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Kainic Acid Lesions to the Lateral Tegmental Field of Medulla: Effects on Cough, Expiration and Aspiration Reflexes in Anesthetized Cats

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Summary

We have tested the hypothesis that neurons of both the ventral reticular nucleus and the adjacent parts of the lateral tegmental field (LTF) may be important for the production of motor programs associated with cough, expiration and aspiration reflexes. Our studies were conducted on non-decerebrate, spontaneously breathing cats under pentobarbitone anesthesia. Dysfunction of the medullary LTF region above the obex, produced by uni- or bilateral injections of kainic acid (a neurotoxin), regularly abolished the cough reflex evoked by mechanical stimulation of both the tracheobronchial and laryngeal regions and in most cases also the expiration reflex induced from the glottal area. However, some electrical activity still occurred in the neurogram of the recurrent laryngeal nerve during probing the laryngeal and glottal regions. Interestingly, the aspiration reflex elicited from the nasopharynx regularly persisted, although with lower intensity after the LTF lesion. Nevertheless, successive midcollicular decerebration performed in four cats also abolished the aspiration reflex. These experiments demonstrate the importance of medullary LTF neurons for the normal occurrence of cough and expiration reflexes. One possible explanation for the elimination of these expulsive processes is that the blockade of the LTF neurons may remove an important source of a facilitatory input to the brainstem circuitries that mediate cough and expiration reflexes. In addition, the potential importance of the mesencephalic reticular formation for the occurrence of the aspiration reflex and the role of the LTF in modulating both the eupnoeic breathing and the blood pressure are also discussed.

Key words

Lateral tegmental field of the medulla • Cough • Expiration and aspiration reflexes • Kainic acid lesions • Midbrain transections • Cat

Introduction

The last decade, which has been dedicated to the brain research, has also brought considerable progress in the field of respiratory neurophysiology. However, compared to the recent discoveries linked to normal respiratory rhythmogenesis, the brainstem structures and central mechanisms involved in the production of airway reflexes such as the tracheobronchial (TB) and laryngo-pharyngeal (LPh) coughs, expiration (ER) and aspiration (AR) reflexes are far from being understood sufficiently. Recently, Shannon *et al.* (1996, 1998) have proposed the

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first model of the central neural circuitry responsible for the cough motor pattern in the cat. The principal feature of this model is that the motor pattern of both eupnoeic breathing and the cough motor pattern is produced by the same respiratory neuronal networks in the Bötzinger complex/rostral ventral respiratory group (VRG) of the medulla (see also Bongianni *et al.* 1998). According to this hypothesis, the Central Pattern Generator (CPG) of breathing seems to be modified to produce cough by excitatory inputs from medullary solitary tract nucleus (NTS) of second-order interneurons mediating afferent information from rapidly and slowly adapting pulmonary receptors. However, it seems to be reasonable to suppose that another brainstem neuronal circuits overlapping the main brainstem respiratory networks could play at least a modulatory role in the neural processing of the cough and other defensive airway reflexes. Mapping of the brainstem neurons by using the C-Fos like immunoreactive method in "fictive" laryngeal cough, has revealed an involvement of respiratory neurons localized not only in the dorsal and ventral respiratory groups but also in the medial and lateral reticular, raphe and pontine parabrachial nuclei (Gestreau *et al.* 1997). In addition, a modulatory role of the interposed cerebellar nuclei on the cough motor pattern was also described in decerebrate cats (Xu *et al.* 1997). Moreover, our recent experiments in non-paralyzed cats with kainic acid lesions to the raphe nuclei and to the adjacent medial reticular formation confirmed the importance of the medullary midline region for producing the cough and expiration reflex motor patterns (Jakuš *et al.* 1998). Thus, it seems that the rapheal and medial systems of the medullary reticular formation may have some facilitatory effects on the brainstem circuitries mediating these reflex behaviors. However, the proper location of the second- and higher-order neurons in processing the aformentioned airway reflexes still remains unknown. In addition, previous studies in decerebrate, vagotomized and paralyzed cats suggested that the reticular neurons of the medullary LTF may be critically involved in generation of complex motor patterns such as gasping and the "gasp-like" aspiration reflex (St. John 1990, Fung *et al.* 1994, 1995), vomiting (Miller and Rugiero 1994), sneezing (Wallois *et al.* 1995), as well as in the sympathetic and baroreceptor reflex control (Gebber and Barman 1985, Clement and McCall 1993) and in the production of vestibulo-sympathetic reflexes (Yates *et al.* 1995). However, the LTF neurons appear to play no role in the neurogenesis of eupnoea in the cat.

The present study performed in anesthetized, spontaneously breathing cats was designed to examine the effects of kainic acid lesions to the area of the nucleus reticularis ventralis and the adjacent parts of the rostral medullary LTF on the elicitability, character and intensity of the TB and LPh coughs as well as the expiration and aspiration reflexes, evoked by mechanical stimulation of the particular airway regions. The changes in both the eupnoeic breathing pattern and the systemic blood pressure following kainic acid injections into the LTF and the effects of successive mesencephalic transections are also described.

Methods

The experiments were performed on seventeen non-decerebrate, non-paralyzed adult cats of either sex (mean body weight 2.74±0.38 kg). The animals were anesthetized with sodium pentobarbitone (Pentobarbital, Spofa) by an initial intraperitoneal (i.p.) dose of 35 mg. kg^{-1} and additional maintenance doses of 2 $mg.kg^{-1}.h^{-1}$ given intravenously (i.v.).

