NEURONAL ORGANIZATION OF THE CENTRAL RESPIRATORY MECHANISMS IN THE BRAIN STEM OF THE CAT

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Abstract. In order to obtain basic evidence on the neuronal organization and localization of the central respiratory mechanism in the brain stem of the cat, the following experiments were undertaken: reexamination of the physiological meaning of apneusis and the gasp; differences in stability of spontaneous burst activity of respiratory neurons in the brain stem reticular formation; changes of spontaneous firing pattern of bulbar respiratory neurons in the isolated brain stem of the cat; changes of firing pattern of a pontine and a bulbar respiratory neuron during continuous recording of both unitary activities after brain stem transection at the pontomedullary junction; responses of respiratory neurons to electrical stimulation of the spinal cord; responses of respiratory neurons to electrical stimulation of the central cut end of the vagus nerve; effects of pentobarbitone on unitary activity of pontine and bulbar respiratory neurons. Based on the experimental result a preliminary scheme of a neuronal network of the central respiratory mechanism consisting of four subsystems of respiratory neurons: a primary neuron system; a satellite neuron system; an input neuron system; an output neuron system.

There remain still many controversial points of view on the neuronal organization of the central respiratory mechanisms and the localization of the mechanisms in the brain stem. The neuronal organization which is primarily responsible for the genesis of the respiratory rhythm is also not fully understood (Wang and Ngai 1964, Wyss 1964). The following experiments were, therefore, undertaken in order to obtain basic findings for further analysis. A large number of neural and humoral factors are involved in the regulation of the respiratory control system. However, depending on the purpose of experimental analysis some artificial com-

partmentation of the respiratory control system is innevitable and would
be allowed within reasonable limits.

Single respiratory unitary discharges were identified on the basis of
phase relation to that of the phrenic nerve discharge (Fig. 1). Three main
populations of respiratory unitary discharges, inspiratory, expiratory and
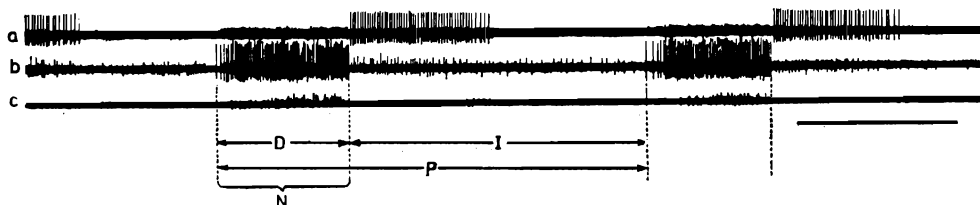another broader group not so clearly related to the respiratory phases,

Fig. 1. Simultaneous recording of an inspiratory (b) and an expiratory (a) unitary
discharge and the phrenic nerve efferent discharge (c) in a cat showing phase
relation between them. Vagotomized cat, paralysed and ventilated artificially. Short
horizontal bar on the right indicates 1 sec. D, duration of volley; N, number
of spikes in volley; I, interval between volleys; P, period of volley. (From Huku-
hara 1966a.)

were scattered and intermingled throughout the bulbar reticular forma-
tion in deeply anaesthetized (pentobarbitone) cats and in the bulbar and
pontine reticular formation of the unanaesthetized, immobilized and arti-
ficially ventilated cat (Fig. 2) (Hukuhara et al. 1969). Here arises an
important problem whether the functional significance of respiratory
neurons in the reticular formation which belong to the same populations
can be regarded as homogeneous or not, although the co-ordinative rela-
tion to the respiratory phases is similar (Hukuhara 1966ab). To obtain
more detailed findings on this essential question, further experimental
analysis of the functional significance of respiratory neurons in the reti-
cular formation was carried out.

1. Experimental methods

Experiments were performed in adult cats. The cats were maintained
by artificial ventilation with local anaesthesia and immobilization with
d-tubocurarine chloride (0.5–1.0 mg/kg/hr, i.v.) or gallamine triethiodide
(1.0–3.0 mg/kg/hr, i.v.) There is a controversy about the central effects
of d-tubocurarine and gallamine, injected intravenously, whether they
exert excitatory effects on the central nervous system (Ellis et al. 1952,
Halpern and Black 1968) or not (Sakuma 1959). In following experiments,
therefore, the intravenous dose of both muscle relaxants was minimized
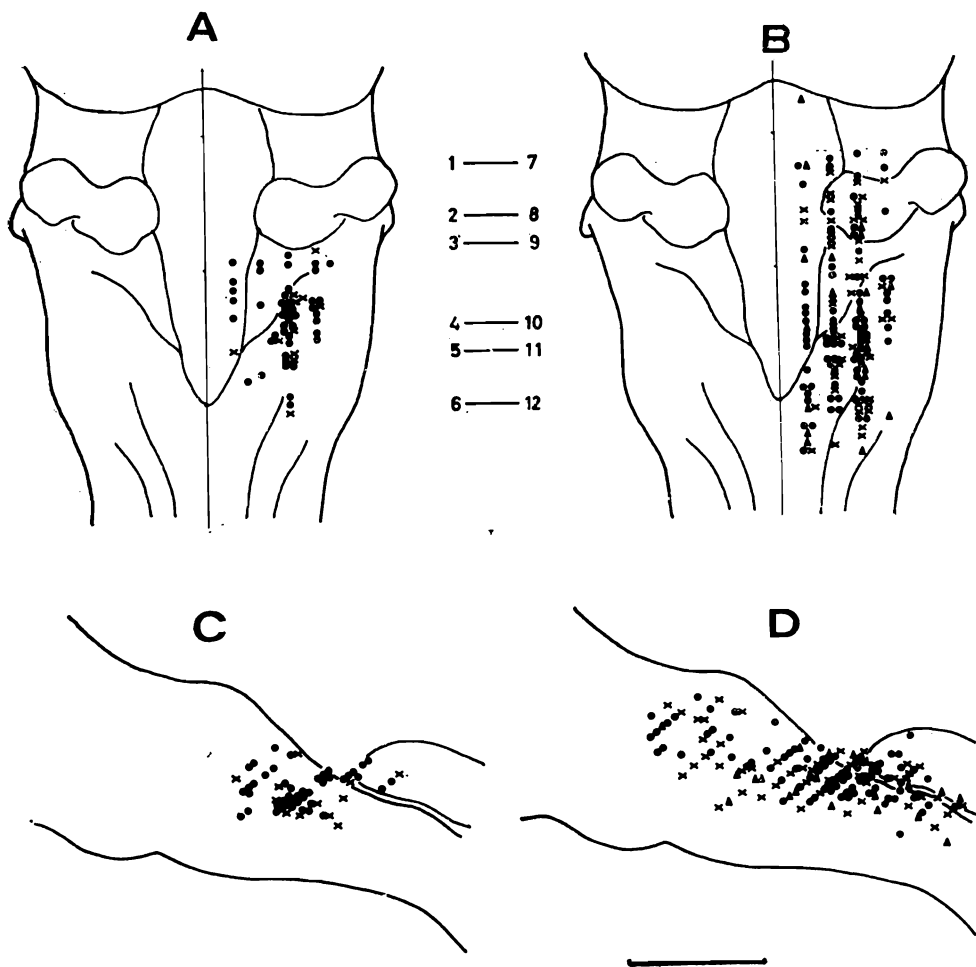as far as possible on the basis of findings by Cohen (1963) and Halpern

Fig. 2. Localization of respiratory neurons in dorsal projections and sagittal sections of the brain stem of the cat obtained by systematic exploration experiments. A and C: 14 anaesthetized cats; B and D: 15 cats paralysed, vagotomized and ventilated artificially. ●, X and ▲ indicate respectively sites where an inspiratory, an expiratory, and a phase-spanning unitary discharge were recorded. (From Hukuhara et al. 1969.)

and Black (1968). The vagi were left intact or were cut in different types of experiments. The arterial blood pressure was recorded from the femoral artery.

