AXONAL PROJECTIONS AND PRIMARY AFFERENTS OF BULBAR RESPIRATORY MODULATED AND UNMODULATED NEURONS TRAVELLING WITH VAGAL NERVE OR SPINAL CORD IN THE RABBIT

G. BÖHMER, M. FALLERT AND H. R. O. DINSE [1]

Department of Physiology, University of Mainz, Saarstrasse 21, D - 6500 Mainz, Federal Republic of Germany

INTRODUCTION

Antidromicity of neuronal response to nerve stimulation is generally assumed when the neuron faithfully follows high stimulus frequencies, threshold voltage is stable and collision of evoked and spontaneously generated action potentials occurs. For neurons the discharge of which is not modulated with respiration (UMN), located in the region of the nucleus ambiguus of cats, latencies of 1-3 msec and maximum frequencies up to 200-300 pulses per sec were reported (20, 21). In contrast orthodromic excitation of neurons by primary afferents is assumed when variability of latency is greater and maximum stimulus frequencies the neuron responds to remain modest. In bulbar respiratory modulated neurons (RMN), however, two facts must be considered when differentiating antidromic from orthodromic activation; threshold voltage (1) and invasion latency vary throughout the respiratory cycle (15). In the present study, latencies were compared to the variability of latency. The data from literature concerning the proportions of RMN which possess efferents along with the vagal nerves or down the spinal cord vary considerably in cats (4, 5, 7, 12, 17, 18, 23). It was therefore of interest to compare

[1] Present address: Department of Zoology, Biophysical Section, University of Mainz.

the proportions of RMN in the rabbit possessing efferents or vagal or spinal afferents with those of the cat.

Methods

Experiments were performed on 36 urethane-anesthetized rabbits. After dorsal laminectomy of cervical vertebra two, steel needle electrodes of 0.3 mm diameter spaced by 2 mm were inserted into the spinal cord, one on each side. Test pulses delivered in a bipolar arrangement prior to immobilisation were set to 15-20 V and pulse duration to 100 μsec in order to elicit distinct somatomor effects. The distance between electrode location and the caudal end of the fourth ventricle where the units were located was 21 mm. Both intact cervical vagal nerve trunks (and with them fibres passing into the recurrent nerves) were stimulated simultaneously. The vagal nerves were dissected over 3 cm and put on two platinum wires spaced by 3.5 mm. Nerves and electrodes were embedded into synthetic caoutchouc to avoid current spread. The distance between stimulating electrode location and the bulb was 50 mm. Duration of vagal pulses was 50 or 500 μsec; strength of stimuli was ten times threshold voltage for evoked responses, *i.e.* voltages from 7-20 V were used. Frequencies of spinal or vagal pulses were 10-1000 pps. Efferent effects of vagal excitation were blocked with 20 μg Oxyphenonium bromide per kg body weight. The animals were immobilized with 5 mg Flaxedil per kg body weight and artificially ventilated.

Bulbar unit activity was recorded by means of glass micropipettes filled with 2 M KCl. For DC amplification of unit potentials a WPI M 701 micro-probe system was used. Superposed traces of evoked responses were photographed from the screen of the oscilloscope. DC unit discharge and electroneurogram of the phrenic nerve were stored by means of a Philips Analog 7 tape recorder (speed deviation is within 0.2% of the nominal value). Continued stimulation progressively modified respiration and inhibited overall burst activity of many RMN. In order to measure latency of responses during quasi-normal bursting activity and interburst intervals, stimulation was discontinued as soon as discharge patterns of RMN became markedly altered. To estimate variability of latency, at least ten successive poststimulus times were measured with the pulses being delivered throughout inspiration and expiration and the differences between the single actual latencies and the arithmetic mean of latencies were averaged. The tape recordings were used to recognize spike collision.