A supplemental dose of pentobarbitone was given if the blood pressure or the respiratory rate increased spontaneously or in response to surgical procedures or squeezing a paw. Airway reflexes were evoked at least 20 min after the administration of an additional dose of anesthetic.

The general surgical preparation has been described in detail previously (Jakuš *et al.* 1998). Briefly, after a tracheostomy the right femoral vein and artery were cannulated for further intravenous injections and measurement of arterial blood pressure (BP), respectively. A cannula for recording pleural pressure (P_{pl}) was introduced into the right pleural cavity. Bipolar teflon-coated stainless steel wire electrodes (50-150 µm in diameter) were introduced into the sternal part of the diaphragm (Dia) and the abdominal (rectus or external obliquus) muscles (Abd) for recording of their electromyographic activities. In twelve cats, the electroneurogram of the right or left recurrent laryngeal nerves (Rec) was also recorded. The animal was then placed in a stereotaxic frame. The dorsal surface of the medulla was exposed by occipital craniotomy and partial cerebellectomy. In four cats, the tectum of the mesencephalon and corpora quadrigemina were disclosed by a wide parietal craniotomy with ablation of the bony tentorium, completed by suction of the overlying parts of the cerebral cortex and cerebellum. After performing a kainic acid lesion into the LTF, successive midbrain surgical transections were performed above and below the superior colliculi (supra- and midcollicular decerebrations), respectively.

The electromyographic (EMG) activities (Dia, Abd) and *electroneurogram of the recurrent laryngeal nerve* (ENG_{Rec}) were amplified, high and low pass filtered (Iso-Dam8 Amplifier, WPI), integrated with resistance-capacitance circuits (time constant 0.05 s), and then displayed on the oscilloscopes (Tektronix-5223, Tesla OPD 609). The raw or integrated electrical activities were digitized and recorded together with BP and P_{pl} signals on-line at 2 kHz using PC software (Lab View, National Instruments).

The defensive airway reflexes – TB and LPh coughs, ER and AR were elicited by mechanical stimulation of the tracheobronchial, laryngopharyngeal, glottal and nasopharyngeal regions *(via* a ventrolateral pharyngostomy), respectively, with a nylon fibre (diameter 0.3-0.5 mm). Typically, the mechanical probing in the tracheobronchial tree and laryngo-pharyngeal area was performed at the inspiratory phase of breathing, the glottal region was stimulated mostly during the expiratory phase and the nasopharynx during both phases of the breathing cycle.

In the course of the experiments, end-tidal CO_2 ($ETCO_2$) was continuously measured by a capnograph (Capnogard, Novametrix). Normocapnia was considered as an $ETCO_2$ fractional concentration of approximately 0.045. In addition, arterial blood samples were analyzed for PO_2, PCO_2 and pH using a blood gas analyzer (AVL - Compact 2). Metabolic acidosis, when it occurred sporadically, was corrected by intravenous administration of 8.4 % sodium bicarbonate. The animals received Hydrocortison (VUAB, Prague) in a single dose 3.0 mg. kg^{-1} i.v. to minimize brainstem swelling. Rectal temperature was maintained at 37.5 °C using a servo controlled heating lamp. At the end of the experiments the animals were killed by an overdose of Pentobarbital.

Chemical lesions of the neurons in the lateral tegmental field were induce by microinjections (0.03-0.1 μl) of the excitotoxin, kainic acid (Sigma, 2.0 mg/ml), dissolved in phosphate buffered saline (pH=7.4), using a glass micropipette with a tip diameter of 20–50 μm. The pipette contained a solution of both kainic acid to destroy the neurons and a Fast Green dye to localize the spreading of the solution. Similarly as was described by Fung *et al.* (1994), the pipette was pressurized in order to eject the solution. The ejected amount was defined by reference to calibrations which had been established for similar micropipettes. Thus, it was possible to determine the amount of solution which was ejected at different pressures for discrete time periods. This amount was determined by direct observation of the tip of the micropipette through a calibrated grid of the eye-piece. The micropipette was mounted in a stereotaxic manipulator and its tip inserted into the medulla 2.2-2.5 mm rostral to the obex, 2.2-2.5 mm lateral to the midline and 3.0-3.3 mm below the dorsal surface. Uni- or bilateral microinjections were then performed. Kainic acid as an excitatory L-glutamic acid agonist produces functional inactivation of cell bodies within 30 min while sparing fibers of passage (Coyle *et al.* 1978). Therefore, we waited 30-40 min following the injections before testing their effects. After completing the experiment, the brainstem was removed for histological processing, using transverseal sections 100 μm thick.

Measurement of variables

Computer-assisted processing of the above mentioned recorded signals was performed. Under conditions of eupnoea, the respiratory rate and the time of inspiratory (t_I), postinspiratory (t_{E1}) and expiratory (t_{E2}) phases was derived from the duration of integrated (∫) EMG activity of the diaphragm (Richter and Ballantyne 1983). Similarly, in provoked airway reflexes the particular inspiratory and expiratory durations were detected from the corresponding ∫ EGM of the diaphragm and abdominal muscles. The intensity of eupnoeic breathing and the strength of particular airway reflexes were assessed from the maximal inspiratory and expiratory pleural pressures ($P_{pl\ I}$ and $P_{pl\ E}$). For systemic arterial blood pressure, the systolic and diastolic levels were evaluated. The above specified variables for eupnoeic breathing were defined from at least five respiratory cycles during the pre- and postinjection states and the averages were thus obtained. Similarly, the airway reflexes were elicited both under control conditions and following kainic acid injections, and their variables were evaluated and averaged. The pattern of electrical activity in the recurrent laryngeal nerve was followed only qualitatively.