The efferent discharge of the phrenic nerve cut in the neck was recorded from its central end with a bipolar hook electrode. After exposure of the dorsal surface of the brain stem, unitary discharges of respiratory neurons were recorded using either a steel microelectrode (1–5 μ

tip diameter) which was insulated except for its tip (Green 1958) or a glass micropipette filled with 3 M KCl having 10–20 MΩ resistance. The data were recorded on magnetic tape for subsequent photography and computer analysis. Furthermore, each recorded spike potential was shown to arise from a cell soma and not from a fibre (Brookhart et al. 1950, Brock et al. 1952, Tasaki et al. 1954, Bishop 1964).

In some series of experiments the unitary spike potentials were processed through and amplitude discriminator to give a standard pulse corresponding to each spike, and subsequently the pulses were integrated and transformed to a pulse at different ratios (see 2 in Fig. 3).

2. Physiological significance of apneusis and gasp

The physiological meaning of the apneusis and gasp, which were defined by Lumsden (1923) on the basis of serial brain stem transections and subsequently regarded as the basic patterns in relation to the organization of the respiratory subcentres (Wang and Ngai 1964, Wyss 1964), has been reexamined (Hukuhara 1970b).

The phrenic nerve efferent discharge as an indicator of the overall output of central respiratory mechanisms and the bulbar respiratory unitary discharge were simultaneously recorded in vagotomized and immobilized cats. Ventilation was regulated by reference to the phrenic discharge and the corticogram of the motor area of the cerebral cortex, which change in close relation to arterial PCO_2 and PO_2 (Sakai et al. 1962, Abeles et al. 1964, Aoyagi and Piiper 1965). The effects of asphyxia due to cessation of artificial ventilation were investigated (Fig. 3).

The eupneic pattern of the phrenic (E) (Fig. 41) and the unitary discharge (Fig. 3A2) changed into manifold abnormal patterns after a lapse of time (mean, 122 ± 9 sec) after cessation of ventilation. Three basic and typical patterns were distinguished among various abnormal patterns. They were an apneustic (A) (Fig. 3B2 and Fig. 42), a gasp (G) (Fig. 3C1,2 and Fig. 44) and an intermingled (apneustic and gasp) pattern (A + G) (Fig. 3B1 and Fig. 43). These three patterns occurred in four types of sequences in the course from eupneic (E) to cessation of discharge (S) after evoking ventilation, in 178 experiments. These were E-A-(A+G)-G-S, E-A-G-S, E-(A+G)-G-S and E-G-S (Fig. 4). A common feature of these sequences was the gasp pattern appearing before the end of discharge.

There should be also noted: (i) inconsistency of the appearance of the apneustic pattern (23% of the experiments); (ii) a remarkable multiplicity of changes of the phrenic discharge patterns (there were various transitional patterns); (iii) the correspondence of pattern change between the
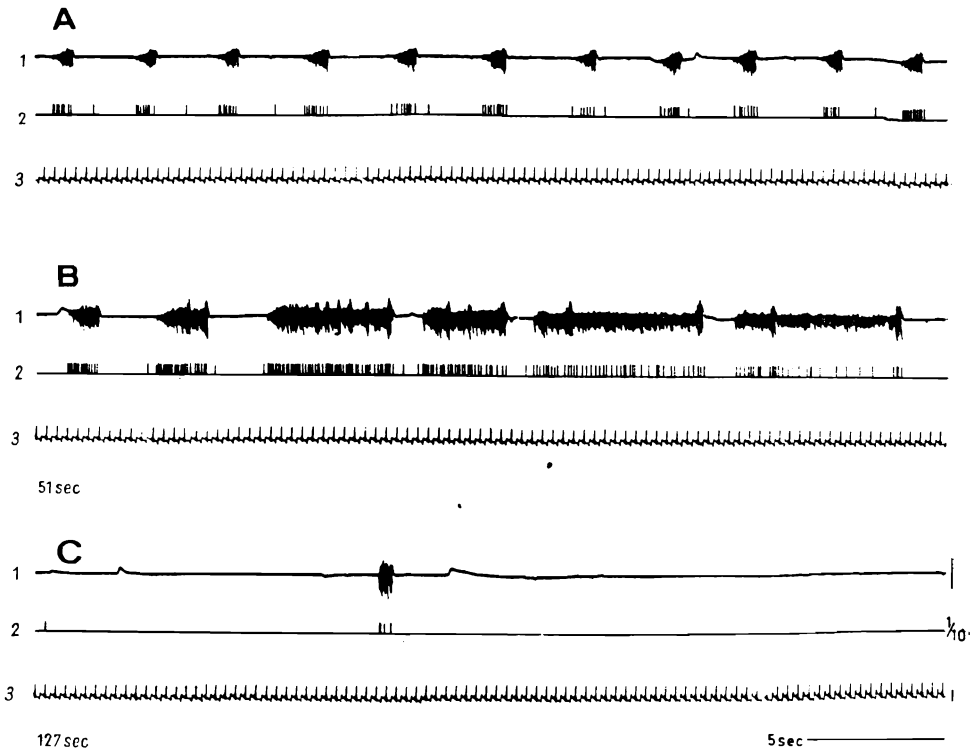
Fig. 3. Changes of discharge pattern of the phrenic nerve discharge and a bulbar inspiratory unitary discharge by asphyxiation. *1*, phrenic nerve discharge; *2*, inspiratory unitary discharge, 10 spikes of the unitary discharge were transformed to a pulse by a counting circuit device; *3*, electrocardiogram. *A*, before asphyxiation, *B*, 51 sec after cessation of artificial ventilation. The eupnoeic patterns of the unitary and the phrenic discharges change to an apneustic pattern; *C*, 127 sec after cessation of artificial ventilation. Note the gasp pattern in both discharges. Calibration: 50 μv for phrenic discharge, 500 μv for ECG.

phrenic nerve and the bulbar unitary discharges (inspiratory as well as expiratory); (iv) that the apneustic as well as the gasp pattern can be readily evoked without brain stem transection. Central mechanisms responsible for these abnormal patterns and their significance in relation to possible graded changes of the level of activity of the central respiratory mechanisms will be discussed in Section 4.