Results and Discussion

Medulla oblongata was sounded stereotaxically from 2 mm rostrally to 4 mm caudally of the promontorium gliosum and 3 mm laterally from the midline, down to 3.5 mm from the dorsal surface. RMN were mainly found near the solitary tract and in the neighbourhood of the nucleus ambiguus, as was previously described (10). Activity was recorded from 84 RMN. 27 units discharged during inspiration (I neurons), 35 neurons during expiration (E neurons), 9 cells exhibited phase-spanning inspiratory-expiratory discharge (IE neurons)

and 13 units expiratory-inspiratory discharge (EI neurons). The commonly used criteria for recognition were adopted, *i.e.*, periodic bursting discharge alternating with interburst intervals in phase with a given fraction of the cycle, stability and independency from the respirator action (for detailed description of patterns, see Fallert and Baum (9) and Fallert and Wassermeyer (10)). In addition the activity of 120 UMN was also recorded. They were found to be scattered all over the area investigated.

Antidromic responses of an UMN to spinal cord stimulation (SCS) are illustrated in Fig. 1 *A*, orthodromic responses in Fig. 1 *B*. In the latter case latency was longer and variability of latency was larger with latencies being distributed in such a way that short latencies occured more frequently than longer ones. A diagram was established by plotting mean latencies of spike responses obtained from all neurons versus variability of latency (Fig. 2 *A*; note semilogarithmic scale). Both variables proved to be stongly correlated (correlation coefficient = 0.84, probability of error < 0.001), and this was true for RMN and UMN, for vagal nerve stimulation (VNS) and SCS and for all stimulation frequencies and pulse durations tested. When stimulation frequencies were raised, however, variability of latency tended to decrease. That is why in a second diagram such tests were considered only in which stimuli were delivered at 10 pps (Fig. 2 *B*; arithmetic scale). This diagram revealed grouping of plotted points in clusters which were clearly separated by spacings in which no points were found. In cluster A 1 upper limit of variability of latency was 100 μsec, a value accepted for antidromic

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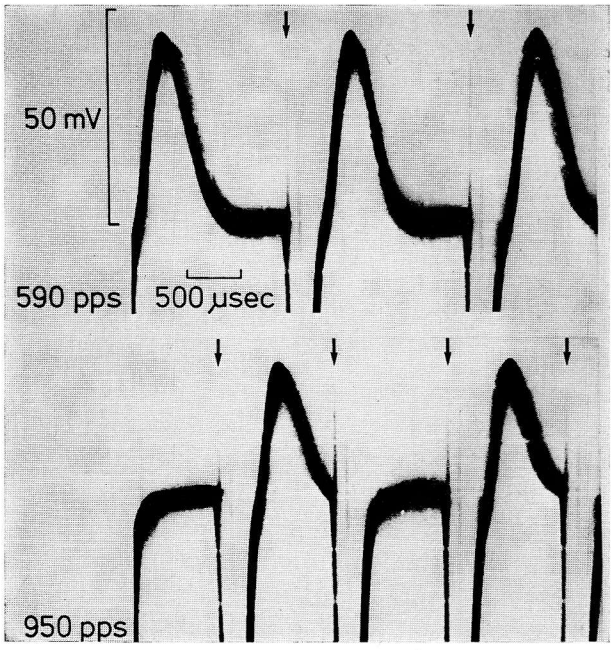
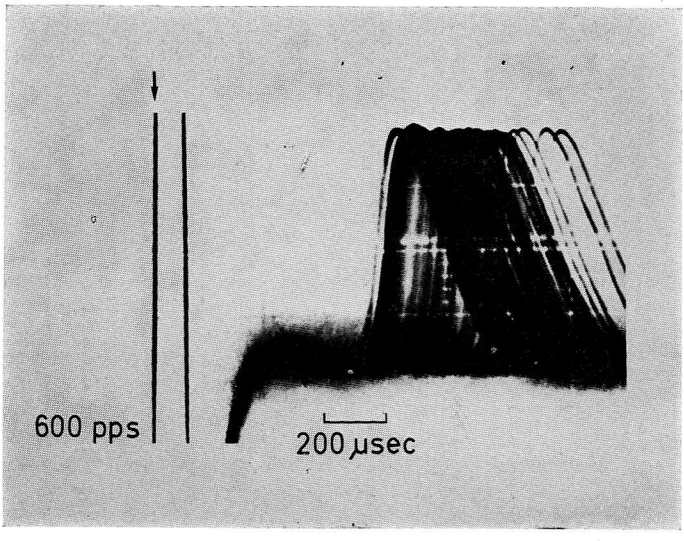
Fig. 1. – *Antidromic and orthodromic responses of UMN.*