The results are expressed as means ± S.E.M. Analysis of variance (ANOVA) or Student´s t-test were used to determine the statistical significance of the differences, as appropriate. $P<0.05$ was considered as significant.

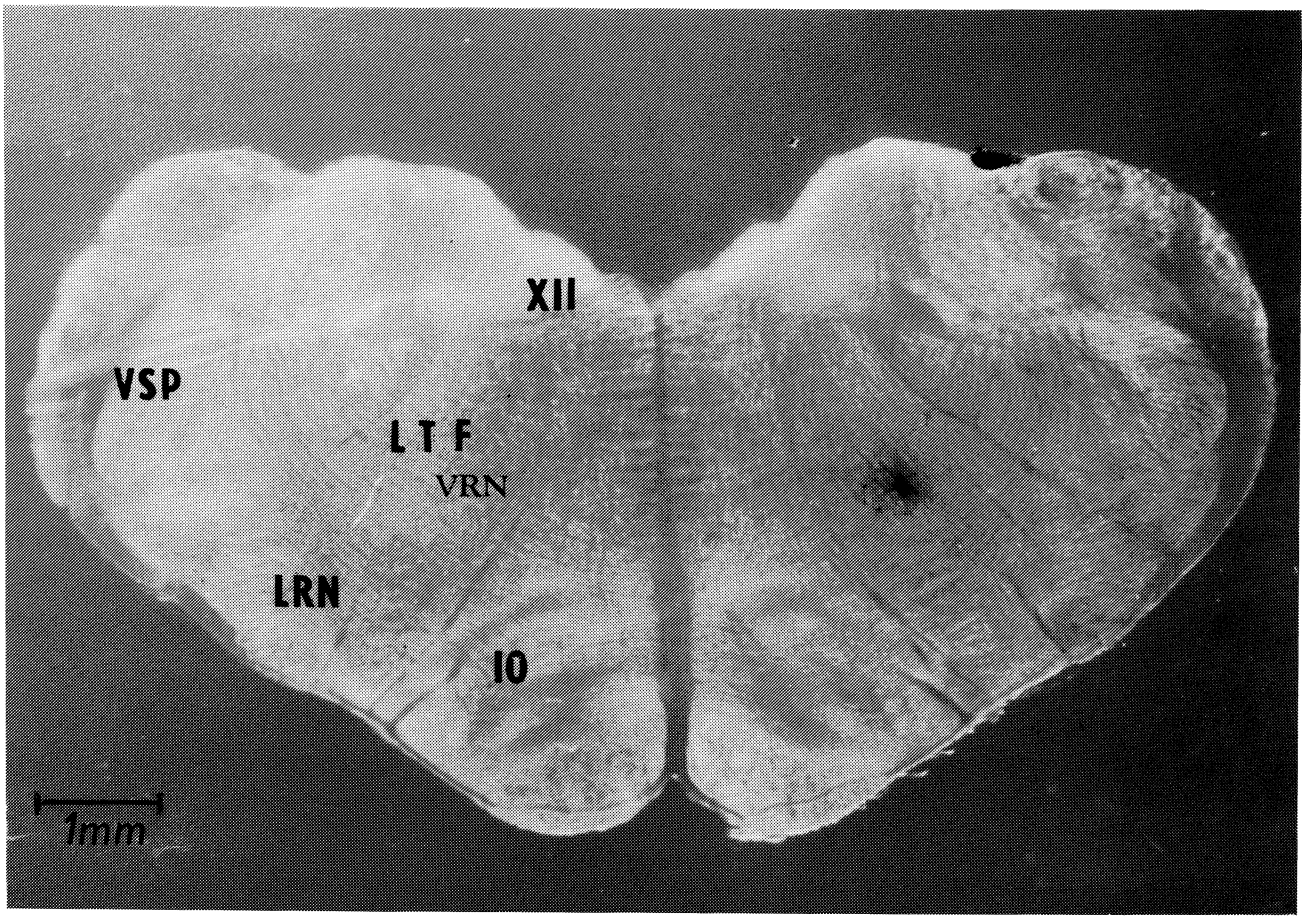

Fig. 1. *Transverse medullary section at the level 2.5 mm rostral to the obex showing the right side unilateral kainic acid lesion to the ventral reticular nucleus (VRN) and the adjacent part of the lateral tegmental field (LTF). O – inferior olivae, LRN – lateral reticular nucleus, VSP – spinal tract of the trigeminal nerve, XII – hypoglossal nucleus.*

Results

Following effects of kainic acid injections into the LTF were observed.

Histology

Unilateral or bilateral kainic acid microinjections into the LTF of the medulla were performed in seven and ten animals, respectively. Histological verification of lesioned sites in the medulla (Berman 1968) proved that the injections mostly infiltrated between 1.6-3.4 mm rostral to the obex (P12.7-P10.8), 1.6-2.7 mm lateral to the midline and 2.7-3.7 mm below the dorsal surface. The lesions mainly affected the ventromedial part of the LTF with the ventral reticular nucleus (Brodal 1957) and the adjacent part of the medial and lateral systems of the reticular formation (Petrovický 1980) above the obex (Fig. 1). Thus, the critical injection site was between the nucleus tractus solitarius (of the DRG) and the nucleus ambiguus (as a part of the VRG), i.e. in the region of the reticular formation known to contain mainly populations of non-respiratory related neurons (Fung *et al.* 1995).