3. Differences in stability of spontaneous discharge between respiratory neurons in the brain stem

Many authors (Dirken and Woldring 1951, Hukuhara et al. 1954, Nelson 1959, Salmoiraghi and Burns 1960, Trzebski and Peterson 1964) have
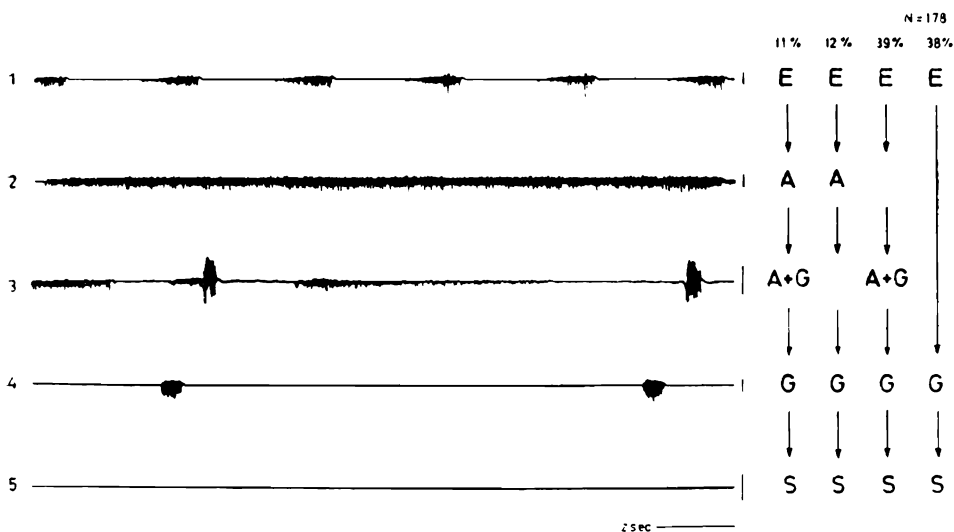
Fig. 4. Various patterns of phrenic nerve discharge showing four sequences of changes due to asphyxiation. *1*, eupnoeic pattern (E); *2*, apneustic pattern (A); *3*, intermingled pattern consisting of apneustic and gasp patterns (A+G); *4*, gasp pattern (G); *5*, cessation of discharge (S). Calibration: 100 μv for *1*, *2* and *3*, 50 μv for *4* and *5*. At the right four sequences of changes are shown in the columns. The percentage of occurrence of each sequence in 178 experiments is indicated at the top of each column. (From Hukuhara 1970*b*.)
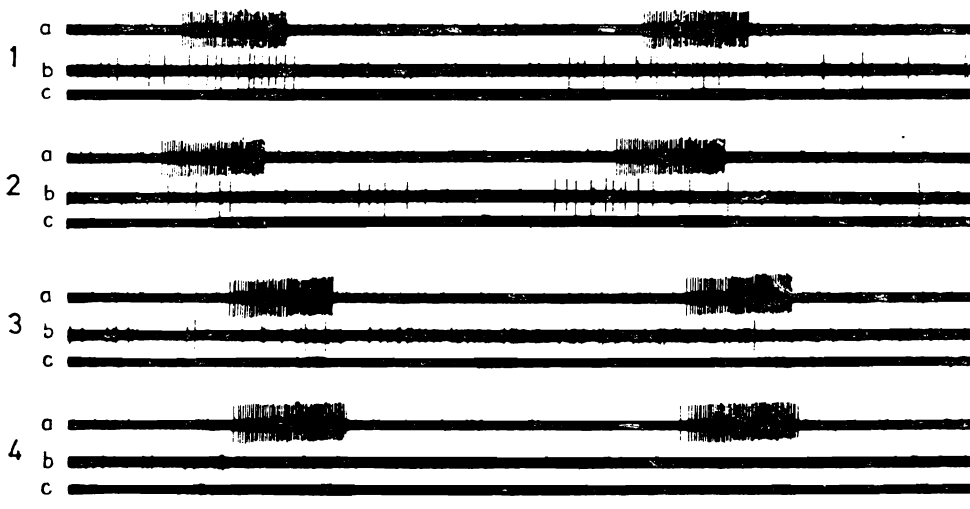

Fig. 5. Comparison of stability of neuronal discharge by stimultaneous recording of a bulbar (*a*) and a pontine (*b*) respiratory unitary discharge, *c*, phrenic nerve discharge. The horizontal bar on the right indicates 1 sec. *1* and *2*, and *3* and *4* are continuous records. *1* and *2* before, and *3* and *4* after intravenous injection of 2 mg/kg pentobarbitone. Note unit *b* which was discharging unstably ceased its spontaneous discharge after pentobarbitone.
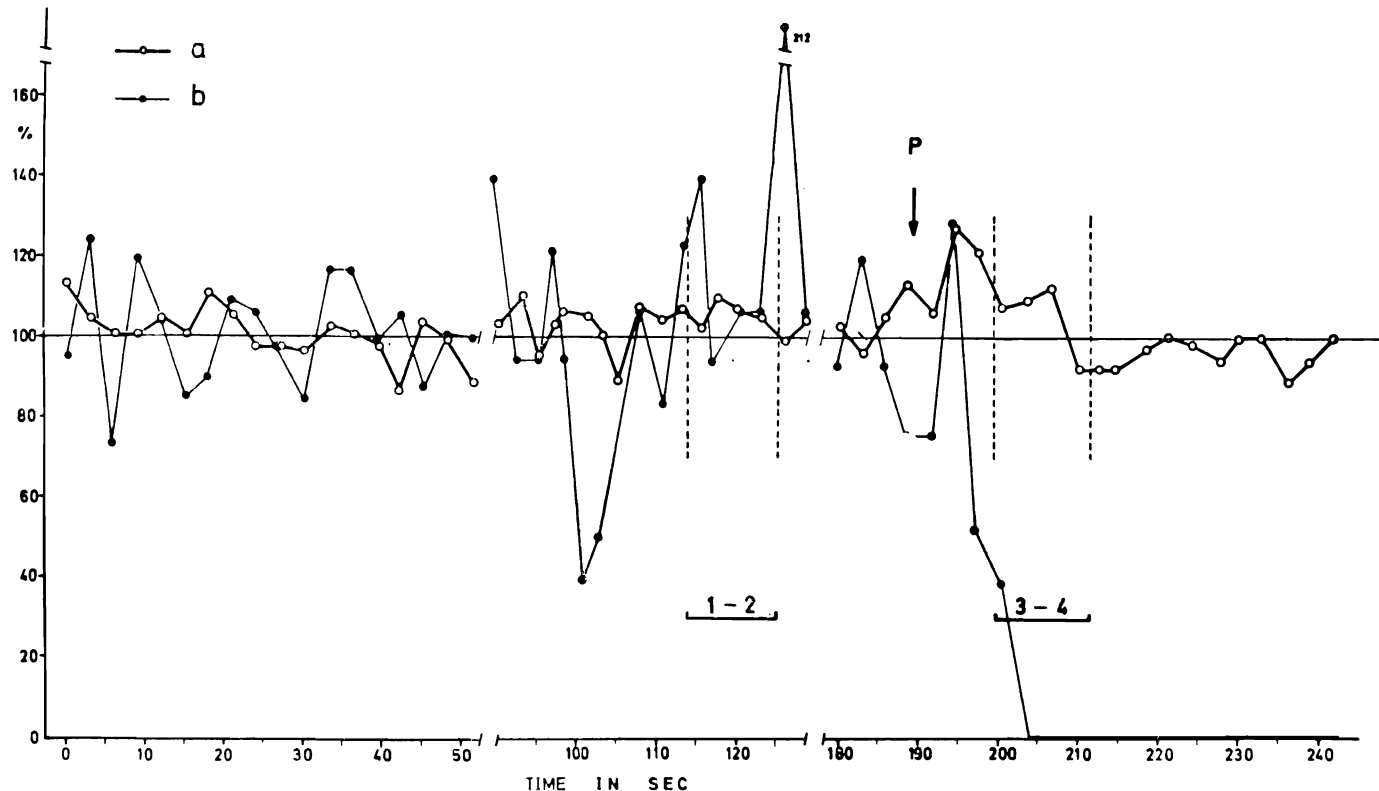
Fig. 6. Difference of stability of respiratory unitary discharges. Graphic presentation of the stability of the discharge of the units in Fig. 5 as expressed by variation of mean frequency in volleys. Abscissa, time in seconds; ordinate, mean frequency in volley. *P*, intravenous injection of 2 mg/kg pentobarbitone. The times during which the tracing of *1* and *2*, and and *3* and *4* in Fig. 5. were taken, are indicated.

shown that the spontaneous burst activity of bulbar respiratory neurons
varies, but the variation was not analysed quantitatively.

The stability has been related to the localization, as well as the
discharge type. In Fig. 5 shows an experiment in which a bulbar (*a*) and
a pontine inspiratory unitary discharge (*b*) were simultaneously recorded.
Changes of the mean frequency in the volleys of both discharges in Fig. 5
are plotted against time (Fig. 6), showing remarkably different stabilities.