A. Antidromic response of an UMN to SCS at cut-off frequency. Unretouched intracellular recordings. Photographic exposure was 1 sec; 250 stimulus-triggered sweeps are superposed. At 590 pps (upper trace) the unit still faithfully followed the stimuli (at arrows); mean latency (to peak) was 600 μsec. Small variability of latency is visible at peaks of action potentials. At 950 pps (lower trace) alternating response occured; each second pulse was ineffectual, the neuron still being refractory. Mean latency was 560 μsec. *B*. Orthodromic response of an UMN to SCS at 600 pps. Extracellular recording. Exposure was 1 sec; 600 sweeps are superposed. The unit did not respond faithfully to the stimuli. Note distribution of latencies with predominance of short latencies. Shortest latency was 788 μsec and longest one was 1320 μsec.

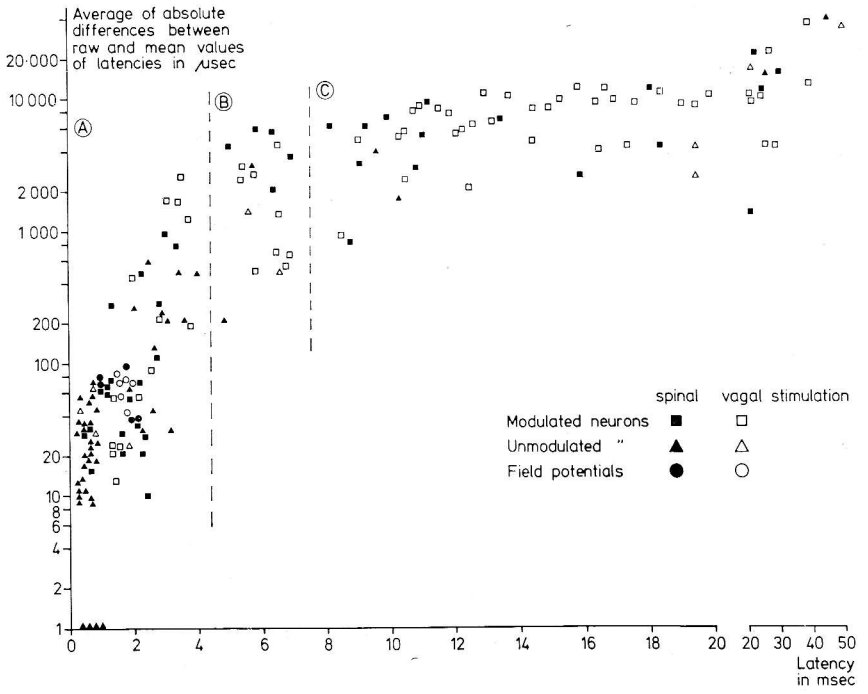


A



B

Fig. 1.


Average of absolute differences between raw and mean values of latencies in µsec
20·000
10 000
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8
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1
A
B
C
spinal
vagal stimulation
Modulated neurons
Unmodulated "
Field potentials
0
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12
14
16
18
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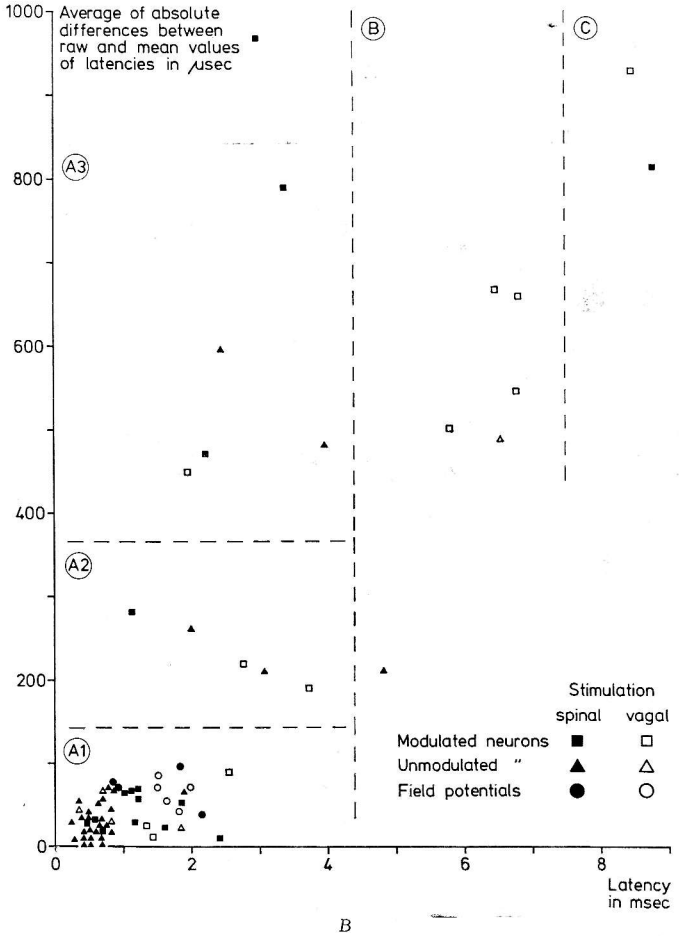
20
30
40
50
Latency in msec