Cough reflex

Under the pre-injection conditions, the cough response to mechanical stimulation of the tracheobronchial or laryngopharyngeal mucosae was characterized by typical sequential changes in the integrated EMG of the diaphragm and abdominal muscles with complex inspiro-expiratory pattern of electrical activity in the recurrent laryngeal nerve and particular inspiro-expiratory deflections of pleural pressure (Fig. 2, Control). In all trials, coughing could be reliably induced before the lesions. When comparing both kinds of cough, it was typical that the mean values of inspiratory duration and maximal inspiratory pleural pressures were higher in the LPh cough ($p<0.01$ and $p<0.02$, respectively) than the corresponding values of the TB cough (Table 1). The kainic acid injections (even in a single dose 0.06 μl given unilaterally) abolished both the inspiratory and expiratory signs of TB and LPh coughs in all the animals tested (Fig. 2, Kainic Acid). Nevertheless, in order to destroy the critical region in both halves of the medulla, the kainic acid was injected bilaterally in 10 of 17 cats. Hence, after both unilateral or bilateral injections, there was no residual diaphragmatic and abdominal electrical activities, or pleural pressure alterations resembling the TB or LPh coughs. However, following the kainic acid injections, some electrical activity provoked by mechanical laryngopharyngeal stimulation was still seen in the raw and integrated electroneurograms of recurrent laryngeal nerves, while those in the diaphragm and abdominal muscles were absent (Fig. 2, LPH-Kainic Acid).

Table 1. Mean values of neural inspiratory and expiratory durations (t_I, t_E), and maximal inspiratory and expiratory pleural pressures ($P_{pl\,I}$, $P_{pl\,E}$) in tracheobronchial (TB) and laryngopharyngeal (LPh) coughs under control conditions and after kainic acid injections into the medullary LTF.

	Control					Kainic acid	
	n	t_I **[s]**	t_E **[s]**	$P_{pl\,I}$ **[kPa]**	$P_{pl\,E}$ **[kPa]**	**n**	**cough**
TB cough	15/89	1.30±0.05	0.39±0.02	−1.33±0.05	1.65±0.14	15/150	absent
LPh cough	10/56	1.55±0.08***	0.37±0.03	−1.53±0.06**	1.47±0.20	10/52	absent

Values are means ± S.E.M., n gives the actual number of animals and the number of evaluated tests. Significant differences between the TB and LPh cough values in control conditions: ** *$p<0.02$,* *** *$p<0.01$. Absent indicates no cough response following kainic acid injections.*

Table 2. The effects of kainic acid injections into the LTF of the medulla on expiration and aspiration reflexes

	Control			Kainic acid		
	n	**t [ms]**	P_{pl} **[kPa]**	**n**	**t [ms]**	P_{pl} **[kPa]**
ER	11/66	85±3	1.2±0.1	11/189	absent	
				3/6	65±6*	0.1±0.1***
AR	16/128	75±2	−2.2±0.1	16/128	74±2	-1.7±0.1****

Significant effects of kainic acid compared to control pre-lesion conditions: * *$p<0.05$,* *** *$p<0.01$,* **** *$p<0.001$. For explanation of other symbols see Table 1.*

Expiration reflex

As shown in Figure 2, (ER-Control), mechanical stimulation of the vocal folds applied mostly at the beginning or during the control expiration regularly evoked the expiration reflex. It was characterized by brief, forceful expiratory effort with a short burst of electrical activity in both the ∫ Abd EMG and the Rec_{ENG}, accompanied by a prompt and large positive swing in pleural pressure without preceding inspiration. After kainic acid injections, mechanical probing failed to elicit any sign of ER in 96.9 % of stimulations in 11 cats (Fig. 2, ER-Kainic Acid, Table 2). Weak expiration reflexes with discernible burst of abdominal EMG activity still persisted in 6 tests (3.1 %) on three cats, however, with a 1.3-fold drop in their duration ($p<0.05$) and a 12-fold decrease in their maximal pleural pressure values ($p<0.01$), compared to the pre-injection controls (Table 2). In addition, the kainic acid injections did not abolish the response of the laryngeal motoneurons that was seen upon probing in the glottal region of all tested cats (Fig. 2, ER-Kainic Acid).