In order to obtain a quantitative measure of stability the standard
errors of five variables were calculated for individual neurons (Fig. 1):
number of spikes (*N*), mean frequency in volley, duration of volley (*D*),

<div align="center">TABLE I</div>

Comparison of stability as expressed by the ratio of the standard error to the mean for each variable
of volley for bulbar and pontine populations of respiratory unitary discharges with respect to local-
ization in vagatomized paralysed and ventilated artificially cats. Numbers in parentheses represent
the number of units. The values of the pontine populations are significantly larger than those of the
bulbar populations for all variables

	Substantia reticularis lateralis bulbi		Substantia reticularis pontis		
	Exp (12)	Insp (15)	Exp (9)	Insp (11)	P-S (9)
N	6.8	4.7	14.3	9.0	8.9
MF	5.1	4.0	10.2	6.6	9.4
D	5.3	3.7	13.1	8.3	8.8
I	4.2	3.3	11.1	7.0	14.7
P	2.5	2.8	6.5	4.2	4.7

Abbreviations: *N*, number of spikes in a volley; *MF*, mean frequency of discharge in volley; *D*, duration of volley;
I, interval between volleys; *P*, period of volley. Exp, expiratory unitary discharge; Insp, inspiratory unitary discharge;
P-S, phase-spanning unitary discharge.

interval between volleys (*I*) and period of volley (*P*). The standard error
was then expressed as a percentage of the mean. These percentages were
pooled for each population of neurons and are given in Table I. Statistical
analysis showed that the percentage standard errors of all variables of
the pontine population were significantly larger than those of the bulbar
population. Between anaesthetized and unanaesthetized preparations no
significant differences in the degrees of fluctuation were found.

Figure 7 shows the relation between stability of neuron discharge and
their localization. The majority of unstable neurons was found in the
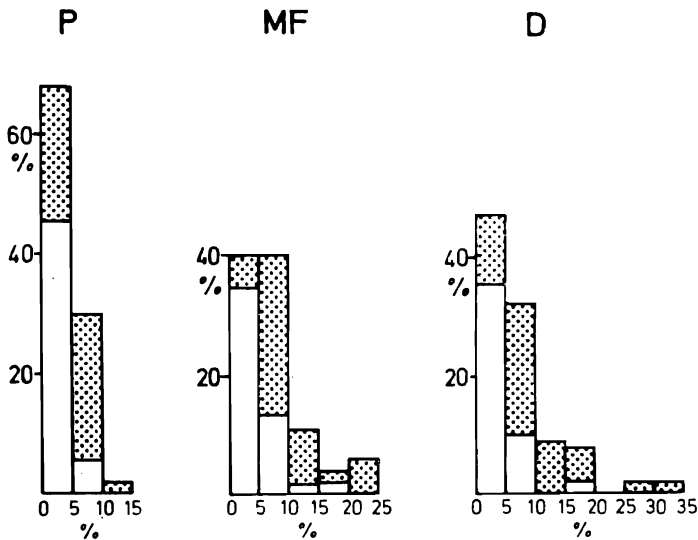pontine reticular formation in respect to stability of period (*P*), mean

Fig. 7. Distribution of the ratios of the standard error to the mean (abscissa) for three variables of respiratory unitary discharge, for vagotomized cats, paralysed and ventilated artificially. Ordinate, percentage of units ($N = 53$). White area, bulbar unitary discharge; stippled area, pontine unitary discharge. P, period; MF, mean frequency; D, duration.

frequency (*MF*) and duration of volley (*D*). However a few unstable neurons were found in the bulbar reticular formation.

These results suggest that the mechanisms inducing spontaneous burst activity of respiratory neurons are not necessarily uniform and that they may have functional differences.

4. Spontaneous rhythmic activity of respiratory neurons in the isolated brain stem

It is still an important question whether the neuronal mechanisms primarily responsible for the genesis of respiratory rhythm are located in the upper pons (Lumsden 1923, Pitts 1946, Wang and Ngai 1964, Cohen 1970, Bertrand and Hugelin 1971), or in the bulbar structure (Hoff and Breckenridge 1949, Breckenridge and Hoff 1950, Hukuhara et al. 1951, Salmoiraghi and Baumgarten 1961, Salmoiraghi 1963, Hukuhara et al. 1966a, Hukuhara 1966a), and whether coexistance of the pontine and bulbar structures with intact neural connections between them (Wyss

1954, 1964, Cohen 1970) is necessary for the genesis of the respiratory rhythm. The meaning of "genesis" should be understood as an "intrinsic production of respiratory rhythm" and, consequently, would be discerned from the processes of modulation and maintenance of respiratory rhythm involving many hypothetical respiratory oscillatory loops (Hugelin and Cohen 1963, Wyss 1964, Cohen 1970, Bertrand and Hugelin 1971).

The mechanism for the genesis of this spontaneous rhythm has been investigated by means of the complete isolation procedures of the brain stem or of the medulla in the cat (Euler and Söderberg 1952, Cohen 1958, Hukuhara 1959, Salmoiraghi and Burns 1960) and in various fishes (Adrian and Buytendijk 1931, Hukuhara and Okada 1956ab). It seems very likely that the unitary discharges observed in these experiments were from the so-called respiratory centres, but this was not established as no comparison was made on one and the same unit before and after the isolation procedures. In fact, some neurons firing spontaneously in bursts and independently of the respiratory rhythm have been identified in the bulbar, pontine and mesencephalic reticular formation (Machne et al. 1955, Scheibel et al. 1955, Moruzzi 1956). Therefore experiments in which the activities of the same neuron are recorded before and after the isolation procedures are indespensable to clarify the location of the mechanism responsible for the genesis of respiratory rhythm.

Therefore, we carried out the following experiment using a floating electrode technique in 65 cats, unanaesthetized, paralysed and artificially ventilated. While the inspiratory unitary discharges (Fig. 8ab) from a neuron soma were being recorded from the lateral bulbar reticular formation (Fig. 81), the cervical vagosympathetic nerve trunks and the carotid sinus nerves were cut (Fig. 82). The XI and XII cranial nerves were also cut. Then the brain stem was transected at the level of the pontomedullary junction and, furthermore, the spinal cord was cut at C_{6-7} with fine otology scissors (length of blades, 3 mm) (Fig. 83). Spontaneous discharges in bursts were maintained for several hours with both neurons showing neither the apneustic nor the gasp pattern as described in Section 2. Later, at the end of the experiments in most cases, the firing pattern of the two neurons spontaneously changed into the apneustic and subsequently into the gasp pattern before cessation of activity. We recorded the same neuronal discharge in 18 successful experiments (16 inspiratory and 5 expiratory neurons) out of 65 cases before and after the isolation procedures. In some experiments, in which an abrupt fall of arterial blood pressure occurred immediately after brain stem transection, the discharge pattern changed into an apneustic or a gasp pattern before cessation of discharges. During the isolation procedures, a few
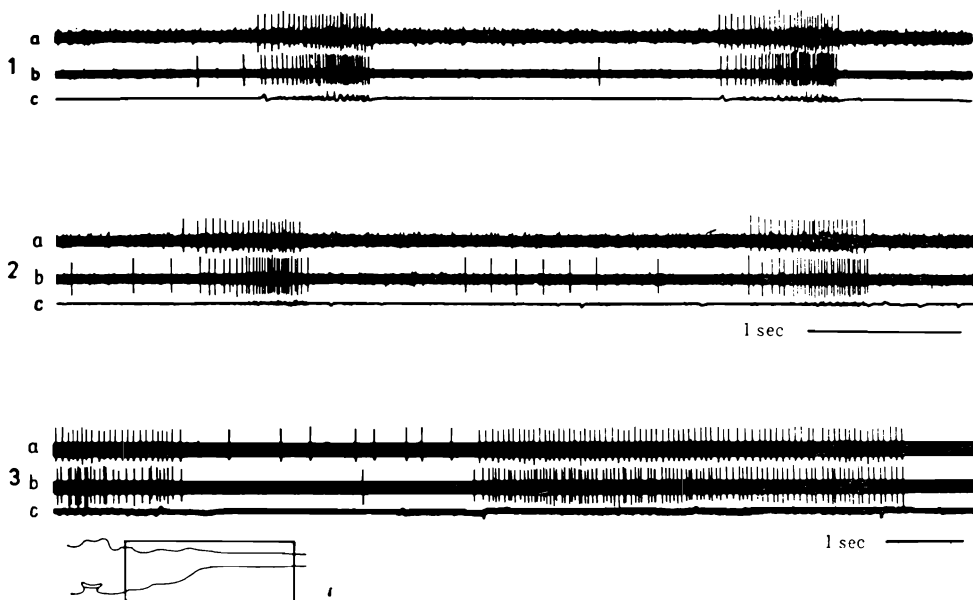
Fig. 8. Changes of bulbar inspiratory discharges caused by transection of the brain stem at the pontomedullary junction and of the cervical segment of the spinal cord at C_{6-7} in the paralysed, artificially ventilated cat. *a* and *b*, bulbar inspiratory discharges in the lateral region of the reticular formation; *c*, phrenic nerve discharge. Levels of the transection of the brain stem and of the cervical segment are indicated in the diagram at the bottom. *1*, before isolation; *2*, after section of the vagosympathetic trunks and the carotid sinus nerves; *3*, after transection of the brain stem at the pontomedullary junction and of the cervical segment between C_{6-7}. In *1*, *2* and *3*, *a* and *b* come from the same two units.