A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Latency in msec

B

Fig. 2.

invasion of callosal efferents (24). Within the range of 100 μsec latencies of RMN oscillated, being short during burst discharge and longer during interburst intervals. A further support for antidromicity was occurrence of collision and faithful response at elevated stimulus frequencies in all tests located in cluster A 1. In some instances, VNS or SCS evoked long-lasting potentials which were positive-negative in polarity and the amplitude of which was comparable to that of extracellular recordings of action potentials (so-called field potentials). The latency of these graded potentials was mostly short and the variability of latency was small which indicated that they were conducted by fast axons without interposed synapse. Lower limit of cluster A 2 was 190 μsec variability of latency and upper limit was 280 μsec. Lower limit of cluster A 3 was 450 μsec. Clusters A 2 and A 3 probably represent mono- and disynaptic responses, respectively. Clusters B and C were made up of points exhibiting both distinctly longer latencies and higher variability of latency. From this it was concluded that number of synaptic delays stepwise increased by one from cluster to cluster.

When activation was orthodromic, latency did not depend on timing of the stimulus within the interspike interval. Latency of action potentials depended on duration of interpulse intervals in a rather individual way. With decreasing intervals in some UMN latency became shorter, in others, however, longer with the cri-

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Fig. 2. – *Relation between latencies of action potentials and variability of latency.*

A. 172 tests performed at various stimulus parameters. Latencies are mean values. Variability of latency grew with increasing latencies in both RMN and UMN. The values appeared to be unevenly distributed along the abscissa. Three clusters (A-C) were spaced from each other at latencies of 4.4 and 7.5 msec. Either of these spacings may reflect a synaptic delay.

B. 76 tests performed at 10 pps on one cell each. Areas A-C are shown again. Within area A three more clusters of values could be distinguished spaced from each other at variability of latency of 142 μsec and 364 μsec. All 14 RMN and 29 UMN situated within area A 1 followed faithfully stimulus frequencies of more than 100 pps and exhibited collision which suggested antidromicity. 5 neurons located within area A 2 exhibited somewhat longer latencies but distinctly larger variability of latency; together with the finding that maximum pulse frequencies for faithful response hardly exceeded 100 pps it must be assumed that activation occured by orthodromic and probably monosynaptic way. 6 neurons located within area A 3 exhibited larger variability of latency and maximum stimulus frequencies for faithful responses were low suggesting disynaptic activation. Excitation of 6 units situated within area B would hence be polysynaptic.

tical interpulse interval duration in the latter case being 1-10 msec when activation was antidromic, but 10-100 msec when excitation was orthodromic (Fig. 3). In some RMN antidromically excited by VNS, latency increased when intervals lasted 3-8 msec, but not