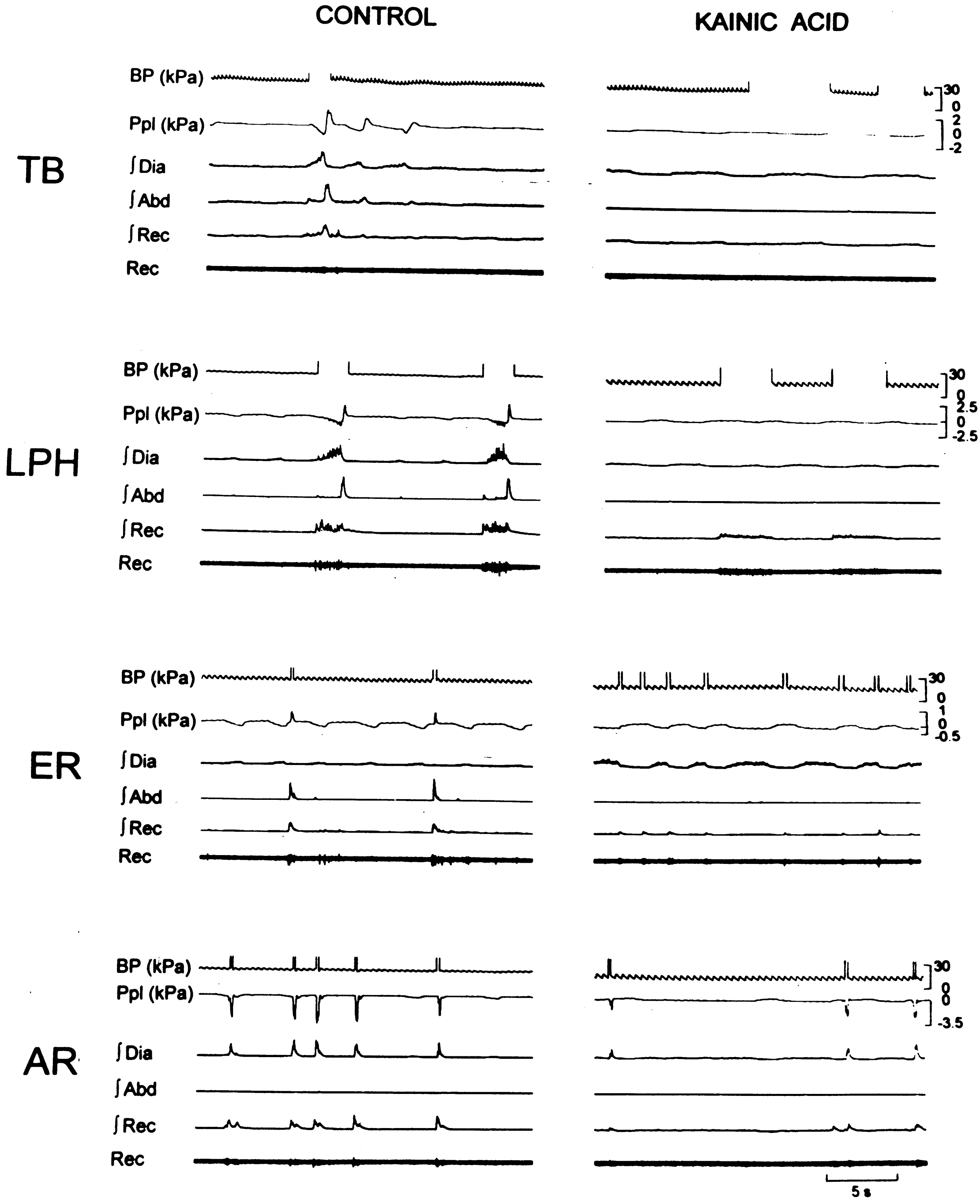

Fig. 2. *Tracheobronchial cough (TB), laryngopharyngeal cough (LPH), expiration reflex (ER) and aspiration reflex (AR) in CONTROL conditions and after the KAINIC ACID injections to the LTF of the medulla, applied unilaterally. The interruptions in blood pressure record indicate mechanical stimulations. For further explanation see the text.*

Aspiration reflex

Mechanical probing in the oro- and nasopharynx performed through a small pharyngostomic opening during control inspiration or expiration evoked an aspiration reflex. It consisted of a brief, but intense and rapid burst of ∫ Dia EMG activity, with a short, abrupt and strongly negative swing of pleural pressure, accompanied by a complex burst of activity in the raw and integrated recurrent laryngeal nerve ENGs (Fig. 2, AR-Control). Kainic acid injections did not abolish the signs of AR in our experiments on spontaneously breathing cats without decerebration. Thus, the AR persisted in all 128 tests (100 %) on 16 tested animals, without any change in its duration, but with a significant drop by 22.7 % in the negativity of maximal pleural pressure values ($p<0.001$), compared to pre-injection controls (Table 2). In contrast to these data, Fung *et al.* (1994) reported that injections of kainic acid into the same LTF area completely abolished both gasping and the "gasp-like" aspiration reflex, but this was seen in decerebrate, vagotomized, paralyzed and artificially ventilated cats. In an attempt to explain this discrepancy, successive midbrain surgical transections were performed in four cats 45-60 min after kainic acid injections into the LTF. The supracollicular transection at the level A 5.2 did not affect the elicitability as well as duration of AR and the maximal pleural pressure values in all 32 tested ARs (Fig. 3a). On the other hand, successive midcollicular transection performed at the level A 0.6 regularly eliminated any signs of the reflex (Fig. 3b).

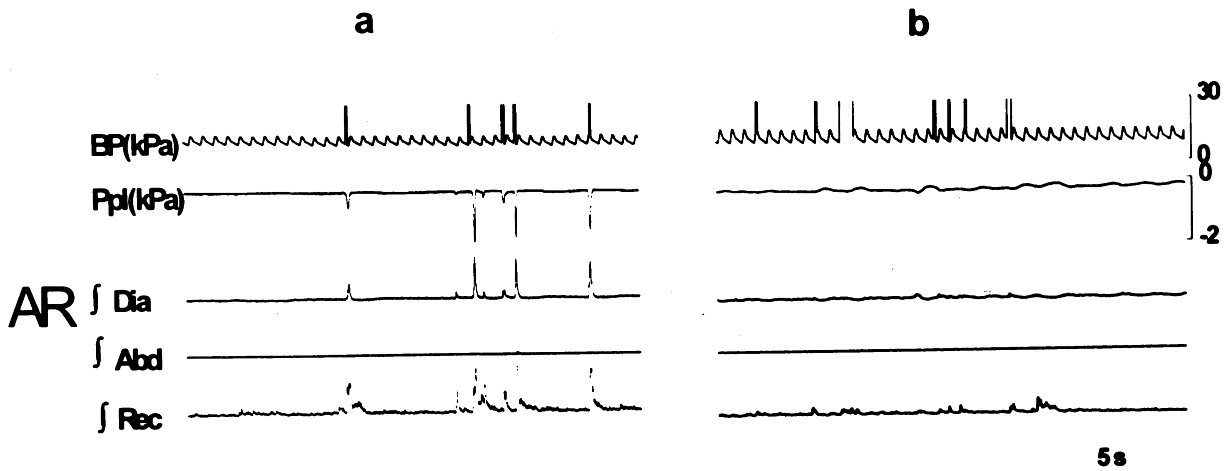

Fig. 3. *Persistence of the aspiration reflex in a cat exposed to midbrain transection above superior colliculi (record **a**) and its elimination by midcollicular decerebration (record **b**). Recorded parameters as in Figure 2.*

Table 3. Mean values of respiratory rate, neural inspiratory (t_I), postinspiratory (t_{E1}) and expiratory (t_{E2}) time durations, maximal inspiratory and expiratory pleural pressures ($P_{pl\,I}$, $P_{pl\,E}$), and systolic and diastolic blood pressures (BP_s, BP_d) during eupnoeic breathing under control conditions and following kainic acid injection into the LTF of the medulla.