neurons lost their periodicity of discharge and fired continuously, and some neurons became silent after the section of the vagus nerves and/or the carotid sinus nerves. It is significant that the periodic burst activity recorded before and after isolation was obtained from one and the same unit and was spontaneous even under such great interventions as shock, haemorrhage, changing blood flow, and hypotension which accompany brain stem transection.

From these results it is evident that the burst activity of the bulbar respiratory units after the isolation procedures was not attributable to the action of vagal afferent inputs or to influences from the area rostral to the pontomedullary junction. It can be concluded therefore that the bulbar respiratory rhythm can be generated intrinsically in the bulbar structure.

5. *Changes of spontaneous discharge of respiratory neurons in the pons and the medulla with simultaneous recording before and after brain stem transection*

There are many differences between bulbar and pontine respiratory discharges: the composition of discharge types among neurons (Cohen and Wang 1959, Hukuhara et al. 1969, Cohen 1970), the stability of spontaneous burst activity (Section 2), and the responses to anaesthetics (Section 8). This finding suggests a different functional significance between bulbar and pontine respiratory neurons. Furthermore it was described in Section 4 that some bulbar neurons continue to fire in bursts with neither the apneustic nor the gasp pattern in the isolated bulbar structure. Therefore we conclude that a bulbar mechanism for the genesis of an intrinsic rhythm is independent of the pontine rhythm (Pitts 1946, Wang and Ngai 1964, Bertrand and Hugelin 1971) and different from the bulbar activity related to the gasp (Hoff and Breckenridge 1949, Breckenridge and Hoff 1950).

The following experiment provides a better understanding of the relationship between the pontine rhythm and the bulbar intrinsic activity. Bulbar and a pontine respiratory discharges were recorded simultaneously by means of a floating electrode technique and subsequently the brain stem was transected at the pontomedullary junction with continued recording of both discharges. There are three possible results of such an experiment.

1. Both unitary discharges continue to fire after transection, but the timing of their bursts becomes independent. This would suggest that two different rhythm-producing mechanisms are located respectively in the bulbar and in the pontine structures.

2. The pontine unit fires continuously after transection, while the bulbar unit ceases spontaneous firing irreversibly. This would indicate that a rhythm-producing mechanism resides in the pons, but not in the bulbar structure.

3. The bulbar respiratory discharge is still spontaneously active in phase with the phrenic nerve discharge after transection, whereas the pontine discharge ceases spontaneous firing irreversibly. This would indicates the existance of a rhythm-producing mechanism in the bulbar structure, but not in the pons as far as the primary respiratory rhythm is concerned.

The described experiment was therefore carried out in vagotomized cats paralysed and ventilated artificially (Fig. 91). It was first established that both the discharges responded in parallel to various respiratory
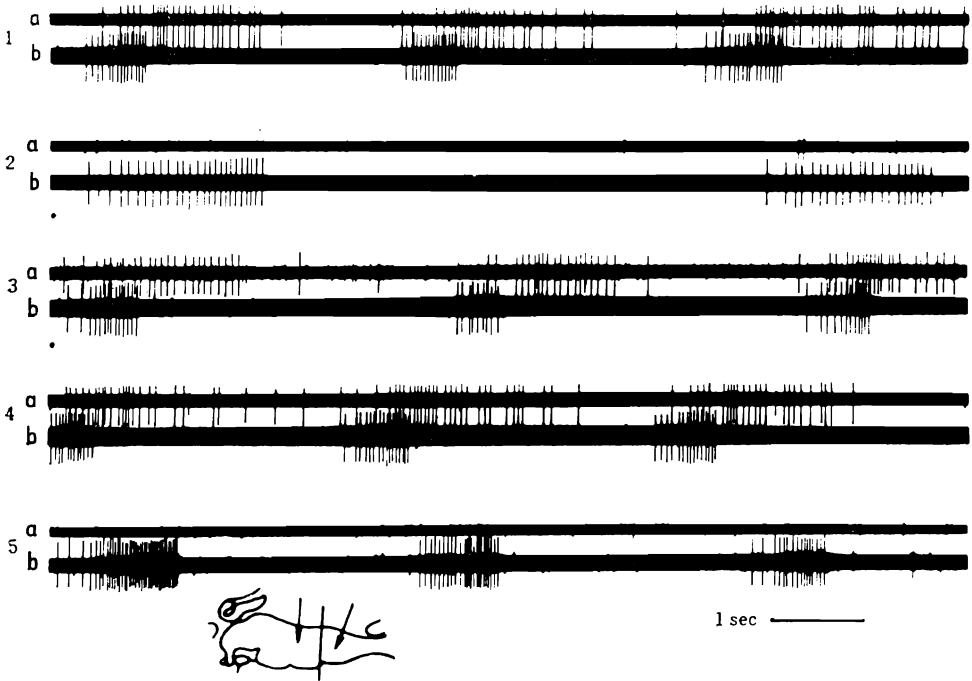
Fig. 9. Changes of unitary activity of bulbar and pontine respiratory neurons by brain stem transection at the pontomedullary junction in mid-course of simultaneous recording in a paralysed and artificially ventilated cat. *a*, pontine unitary discharge (phase-spanning type); *b*, bulbar inspiratory discharge in the lateral region of the bulbar reticular formation. *1*, intact; *2*, 60 sec (at the dot on the left of the tracing) after intravenous injection of 2 mg/kg of pentobarbitone; *3*, 60 min after pentobarbitone; *4*, after section of the vagosympathetic trunks and the carotid sinus nerves; *5*, after transection of the brain stem. The pontine neuron *(a)* ceased its spontaneous activity after transection, whereas the bulbar neuron *(b)* continued to fire showing neither apneustic nor gasp pattern.

stimuli and correlated with changes in phrenic nerve discharge. Pentobarbitone (2 mg/kg) was then injected intravenously. Then pontine discharge (Fig. 9*2*) ceased for 30 min. At 60 min after the injection (Fig. 9*3*), the vagosympathetic and the carotid sinus nerves were cut. No remarkable changes in either discharge pattern were noticed (Fig. 9*4*). Subsequently the brain stem was transected at the pontomedullary junction with continued recording of both the unit discharges. Spontaneous discharges of the pontine unit ceased after the transection, whereas the bulbar discharge continued in bursts without showing the apneustic or the gasp pattern (Fig. 9*5*). Usually the spontaneous burst activity of the bulbar neuron continued for several hours after tran-

section. Then, at the end of most experiments the discharge pattern changed spontaneously into the apneustic (Fig. 3B2) or the gasp pattern (Fig. 3C2) followed by cessation of discharge.

These results show that neither the apneustic nor the gasp pattern of respiratory unitary and phrenic nerve discharges can be regarded as representative of a definite neural organization specified as a "centre" in the brain stem. Together with the results of Sections 2 and 4 they indicate that the patterns are transient activities in relation to the total number of respiratory neurons in full activity among the whole population of the central respiratory mechanism.