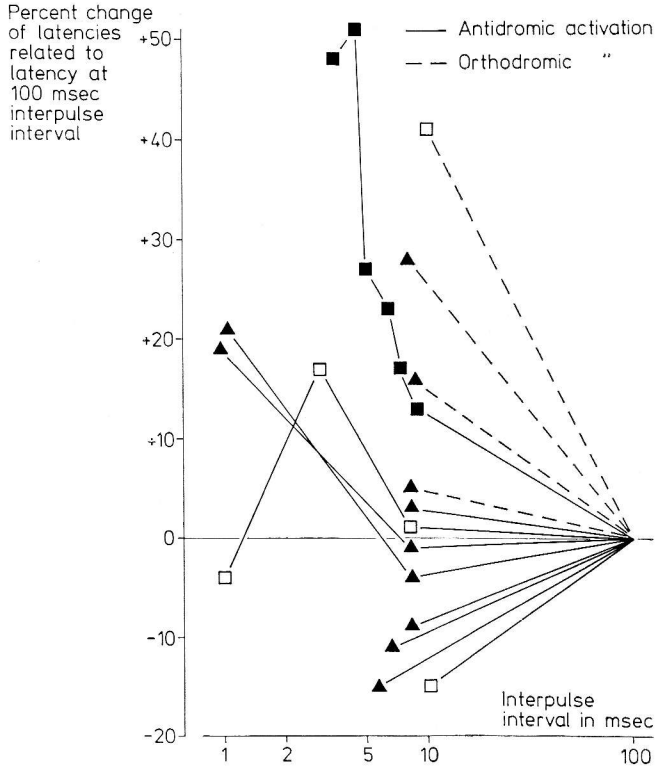

Fig. 3. – *Typical dependencies of latencies (ortho- or antidromically evoked responses) from duration of interstimulus intervals.*

Same symbols as in Fig. 2. Changes are related to interpulse intervals of 100 msec duration. In three UMN and one RMN shown, orthodromically activated by spinal or vagal way, latencies increased at intervals of 8-10 msec duration indicating deficiency of synaptic transmission. Among the antidromically activated units one RMN shown exhibited marked increase in latency during SCS when intervals were about 5 msec. The latency of response of another RMN shown (VNS) was lengthened at 3 msec interval duration but shortened again at 1 msec. In two UMN shown, latencies changed little at 8 msec interval duration during SCS; lengthening of latencies at 1 msec interval duration signalled phase of post-spike unresposiveness. In three UMN shown (SCS) and in one RMN shown (VNS) latencies were shortened at 6-10 msec interval duration. Measurements for shorter interpulse intervals could not be obtained from those cells.

if they were shorter. In other RMN latency increased at elevated frequencies of SCS; this has also been found in cats (1). When activation was orthodromic, maximum stimulation frequencies for faithful unit response hardly exceeded 100 pps but they were higher in cases of antidromic invasion. At cutting-off frequency some UMN exibited discharge alternating in an 1:2 manner; sometimes latency became shorter (Fig. 1 *A*). When stimulus frequency was further increased in a unit exhibiting alternating response, the amplitude of every second action potential became smaller and latency of the latter became longer. At some instances a particular kind of response was observed; when stimuli were delivered during the phase of relative refractoriness originating from the preceding response, latency increased from pulse to pulse and amplitude was reduced and in regular intervals a SD spike failed to be generated. After this a response of normal amplitude appeared and latency was short again. During burst discharge of RMN antidromically evoked spikes often entailed delay of generation of the following spontaneous action potential and thus reduced the spontaneous firing rate. At high stimulus frequencies the probability of collision to occur is thus reduced. This may be the reason why in the test illustrated in Fig. 1 *A* collision did not occur.

Care must be taken in setting the stimulus strength, since threshold of antidromic responses of RMN oscillates throughout the respiratory cycle. In I and E units threshold was lowest during burst discharge, was somewhat higher during the second half of the interburst interval and was highest during the early part of the latter (Fig. 4).

Conduction velocities of vagal and spinal efferents and latencies of orthodromic responses for all types of RMN and UMN as well as velocities calculated for field potentials are listed in Table I; the number of RMN and UMN which were antidromically or orthodromically excited by vagal or spinal way is also indicated. The proportion of RMN having vagal or spinal axonal projections was as low as 15% (13 out of 84 RMN). In the cat the proportion of I neurons sending their axons along with the vagal nerves is reported to be 3-15% (3, 5, 23) and that of E units 8-46% (3, 4, 5, 23). In the rabbit we found a proportion of 8% (3 out of 40 neurons) in EI and I neurons, whereas in IE and E units antidromic responses to VNS did not occur. Results about the proportion of RMN of the cat sending their axons down the spinal cord vary between 15% and