		Breathing						Blood pressure	
	n	**rate [min^{-1}]**	**t_I [s]**	**t_{E1} [s]**	**t_{E2} [s]**	**$P_{pl\,I}$ [kPa]**	**$P_{pl\,E}$ [kPa]**	**BP_S [kPa]**	**BP_d [kPa]**
Control	15/15	22.7	1.18	0.32	1.27	–0.40	–0.14	23.2	16.8
		±1.4	±0.11	±0.05	±0.10	±0.05	±0.04	±0.7	±0.7
Kainic	15/15	19.3	1.85	0.56	1.10	–0.41	–0.14	18.6	12.2
acid		±1.8	±0.24**	±0.06***	±0.19	±0.05	±0.04	±1.0****	±0.9****

For symbols and significances see Tables 1 and 2.

Breathing pattern and systemic blood pressure

Table 3 summarizes the mean values of respiratory parameters, the systolic and diastolic blood pressures in fifteen cats before and after kainic acid injections into the LTF.

Since the differences between the control and post-lesion values for the investigated parameters appeared to be statistically significant in the group of animals subjected either to unilateral or bilateral injections, the data collected from both groups were pooled.

Compared to the control, the kainic acid lesions only slightly altered the pattern of eupnoeic breathing. Nevertheless, the inspiratory and postinspiratory durations were somewhat prolonged ($p<0.02$ and $p<0.01$, respectively). The evaluation of $ETCO_2$ and arterial blood gas tensions revealed no significant changes in PaO_2 and $PaCO_2$ following kainic acid injections. In spite of the fact that the blood gas tensions remained within normal limits throughout the experiment, the pH values dropped (7.291 ± 0.003) indicating that metabolic acidosis had developed which was corrected approx. 2-3 times during the experiment. The kainic acid lesions were also accompanied by a consistent drop in systolic and diastolic blood pressures ($p<0.001$) compared to pre-injection values.

Discussion

The present paper provides new and important information that kainic acid lesions in both the ventral reticular nucleus (that belongs to the medial system of the medullary reticular formation) and to the adjacent parts of the rostral medullary LTF (localized between the DRG and VRG) abolish both the tracheobronchial and laryngopharyngeal cough responses and mostly also the expiration reflex, while preserving the aspiration reflex. The brainstem contains neuronal populations extending from the level of the medulla (DRG, VRG, rapheal, medial, and lateral systems of the reticular formation) to the pons (the pontine respiratory group). These neuronal populations are important for mediating airway reflexes such as the cough (Dubi 1959, Engelhorn and Weller 1965, Kasé 1980, Jakuš *et al.* 1985, 1987, Oku *et al.* 1994, Grélot and Bianchi 1996, Shannon *et al.* 1996, Gestreau *et al.* 1997, Bongianni *et al.* 1998, Shannon *et al.* 1998, Jakuš *et al.* 1998), the expiration reflex (Bongianni *et al.* 1988, Dyachenko 1990, Jakuš *et al.* 1996) as well as the aspiration reflex (Batsel and Lines 1973, du Pont 1987, Fung *et al.* 1994, 1995). Recent studies have demonstrated that the medullary LTF plays an important integrative role in the mediation of such complex reactions as the production of gasping, aspiration reflex, vomiting, sneezing as well as in cardiovascular control and in the mediation of vestibulosympathetic reflexes (for individual references see Introduction). However, the role of the LTF in the genesis of the cough and expiration reflexes has not yet been established.

Since the nomenclature of reticular nuclei lacks uniformity, the "critical region" targeted by kainic acid injections was described according to the stereotaxic atlas of Berman (1968) and we used the terminology of Brodal (1957) and Petrovický (1980). The somata of LTF neurons in that region contain a high density of reticulo-nuclear and reticulo-reticular (ladder-like) connections with variety of nuclei in the mesencephalon, pons, and medulla (Scheibel 1984). However, their morphological and functional connections with the second- and higher-order neurons of the cough, expiration and aspiration reflexes are still unknown. Nevertheless, as has been reported previously (Brodal 1957, Pavlásek and Petrovický 1994), the medioventral part of the rostral reticular formation, being mostly affected in our cats, may transmit the received information mainly in the descendent direction, e.g. from the suprapontine, pontine and the most rostral medullary areas to the respiratory VRG premotor neurons and spinal respiratory motoneurons (Feldman 1986). On the other hand, the laterodorsal part, which is only partly affected by our lesions, mediates signals mainly in the ascending direction, e.g. from receptors in the airways and medullary NTS input neurons to the raphe, pontine and midbrain reticular neurons. In this study we recorded neither the electrical activity of neurons located in the medial part of the LTF, nor we have evaluated their anatomical and functional connections. We therefore could not define the type of neurons (respiratory-related or non-respiratory), their connections (excitatory or inhibitory) and the sites of their targets (the spinal cord, medulla, pons) which were actually affected by our lesions. Thus, the mechanisms by which the LTF neurons may contribute in mediating the information, e.g. from the NTS second-order "cough" neurons finally to the medullary Bötzinger complex/VRG areas (Shannon *et al* 1997), could not be answered .