The bulbar discharge responded in the same direction to various respiratory stimuli before and after the transection. When the pontine reticular formation was explored with another microelectrode after the transection, many unitary discharge were recorded, but no discharge showing periodicity consistent with respiratory phases was detected. It is therefore difficult to accept that cessation of the periodic burst activity of the pontine discharge is due to the overall reduction of the activity of the pontine structure by non-specific effects of transection.

In such experiments it is crucial that the floating electrode tip remains in the same position in the course of brain stem transection. The spontaneous activity of the pontine respiratory neurons was recorded during all the experiments on electromagnetic tape, and the course of changes of activity on brain stem transection was displayed repeatedly on a cathode-ray oscilloscope and photographed. In addition, computer analysis was carried out by means of an amplitude variation, as well as a pulse density variation program for confirmation of the nature of the cessation of the pontine discharges. These procedures allowed detection of successful (Fig. 10A) and unsuccessful experiments (Fig. 10B). The doubtful cases were discarded from the evaluation of the experiment.

Of a total of 40 pontine neurons spontaneous discharge ceased after the transection in 39 successful experiments; in one successful case the pontine unit continued its spontaneous firing but it lost its respiratory rhythm changing its firing pattern into an irregular one in 40 successful experiments out of 87 cases.

Thus the periodic burst activity of the pontine respiratory discharge is secondary to and produced by ascending influences from the structures below the pontomedullary junction. Other evidence supports the idea of an ascending activating influence from the bulbar structure. A periodic burst activity has been observed in the cortical and subcortical EEG whose periodicity synchronized with that of phrenic nerve discharge in the curarized cat (Kumagai et al. 1966) and in EEG recording in man (Bülow and Ingvar 1961). This respiratory periodicity of the EEG was

abolished by brain stem transection at the midpontine level (Fig. 11B) in 19 cats (T. Hukuhara, Jr. et al., unpublished data).
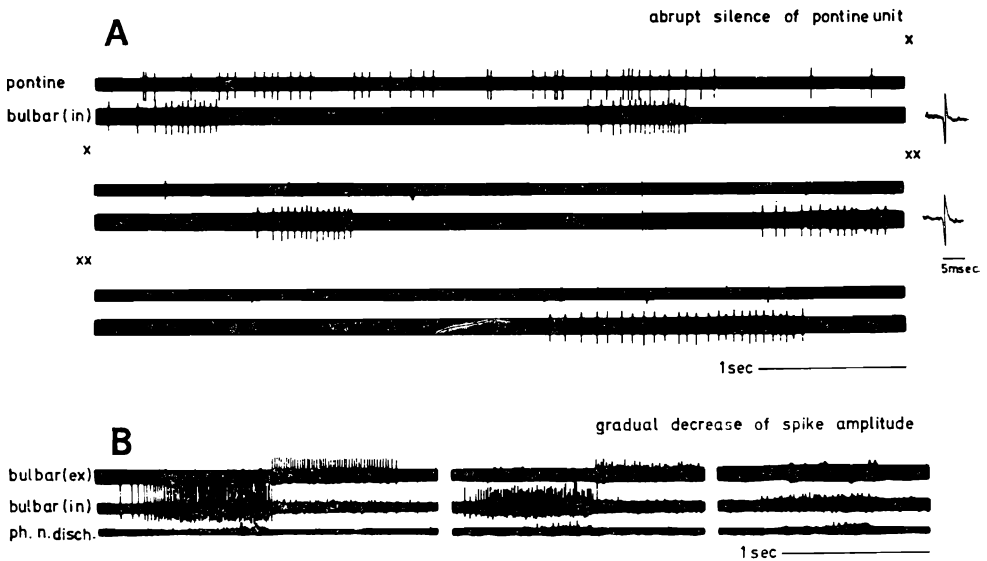

Fig. 10. Changes of discharge pattern of a respiratory neuron by brain stem transection in mid-course of recording with a floating electrode technique. A: changes for a unit when the brain stem was transected with success, maintaining the recording of both unitary discharges. At marks X and XX the tracing is continuous. Two insets on the righthand show the spike-form of the bulbar discharge before (above) and after (bottom) transection. Note an abrupt cessation of spontaneous discharge of the pontine neuron after transection. In such case the possibility of dislocation of the recording electrodes by the procedures for brain stem transection could be excluded. B: changes in an experiment with failure to maintain recordings of the unitary discharges of both neurons in the course of transection, due to dislocation of electrodes. Note the gradual decrease of the spike amplitudes of discharges in the middle and right panel of the tracings after transection.

6. Response of respiratory neurons to stimulation of the spinal cord

In order more specifically to localize respiratory neurons sending their axons to the spinal cord or being activated synaptically by spinal cord stimulation, responses of neurons were examined to electrical stimulation of the cervical segment (C_{2-4}) with a stimulating microelectrode with a tip diameter of 1–5 μ in the course of systematic exploration in the whole area of the reticular formation of the pons and the medulla in vagotomized cats, paralysed and ventilated artificially (Hukuhara et al. 1968ab).

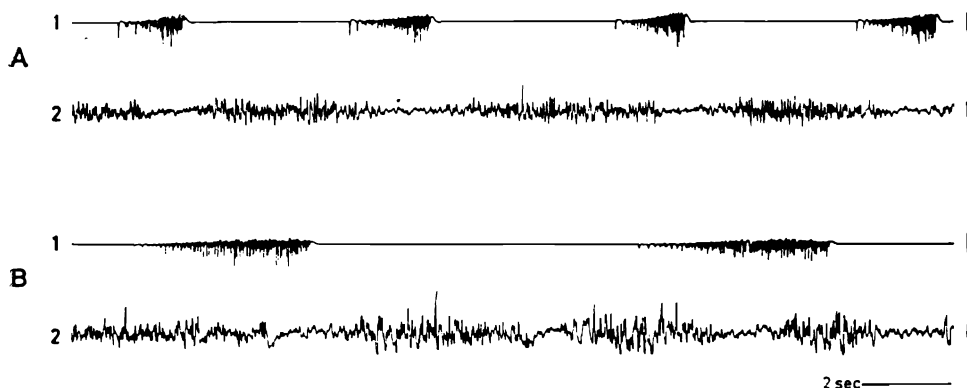The responses of two respiratory neuron populations (inspiratory and

Fig. 11. Changes of periodic burst activity in neocortical EEG and phrenic discharge by midpontine transection in the paralysed, artificially ventilated cat. A, before transection; B, after transection. 1, phrenic nerve discharge; 2, EEG in the motor area of the cerebral cortex. Calibration: 50 μv is indicated by the vertical short bars on the right. Note periodicity of periodic burst activity in the EEG synchronizes with that of the volley of the phrenic nerve discharge in A, whereas the synchrony is abolished after midpontine transection in B.

expiratory) have been investigated in anaesthetized cats, although the antidromic response of neurons have been examined only in the relatively circumscribed region of the caudal part of the medulla (Baumgarten and Nakayama 1964, Nakayama and Baumgarten 1964, Bianchi 1969, 1971, Merrill 1970).

The soma- spike was differentiated from an axonal one on the basis of the following criteria: (i) wave form, polarity and duration of the evoked response were identical to those of the spontaneous spikes; (ii) responses were elicited in an "all or none" fashion depending on stimulus intensity; (iii) the responses were elicited only during the respiratory burst of unitary activity at an adequate stimulation intensity; (iv) collision was observed when the neuron fired spontaneously or when paired shocks with a short interval were applied (Magni and Willis 1963, Wolstencroft 1964); (v) disintegration (fragmentation) of the rising phase of spikes was observed with either high frequency stimulation or paired shocks (Brock et al. 1953, Eccles 1955, Phillips 1956, Phillips et al. 1963, Nakayama and Baumgarten 1964): (vi) the upper limit of stimulation frequency was relatively low, ranging from 250 to 300 Hz (Porter 1963, Nakayama and Baumgarten 1964). In these experiments a steel microelectrode (Green 1958) was used, and doubtful cases in which the multineuronal response (Bishop 1964) could not be distinguished from a single spike were discarded from the evaluation of the experiment.