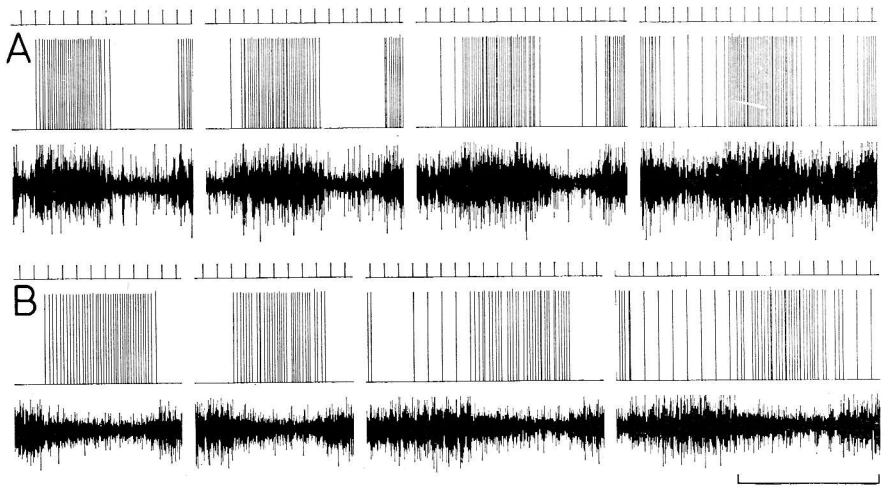

Fig. 4. – *Cyclic oscillation of threshold voltage of antidromical spike invasion.*

SCS delivered at 10 pps. Time base is 1 sec. *A*. I neuron; from left to right side: 15 V, stimuli were below threshold; 16 V, spikes were elicited at the end of the interburst pause and during the first half of the burst discharge; 17 V, spikes were elicited towards the end of the silent period and throughout burst discharge; 20 V, stimuli were supra-threshold throughout the respiratory cycle. Note short inhibition of spike discharge following evoked action potentials during the burst discharge. *B*. E neuron exhibiting similar oscillation of threshold; from left to right side voltages were: 9 V; 11 V; 13 V; 15 V.

96% in I neurons and 19% and 98% in E units (2, 3, 4, 5, 7, 12, 17, 18, 23). In the rabbit we could find such high proportion in UMN only, but not in RMN. Only 12% (10 out of 84 units) of all RMN were found to have spinal descending axons, with the I neurons having 22% (6 out of 27 cells) and the other phase types 6-11%. In the cat all IE units project into the spinal cord (3). It seems that in the rabbit the major part of bulbar RMN are ' propriobulbar ' which are supposed to be mainly involved in central rhythm generation. In the experiments reported above conduction of spinal axons emerging from RMN was found to range from 9-45 m per sec. While comparable values were found by some authors (3, 5, 6, 7), higher values ranging from 30 up to 93 m per sec were described by other investigators (3, 5, 13, 14, 19, 22).

As can be seen from Table I, the proportion of RMN getting vagal or spinal afferents was as low as 14% (12 out of 84 RMN). Spinal

TABLE I. – *Projections and afferents of RMN and UMN.*

Numbers of RMN and UMN which project into the vagal nerves or down the spinal cord or which receive vagal or spinal afferents are shown. Latencies of responses of all four types of RMN and UMN and latencies of field potentials elicited by VNS or SCS are listed. From latencies conduction velocities of vagal and spinal axons were calculated. When latencies of orthodromically evoked responses were short, conduction velocities of afferents were calculated based on monosynaptic unit excitation; 0.5 msec were allowed for synaptic transmission (left hand side values in last column). In addition conduction velocities were calculated taking into account the number of interposed synapses as deduced from cluster formation illustrated in Fig. 2 (right hand side values in last column).