Lack of the cough and expiration reflexes was also seen in our previous experiments in cats with kainic acid lesions to the rapheal nuclei in the medullary midline (Jakuš *et al.* 1998). The similarity of our findings obtained during dysfunction of the medial (LTF) and the rapheal (obscurus and pallidus raphe nuclei) systems of the reticular formation strongly indicates that they both may provide an important source of facilitatory input to the spinal respiratory motoneurons and/or to the brainstem regions that could produce or mediate the cough and ER. The former possibility seems to be more probable for the raphe nuclei, as was also suggested for the modulation of breathing (Holtman *et al.* 1986, Lalley *et al.* 1997) and vomiting (Miller *et al.* 1996). Moreover, in our previous experiments with kainic acid lesions to the raphe nuclei, some signs of a cough-like pattern were still preserved in the ENG of the recurrent laryngeal nerve, indicating that a central program of the cough was probably performed, but it could not be manifested (Jakuš *et al.* 1998). The latter possibility (that the brainstem regions with a central cough-coordinating network or the pre-motor and motor outputs were actually affected) might be more plausible for the LTF, because a residual bursting activity in the recurrent laryngeal nerve ENG did not resemble the cough pattern that had appeared under control conditions before the lesion. However, it should be noted that the recurrent laryngeal nerve ENG recorded in airway reflexes consisted of overlapping bursts of inspiratory, postinspiratory and expiratory laryngeal motor activities. Multifibre recordings did not make it possible to distinguish and then to quantify the laryngeal activity being involved in the particular reflex phases. Despite these uncertainties, our present study indicates that the medial part of the medullary LTF provides an important source of facilitatory input for inducing cough and ER in non-decerebrate, spontaneously breathing cats. Furthermore, the effectiveness of unilateral kainic acid lesion within the "critical" LTF area suggests that these bilateral structures are mutually interconnected and able to work "as an entity", similarly as has been previously suggested for the genesis of the respiratory rhythm (Gromysz and Karczewski 1984, Jakuš *et al.* 1990). Moreover, it seems that the efficacy of our kainic acid injections also depended on "a critical number of neurons" damaged, because the smaller injections (e.g. 0.01-0.03 μl), even when given bilaterally, were unable to abolish all signs of coughing and the ER.

An interesting, but unexpected finding concerned the preservation of the AR (elicited from the nasopharynx) after kainic acid injections into the LTF in our spontaneously breathing non-decerebrate cats. In comparable experiments of Fung *et al.* (1994), the AR was also abolished, but their experiments were performed under different conditions, e.g. after midcollicular decerebration, bilateral vagotomy, muscle paralysis and artificial ventilation. The reason for this discrepancy is still obscure. The possibility that a different locus in the LTF (than that considered by Fung and coworkers as "critical for gasping and the AR") was affected, might be excluded, since the same regions in the LTF were lesioned in both types of experiments. Nevertheless, in our cats the intensity of the AR was significantly reduced after kainic acid lesion, indicating that one of probably multiple brainstem areas involved in the manifestation of AR had been affected. Such possibility is supported by our preliminary finding that, in addition to the LTF lesion, successive surgical transections of the midbrain at the level between the superior and inferior colliculi (midcollicular decerebration) finally abolished the AR, while it persisted after a similar section performed above the superior colliculi. This finding suggests that under some circumstances (e.g. when the critical area for the AR in the LTF is damaged) certain midbrain reticular neurons at the level of the superior colliculi might also affect the appearance of the AR. However, this idea is still hypothetical, because the respective data were obtained in a limited number of animals. Nevertheless, it will be of interest to ascertain whether some of the respiratory related neurons in the central tegmental field of the midbrain (Chen *et al.* 1991), known to receive inputs from the upper airways (Chen and Eldridge 1997), could play such a potential role.

As has been noted in the results, following both uni- or bilateral injections of kainic acid into the medial part of the LTF, the eupnoeic pattern of breathing was still present, although it was moderately modified. Our lesions only slightly increased the inspiratory and postinspiratory durations alone, while the values of $ETCO_2$ and arterial blood gas tensions remained within normal limits. The reason for these moderate changes in inspiratory and postinspiratory timing is not clear, but again it could be explained by a removal of the facilitatory input from the LTF neurons to phrenic motoneurons, or to the medullary regions of the respiratory controller. Thus, the present study corroborates the former findings of St. John (1990, 1996) that the region in the LTF (stated to be critical for gasping) does not appear to play a role in the neurogenesis of eupnoea in animals with intact pons and medulla.

Furthermore, this study also supports the data on the role of LTF neurons in cardiovascular (particularly sympathetic and baroreflex) control. It was thus shown that many LTF neurons have projections to the cardiovascular regulatory region in the rostral ventrolateral medulla (Barman and Gebber 1987) and they can even participate in the generation of some components of spontaneous sympathetic nerve discharges (Barman and Gebber 1993). In this respect, the kainic acid injections in our experiments led to a specific and consistent fall of both systolic and diastolic blood pressures, although their control values were higher, mostly reflecting the level of anesthesia. Such findings were also observed following similar lesions into the raphe nuclei.

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References

BARMAN SM, GEBBER GL: Lateral tegmental field neurons of the cat medulla: a source of basal activity of ventrolateral medullospinal sympathoexcitatory neurons. *J Neurophysiol* **57:** 1410-1424, 1987.