The antidromic response of neurons was differentiated from the orthodromic one on the basis of the following criteria (Porter 1963, Nakayama and Baumgarten 1964, Wolstencroft 1964): (i) short and constant latency

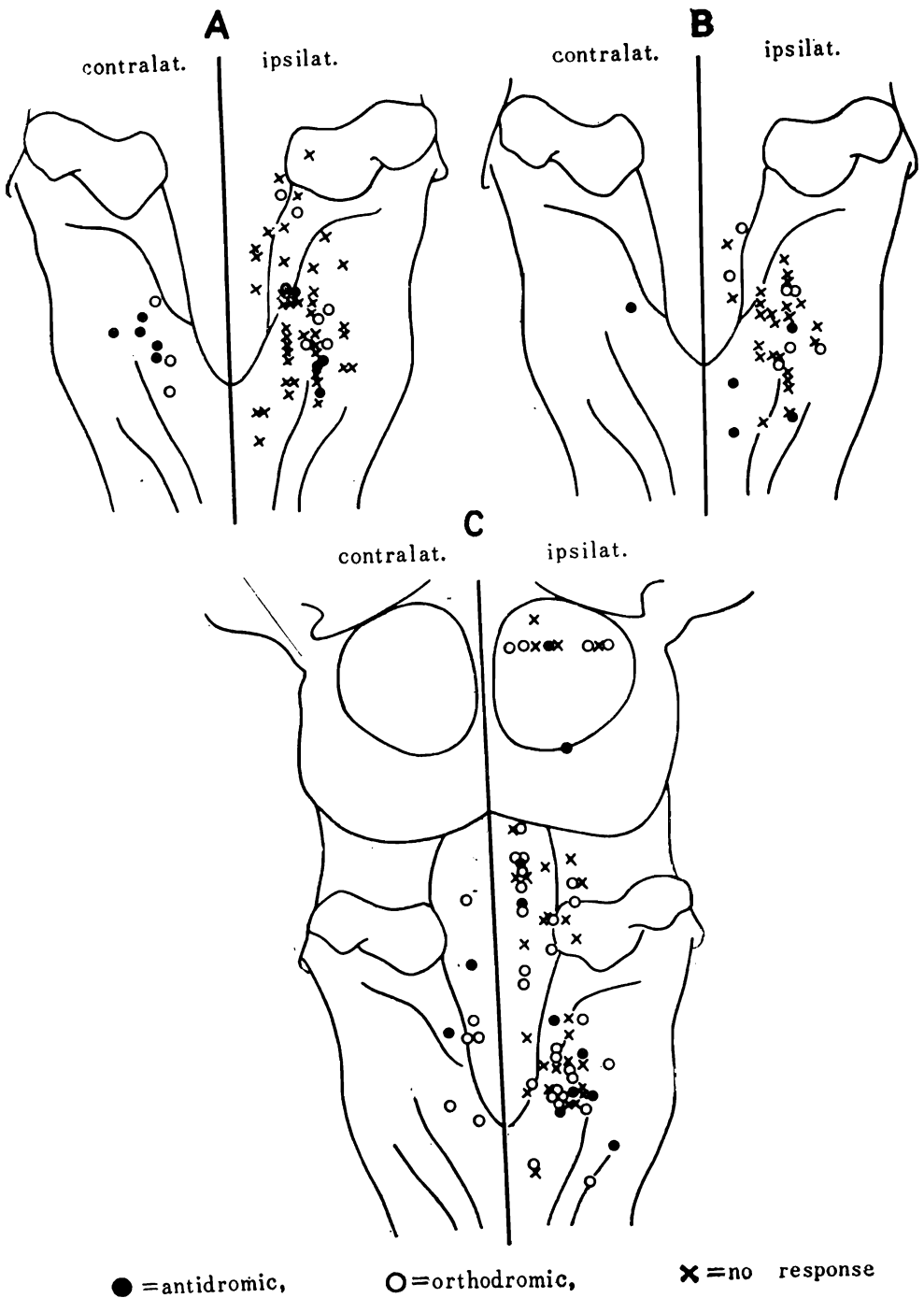Fig. 12. Dorsal projections of the brain stem showing localization of respiratory neurons with respect to type of response to electrical stimulation of the spinal cord. A, distribution of inspiratory neurons; B, distribution of expiratory neurons; C, distribution of another "broader group" of respiratory neurons not so clearly related to respiratory phases, ipsilat., ipsilateral; contralat., contralateral.

of response: (ii) ability of the response to follow increasing frequency of stimulation (loc. cit.); (iii) disintegration of spike (loc. cit.).

A total of 175 respiratory neurons were encountered in the systematic exploration experiment in 36 cats. Two kinds of response were found in each of three groups of discharge type of respiratory neurons; inspiratory (66 neurons), expiratory (36), and another "broader group" not so clearly related to the inspiratory and expiratory phases (73). The proportions of neurons responding antidromically were approximately the same for all three groups (14, 15 and 16%). The proportions of neurons responding orthodromically in the inspiratory (15%) and expiratory (19%) groups were similar; however this proportion in the "broader group" was considerably larger (48%). Figure 12 shows the localization of respiratory neurons displayed in dorsal projections of the brain stem. Inspiratory and expiratory neurons responding antidromically were located ipsi- and contralaterally and diffusely throughout the lateral region of the bulbar reticular formation, but not in the medial part. Inspiratory, as well as expiratory neurons which responded orthodromically were located in both the medial and lateral part of the bulbar reticular formation and in the lower part of the pontine reticular formation. No circumscribed regions in which respiratory neurons were grouped according to their response to antidromic or orthodromic stimulation were found. Neurons in the "broader group" responding antidromically or orthodromically were located throughout the entire reticular formation of the medulla, the pons and the midbrain (Fig. 12C).

Respiratory neurons responding antidromically send axons to the cervical segment, probably to the motoneuron pool. Neurons activated orthodromically may be closely related to the mechanisms which are conveying afferent input from the spinal cord and/or mediating respiratory reflexes from the respiratory muscles, peripheral sensory mechanisms and digestive tract (Widdicombe 1964). Respiratory neurons which fired in a similar relation to the respiratory phases were classified into three groups on the basis of different anatomical relations to the spinal cord. Therefore a considerable complexity of the anatomical relation between respiratory neurons and the spinal respiratory mechanisms should be expected, and some different functional significance among central respiratory neurons may very probably exist.

7. Orthodromic response of respiratory neurons to afferent vagal stimulation

By afferent electrical stimulation of the vagus nerve the majority of bulbar respiratory unitary discharges and the phrenic nerve discharge were facilitated at low frequency and inhibited at high frequency stimu-

lation, at constant, threshold intensity in paralysed cats (Hukuhara et al. 1966b). This reversal of stimulation effect was present after brain stem transection at the pontomedullary junction (Hukuhara 1971ab).

A total of 123 bulbar respiratory neurons were investigated for their response to single orthodromic shocks in order to analyse the mechanism of this reversal phenomenon. The orthodromic unitary spike response (Porter 1963, Nakayama and Baumgarten 1964, Hukuhara et al. 1968ab) was observed in only 20 neurons (17% to the total) including inspiratory, expiratory and the "broader group". These neurons were scattered throughout the lateral bulbar reticular formation. No neuron which responded antidromically was found. The rest of neurons (103) did not respond. The high frequency stimulation by which the orthodromic response of respiratory neurons was abolished caused simultaneously an inhibitory effect in both the phrenic discharge and the unitary discharge of other respiratory neurons. Pentobarbitone (1–5 mg/kg) abolished or depressed the orthodromic response of neurons and the facilitatory effect of the phrenic discharge.

These results lead to the following conclusions: (i) only a few of the bulbar respiratory neurons are directly receiving a vagal afferent input; (ii) the neuronal connexion in the afferent pathway to bulbar respiratory neurons is not necessarily uniform; (iii) the presence of an upper stimulation frequency for the facilitatory effect of the phrenic and unitary discharges is closely related to the limited ability of respiratory neurons to follow orthodromically an increase of frequency of afferent vagal stimulation.