	Cell type (Number of cells tested)	Antidromic activation		Orthodromic activation	
		Latency in msec	Conduction velocity in m/sec	Latency in msec	Conduction velocity in m/sec
Vagal nerve stimul.	EI (13)	1.34 2.55	37.2 19.6	1.98 6.64	33.8 51.0 9.7
	I (27)	1.44	34.8	3.07 3.52	19.5 24.1 16.6 19.8
	IE (9)	—	—	6.87	9.3
	E (35)	—	—	3.70 3.74	15.6 18.5 15.4
	UMN (120)	0.36 0.77 1.86	140.5 64.7 26.9	—	—
	Field pot. (5)	1.66 ± 0.14 (n = 5)	30.1 ± 0.7	—	—
Spinal cord stimul.	EI	1.65	12.7	1.28 2.06	26.9 13.5
	I	1.16 ± 0.51 (n = 6)	21.6 ± 10.1	2.97	8.5 10.7
	IE	2.40	8.73	—	—
	E	0.47 1.19	45.0 17.7	2.24 3.34	12.1 16.9 7.4 9.0
	UMN	0.47 ± 0.25 (n = 80)	45.2 ± 16.3	0.79 3.03 ± 1.08 (n = 8)	72.4 9.6 ± 3.9
	Field pot. (4)	1.24 ± 0.28 (n = 4)	16.1 ± 7.1	—	—

afferents to IE neurons and vagal afferents to UMN were not found. When calculation of conduction velocities of vagal afferents to RMN was based on monosynaptic transmission and 0.5 msec were allowed for synaptic delay, the range was 15-34 m per sec. If the cluster formation illustrated in Fig. 2 was taken into consideration and calculation was based on the corresponding estimated number of synapses, the range was 9-51 m per sec. In rabbits, the B 2 fibre group which mediates lung collapse reflex conducts at 15-17 m per sec and the B 1 fibres originating from lung stretch receptors which mediate the Hering-Breuer inflation reflex conduct at 38-44 m per sec (16). In cats, afferent conduction velocity was found to be 33 m per sec (8). Long latencies noted in an EI and an IE cell strongly suggest that excitation was transmitted over more than the three synapses corresponding to cluster B, unless slow afferent vagal conduction is assumed. Latencies of spinally evoked orthodromic responses of RMN were found to range from 1.3-3.4 msec which is in the order of magnitude of values described for cats (19).

A predilection site of RMN sending their axon with the vagal nerves or down the spinal cord to be located in the solitary tract or in the nucleus ambiguus region could not be found. The same was true for RMN receiving vagal or spinal afferents. In contrast, UMN which were antidromically excited by VNS were located in the nucleus ambiguus region. UMN which project down the spinal cord were found scattered all over the area investigated. The finding that UMN differ not only in the arrangement of efferents and afferents but also in conduction velocities from RMN lends further support to the assumption that RMN represent an own kind of neurons. The data reported above underscore the conception uttered by Gromysz and Karczewski (11) that there are structural differences in the bulbar respiratory complex of rabbits, compared to the cat.

Summary

Respiratory modulated neurons of the rabbit were examined for efferent projections and afferent connections via the spinal cord or the vagal nerves. Variability of latency of responses proved to be related to latencies. When latency of evoked potentials is plotted versus variability of latency, monosynaptic responses can apparently

be discriminated from oligo- or polysynaptic responses. Latency of antidromic responses depends from stimulus frequencies used. Results showed that only 12% of all respiratory modulated neurons have spinal descending axons conducting at 9-45 m per sec and 8% of the inspiratory group of neurons possess axons running along with the vagal nerves conducting at 20-37 m per sec. It is concluded that the respiratory network in the rabbit is essentially different from that in the cat.

