Distribution of respiratory units in pontine structures. Abbreviations the same as in Fig. 1. Above the semicircles: mean cellular density. At the right of the semicircles: % of various types of cells; ★ and dotted areas: inspiratory. ▲ and checked areas: phase spanning IE. ● and black areas: expiratory. ■ and striped areas: phase spanning EI. Ø and white areas: non-modulated units.

but the question still arises as to the meaning of the presence of these few respiratory units.

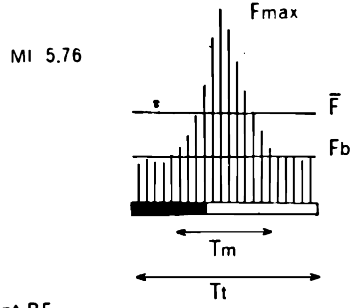
A second problem is brought up by the distribution in the pontine reticular formation (Fig. 3, bottom left), where 30% of the cells are respiratory units with all sub-types represented. This induced us to put the pontine reticular formation in a category by itself and to make further analysis of the total population. We chose four characteristics to classify the activity in the pontine regions and submitted their distribution to statistical tests; they are: (i) the mean cellular density, (ii) the mean respiratory unit density, (iii) the ratio of the mean respiratory unit density to the mean cellular density, and (iv) the mean respiratory modulation index. Since all the tests on all the characteristics led to the same conclusions, the results of only the last will be shown here (Fig. 4).

The respiratory modulation index was defined in order to ascertain the magnitude of the respiratory modulation of any given cell. We have seen that most of the pontine respiratory units (Fig. 4, top right) have a continuous firing activity, whose frequency increases during a certain portion of the respiratory cycle. The importance of the modulation index depends (i) on the duration of the frequency increase, compared to the length of the respiratory cycle and (ii) on the magnitude of the frequency increase, compared to a basal frequency which we had to calculate (Fig. 4, upper right).

This modulation index enabled us to divide the pontine cells in three groups. In the first one fall the units whose modulation index is over 0.70, which are considered as having a definite respiratory discharge pattern. The second group has an index between 0.70 and 0.55; the modulation is not so definite and the cells were not taken into account as respiratory cells. No modulation at all could be detected in the cells of the last group (index under 0.55). The values of the mean modulation index of each pontine structure only confirmed our first classification (Fig. 4*MI*). A first group contains the pneumotaxic system and some elements of the trigeminal system with a mean index above 1. In the second group falls the pontine reticular formation, whose mean index is 0.74 indicating the presence of a certain number of respiratory cells. In the third group the mean modulation index is very low, these structures contain few respiratory units.

Only the statistical analysis of the variance of the modulation index and the significance tests were to bring us further information (Fig. 4, middle and bottom right). It can be seen that the NPBM differs significantly from all the pontine formations but the trigeminal motor nucleus. The trigeminal motor nucleus differs significantly from all pontine formations but the NPBM, the Vth mesencephalic root and the brachium

	MI	SD log MI														
NPBM	1.40	1.12	NPBM													
BC pa	1.05	1.12	**	BC pa												
5M	1.22	1.02	0	0	5 M											
5Mes	1.07	1.10	**	0	0	5 Mes										
Pont.RF	0.74	1.07	***	***	**	***	Pont.RF									
LL	0.65	1.00	***	***	***	***	0	LL								
IC	0.52	1.10	***	***	***	***	0	0	IC							
BP	0.56	1.25	***	**	***	***	0	0	0	BP						
Mes.RF	0.52	0.84	***	***	***	***	***	*	0	0	Mes.RF					
CG	0.41	1.02	***	***	***	***	***	**	0	0	0	CG				
MLF	0.42	0.42	***	**	**	**	0	0	0	0	0	0	MLF			
NPBL	0.41	1.42	***	***	***	***	*	0	0	0	0.	0	0	NPBL		
BC pp	0.43	0.88	***	***	***	***	*	0	0	0	0	0	0	0	BC pp	
LM	0.38	0 96	***	***	***	***	*	0	0	0	0	0	0	0	0	LM
CAE	0.37	0.74	***	***	***	***	**	*	0	0	0	0	0	0	0	0



$$F^+ = \forall F > \bar{F}$$

$$\bar{F}_b = \bar{F} - \frac{\sum_1^n (F^+ - \bar{F})}{n}$$

$$MI = \frac{F_{max} - \bar{F}_b}{\frac{T_m}{T_t} \times \bar{F}_b}$$

Fig. 4. Respiratory modulation index of pontine units. Upper right: calculation of modulation index. *F̄*, mean frequency; *Fb*, basal frequency; *Fmax*, peak frequency; *Tm*, duration of modulation; *Tt*, cycle length. Abbreviations for pontine structures as in Fig. 1. MI, modulation index; SD, standard deviation. Middle and bottom right, significance tests.

conjunctivum. The pontine reticular formation differs significantly from many pontine structures including the mesencephalic reticular formation; it differs also significantly from the respiratory areas. There is no difference between the remaining pontine structures.

These results bring us enough arguments to make a few conclusions concerning:

1. *The NPBM area.* Its great cellular density, its high percentage of various respiratory cells, its high modulation index, its significant difference from all the other pontine areas strengthen the hypothesis of its primacy in the origin of respiratory rhythmicity.

2. *The trigeminal system.* We may distinguish it from the pneumotaxic area, relying on the exclusive discharge pattern of its cells to suppose that its role is restricted to the command of accessory respiratory musculature.

3. *The pontine reticular formation.* We may propose an hypothesis: the presence of only a limited number of well modulated cells suggests that it plays only a secondary role in the origin of rhythmicity. The diffuse projections of the reticular formation to many central structures could account for the presence of the few well modulated cells that are to be found scattered among them. In a more extensive view, it could explain the discrete respiratory modulation one may ascertain in some spinal reflexes, as well as in some activities of the autonomic system (for instance the splanchnic activity).

SUMMARY

A respiratory modulation was ascertained in tonic pontine cells after establishing cycle triggered histograms compared to the discharge of the phrenic nerve. Experiments were performed on 19 normocapnic cats, with a spinal cord section at C_7 level, curarized and vagotomized. 2974 cells were recorded with glass tungsten microelectrodes using a computer to filter the noise, select the spikes, reject respiratory cycles of unsuitable duration and establish histograms. A respiratory modulation index was calculated so as to compare all units.

According to the explored area, the mean cellular density (number of cells in a 1000 μ height) varies from 5 to 17. The percentage of respiratory cells is over 75, the mean modulation index is high in the pneumotaxic system (nucleus parabrachialis medialis) and in trigeminal areas controlling accessory respiratory musculature. 31% of respiratory units and a moderate modulation index are found in pontine reticular formation. Remaining pontine structures contain 8 to 20% respiratory units and a low mean modulation index.

Variance analysis of the modulation index shows the pneumotaxic and trigeminal system to differ very significantly from all other pontine areas. The pontine reticular formation differs significantly from both respiratory areas and other pontine formations. There is no significant difference between these remaining pontine structures.

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