Since the intensity of stimulation was kept at threshold on the basis of previous findings in the cat (Wyss 1943, Paintal 1963), it may be suggested that the pathways mediating the orthodromic response of bulbar respiratory neurons play a role in the facilitatory response of bulbar respiratory discharge caused in decerebrate preparations by slight lung inflation (Hukuhara et al. 1956).

8. Effects of pentobarbitone on spontaneous unitary discharges of respiratory neurons

During simultaneous recording from a bulbar and a pontine or two bulbar respiratory unitary discharges the effects of pentobarbitone (1–5 mg/kg i.v.) were investigated in vagotomized cats, paralysed and ventilated artificially (Hukuhara et al. 1968b). Each neuron showed one of four types of change described below. With simultaneous recording from two neurons both exhibited different types of change at the same time. A total of 365 neurons was studied (249 bulbar and 116 pontine unit).

Type I, 27% of the neurons ceased firing for 30 to 60 min (Fig. 53b,4b
and 92a,3a); type II, in 55% the drug caused a decrease of spikes and of
mean frequency in each volley, and prolongation of burst duration and
of the period of the volley (Fig. 92b); type III, 13% changed their activity
into a continuous firing; type IV, in 3% of neurons there was an increase
of spikes and of mean frequency in each volley, and shortening of the
period. The rest of the neurons (2%) did not show any change. In the
pontine population the proportions of types of change were different
from those for the whole population. Numbered as before they were: I,
52%; II, 22%, III, 26%, IV, 0%. Thus in the pontine population the
changes I and III were more frequent compared with those for the whole
population.

Of these types of change, only type III (Robson et al. 1963) and type
II (Hukuhara et al. 1956, Brodie 1959) by pentobarbitone or morphine,
and types II and III by chloralose-urethan (Bystrzycka et al. 1969) have
previously been reported in bulbar neurons. When two unitary discharges
belonging to the same population were recorded simultaneously, pento-
barbitone injection caused one type of change in one neuron and, con-
comitantly, a different type of change in the other neuron. Therefore the
mechanism of spontaneous firing of respiratory neurons even in the same
population is not necessarily uniform, and different mechanisms can
subserve the generation of spontaneous burst activity.

9. Functional organization of respiratory neurons in the central respiratory mechanisms in the brain stem

The functional basis of the central respiratory mechanism is a net-
work which consists of three populations: inspiratory and expiratory
neurons and third "broader group". This network is thought to be located
in the entire area of the mesencephalic, pontine and bulbar reticular
formation. It may include four neuronal subsystems which are different
in function. The anatomical location of each subsystem, except for the
primary neuron system, is not necessarily confined to a specifically
localized area of the brain stem reticular formation.

The neuronal subsystem are: 1) a primary neuron system, 2) a sat-
ellite neuron system, 3) an input neuron system, and 4) an output neuron
system (Fig. 13).

1. *Primary neuron system* (Fig. 131). The elements of this system are
the inspiratory and expiratory neurons which are localized in the
lateral bulbar reticular formation (Sections 4 and 5) and which fire with
a high stability (Section 3) of burst activity. Explanation of the mecha-
nism of genesis of the spontaneous periodic activity of the system is

HIGHER CENTRAL MECHANISMS

① PRIMARY   NEURON
② SATELLITE NEURON
③ INPUT   NEURON
④ OUTPUT   NEURON

RET. FORM.
-MESENCEPH.
PONTINE
-BULBAR

AFFERENT INPUT RELATING
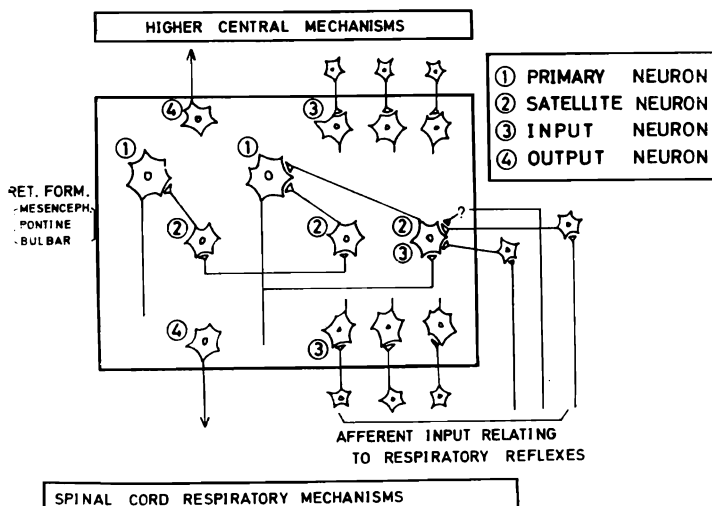TO RESPIRATORY REFLEXES

SPINAL CORD RESPIRATORY MECHANISMS

Fig. 13. Schematic representation for possible functional differentiation of respiratory neurons in the brain stem reticular formation. For explanation, *see* text.

still lacking, although some oscillatory circuit (Cohen 1970, Bertrand and Hugelin 1971) may be present within the subsystem. Possible trigger mechanisms are also unknown at present. Further investigation is needed to see whether a pacemaker cell is present, like those in the abdominal ganglion of *Aplysia* which are spontaneously active, discharging with bursts even after complete isolation of the cell soma from the ganglion (Alving 1968, Baumgarten and Chen 1971), or like a "starter neuron" (Salmoiraghi and Baumgarten 1961) together with neuronal circuits (Salmoiraghi and Baumgarten 1961, Salmoiraghi 1963, Cohen 1970).

2. *Satellite neuron system* (Fig. 132). The central respiratory mechanisms may include various types of internuncial neurons. Respiratory neurons of all discharge types could be the elements of the system. Periodic activity originated by the primary neuron system would be modulated and maintained by these satellite neurons. $R\beta$ neuron (Baumgarten and Kanzow 1958) may be one type of satellite neuron. Respiratory neurons which were facilitated by pentobarbitone (Section 8) may be also a type of such internuncial neuron. The majority of neurons which are less stable in their spontaneous burst activity (Section 2) could belong to this system.

3. *Input neuron system* (Fig. 133). All modalities of afferent input to the respiratory network are mediated by this system. Only specific respiratory neurons receive directly such afferent input. In fact 17% of bulbar respiratory neurons could be activated orthodromically by afferent vagal stimulation (Section 7). In addition, in responses to electrical stimulation

of the cervical cord, specific bulbar and pontine respiratory neurons could be activated orthodromically (Section 6). Furthermore respiratory neurons which responded synaptically to stimulation of the midbrain reticular formation amounted to 20% of the total (T. Hukuhara, Jr., unpublished data). Therefore, there may exist specific input respiratory neurons which are related to central mediation of respiratory reflexes or to modulation of respiratory movement by the higher central mechanism.

4. *Output neuron system* (Fig. 134). The total output of the respiratory neuron network may be transmitted to the spinal respiratory mechanisms, as well as to higher substrates (Kumagai et al. 1966) by specific respiratory neurons. These neurons constitute the output neuron system. Respiratory neurons which send their axons to the spinal cord constitute 15% of the total (Section 6) and those sending their axons to the midbrain reticular formation amounted to 10% (T. Hukuhara Jr., unpublished data). The total output of the network may pass also to circulatory centres (Hukuhara and Takeda 1970), central sympathetic substrates, somatic motor systems and brain stem structures relating to regulation of the waking-sleep cycle. A neuron could also belong to two or three functional subsystems among the (2), (3) and (4) neuron systems.

A scheme of functional and neuronal organization of the central respiratory mechanisms consisting of four subsystems of reticular respiratory neurons has been described. This is formulated mainly on the basis of direct experimental evidence. Although the scheme is still premature and preliminary and is open to further investigation, it serves as a step for further experimental research, as well as for better understanding of the neuronal organization of the central respiratory mechanisms.

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