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REFERENCES

1. Baumgarten, R. von and Nakayama, S. Spontane und reizbedingte Änderungen der antidromen Erregbarkeit von bulbären respiratorischen Nervenzellen der Katze. *Pflügers Arch., 281*: 245-258, 1964.
2. Bianchi, A. L. Activation antidromique des neurones respiratoires bulbaires. *J. Physiologie. 61*:, Suppl. 1: 91, 1969.
3. Bianchi, A. L. Localisation et étude des neurones respiratoires bulbaires. Mise en jeu antidromique par stimulation spinale ou vagale. *J. Physiologie, 63*: 5-40, 1971.
4. Bianchi, A. L. and Barillot, J. C. Modification de l'activité des neurones respiratoires bulbaires au cours des réflexes de Hering-Breuer. *J. Physiologie, 63*: 116 A, 1971.
5. Bianchi, A. L. and Barillot, J. C. Activity of medullary respiratory neurones during reflexes from the lungs in cats. *Resp. Physiol., 25*: 335-352, 1975.
6. Bianchi, A. L. and Barillot, J. C. Mode d'intervention des neurones bulbo-spinaux et des neurones propriobulbaires inspiratoires dans la commande de la respiration. Pp. 31-39. In Duron, B. (Ed.), *Respiratory centres and afferent systems. INSERM Symp. Vol. 59*, 1976.
7. Berger, A. J. Dorsal respiratory group neurons in the medulla of cat: spinal projections, responses to lung inflation and superior laryngeal nerve stimulation. *Brain Res., 135*: 231-254, 1977.
8. Euler, C. von, Hayward, J. N., Marttila, I. and Wyman, R. J. Respiratory neurones of the ventrolateral nucleus of the solitary tract of cat: vagal input, spinal connections and morphological identification. *Brain Res., 61*: 1-22, 1973.
9. Fallert, M. and Baum, K. The bulbar respiratory centre in the rabbit. II. Responses of respiratory neurons to intermittent electrical bulbar stimulation during in- or expiration. *Pflügers Arch., 364*: 269-284, 1976.
10. Fallert, M. and Wassermeyer, B. Spatial distribution of various types of bulbar respiratory neurons in the rabbit. *Exp. Brain Res., 30*: 339-351, 1977.
11. Gromysz, H. and Karczewski, W. A. Responses of the brain stem respiratory neurones to stimulation of the vagal input. Pp. 117-128. In Duron, B. (Ed.), *Respiratory centres and afferent systems. INSERM Symp. Vol. 59*, 1976.

12. HUKUHARA, T. JR. Neuronal organization of the central respiratory mechanisms in the brain stem of the cat. *Acta Neurobiol. Exp., 33*: 219-244, 1973.
13. KIRKWOOD, P. A. and SEARS, T. A. Monosynaptic excitation of the thoracic expiratory motoneurones from lateral respiratory neurones in the medulla of the cat. *J. Physiol., Lond., 234*: 87-89 *P*, 1973.
14. KREUTER, F., RICHTER, D. W., CAMERER, H. and SENEKOWITSCH, R. Morphological and electrical description of medullary respiratory neurons of the cat. *Pflügers Arch., 372*: 7-16, 1977.
15. LIPSKI, J., TRZEBSKI, A. and KUBIN, L. Excitability changes of dorsal inspiratory neurons during lung inflations as studied by measurement of antidromic invasion latencies. *Brain Res., 161*: 25-38, 1979.
16. LÜSCHER, H.-R. *Ein Beitrag zur Selbstssteuerung der Atmung.* Inaugural-Dissertation, Zürich, pp. 1-25 1975.
17. MERRILL, E. G. The lateral respiratory neurones of the medulla: their associations with nucleus ambiguus, nucleus retroambigualis, the spinal accessory nucleus and the spinal cord. *Brain Res., 24*: 11-28, 1970.
18. MERRILL, E. G. Finding a respiratory function for the medullary respiratory neurons. Pp. 451-486. In BELLAIRS, R. and GRAY E. G., (Eds.), *Essays on the nervous system.* Oxford, Clarendon Press, 1974.
19. NAKAYAMA, S. and BAUMGARTEN, R. VON. Lokalisierung absteigender Atmungsbahnen im Rückenmark der Katze mittels antidromer Reizung. *Pflügers Arch. 281*: 231-244, 1964.
20. PORTER, R. Unit responses evoked in the medulla oblongata by vagus nerve stimulation. *J. Physiol., Lond., 168*: 717-735, 1963.
21. PUIZILLOUT, J.-J. and TERNAUX, J.-P. Variations d'activités toniques, phasiques et respiratoires, au niveau bulbaire pendant l'endormement de la préparation encéphale isolé. *Brain Res., 66*: 67-83, 1974.
22. RICHTER, D. W., HEYDE, F. and GABRIEL, M. Intracellular recordings from different types of medullary respiratory neurons of the cat. *J. Neurophysiol., 38*: 1162-1171, 1975.
23. RICHTER, D. W., CAMERER, H., MEESMANN, M. and RÖHRIG, N. Studies on the synaptic interconnection between bulbar respiratory neurones of cats. *Pflügers Arch., 380*: 245-257, 1979.
24. SWADLOW, H. A., WAXMAN, S. G. and ROSENE, D. L. Latency variability and the identification of antidromically activated neurons in mammalian brain. *Exp. Brain Res., 32*: 439-443, 1978.