BARMAN SM, GEBBER GL: Lateral tegmental field neurons play a permissive role in governing the 10-Hz rhythm in sympathetic nerve discharge. *Am J Physiol* **265:** R1006-R1013, 1993.

BATSEL HI, LINES AJ: Bulbar respiratory neurons participating in the sniff reflex in the cat. *Exp Neurol* **39:** 469-481, 1973.

BERMAN AL: *The Brain Stem of The Cat. A Cytoarchitectonic Atlas with Stereotaxic Coordinates.* The University of Wisconsin Press, Madison, 1968, 175 p.

BONGIANNI F, CORDA M, FONTANA G, PANTALEO T: Influences of superior laryngeal afferent stimulation on expiratory activity in cats. *J Appl Physiol* **65:** 385- 392, 1988.

BONGIANNI F, MUTOLO D, FONTANA G, PANTALEO T: Discharge patterns of Bötzinger complex neurons during cough in cat. *Am J Physiol* **274**: R1015-R1024, 1998.

BRODAL A: *The Reticular Formation of the Brain Stem. Anatomical Aspects and Functional Correlations.* Oliver and Boyd, Edinburgh, London, 1957, 87 p.

CHEN Z, ELDRIDGE FL: Imputs from upper airway affect firing of respiratory-associated midbrain neurons. *J Appl Physiol* **83:** 196-203, 1997.

CHEN Z, ELDRIDGE FL, WAGNER PG: Respiratory-associated rhythmic firing of midbrain neurones in cats: relation to level of respiratory drive. *J Physiol Lond* **437:** 305-325, 1991.

CLEMENT ME, MCCALL RB: Impairment of baroreceptor reflexes following kainic acid lesions of the lateral tegmental field. *Brain Res* **618:** 328-332, 1993.

COYLE JT, MOLLIVER ME, KUHAR MJ: In situ injection of kainic acid: a new method selectively lesioning neuronal cell bodies while sparing axons of passage. *J Comp Neurol* **180:** 301-324, 1978.

DUBI A: Zur Bedeutung pontiner Atmungssubstrate für den Husten. *Helv Physiol Acta* **17:** 166-174, 1959.

DU PONT JS: Firing patterns of bulbar respiratory neurons during sniffing in the conscious, non-paralysed rabbit. *Brain Res* **414:** 163-168, 1987.

DYACHENKO YA: Involvement of bulbar respiratory and non-respiratory neurones in onset of expiration reflex. *Neirofiziologia* **22:** 670-680, 1990.

ENGELHORN R, WELLER E: Zentrale Representation hustenwirksamer Afferenzen in der Medulla oblongata der Katze. *Pflügers Arch* **284:** 224-239, 1965.

FELDMAN JL: Neurophysiology of breathing in mammals. In. *Handbook of Physiology. The Neurons System. IV. Intrinsic Regulatory Systems of the Brain.* FE BLOOM (ed), American Physiological Society, Bethesda MD, 1986, pp. 463-524.

FUNG ML, ST. JOHN WM, TOMORI Z: Reflex recruitment of medullary gasping mechanisms in eupnoe by pharyngeal stimulations in cats. *J Physiol Lond* **475:** 519-529, 1994.

FUNG ML, TOMORI Z, ST. JOHN WM: Medullary neuronal activities in gasping induced by pharyngeal stimulation and hypoxia. *Respir Physiol* **100:** 195-202, 1995.

GEBBER GL, BARMAN SM: Lateral tegmental field neurons of cat medulla: a potential source of basal sympathetic nerve discharge. *J Neurophysiol* **54:** 1498-1512, 1985.

GESTREAU C, BIANCHI AL, GRÉLOT L: Differential brainstem Fos-like immunoreactivity after laryngeal induced coughing and its reductions by codeine. *J Neurosci* **17:** 9340-9352, 1997.

GRÉLOT L, BIANCHI AL: Multifunctional medullary respiratory neurons. In: *Neural Control of Respiratory Muscles.* AL BIANCHI, AD MILLER, BP BISHOP (eds), CRC Press, Boca Raton, FL, 1996, pp 297-304.

GROMYSZ H, KARCZEWSKI WA: The split-respiratory centre in the cat: responses to hypercapnia. *Respir Physiol* **57:** 225-234, 1984.

HOLTMAN J Jr, ANASTASI NC, NORMAN WP, DRETCHEN KL: Effect of electrical and chemical stimulation of the raphe obscurus on phrenic activity in the cat. *Brain Res* **362:** 214-220, 1986.

JAKUŠ J, TOMORI Z, STRÁNSKY A: Activity of bulbar respiratory neurones during cough and other respiratory tract reflexes in cats. *Physiol Bohemoslov* **34:** 127-136, 1985.

JAKUŠ J, TOMORI Z, BOŠEĽOVÁ L, NAGYOVÁ B, KUBINEC: Respiration and airway reflexes after transversal brain stem lesions in cats. *Physiol Bohemoslov* **36:** 329-340, 1987.

JAKUŠ J, STRÁNSKY A, NAGYOVÁ B, ORAVEC A, BOŠEĽOVÁ L, BARÁNI H: The effects of focal cooling of medulla oblongata structures on quiet breathing in cats. *Physiol Bohemoslov* **39:** 171-184, 1990.

JAKUŠ J, STRÁNSKY A, POLIAČEK I, BARÁNI H: Laryngeal patency and expiration reflex following focal cold block of the medulla in the cat. *Physiol Res* **45:** 107-116, 1996.

JAKUŠ J, STRÁNSKY A, POLIAČEK I, BARÁNI H, BOŠEĽOVÁ L: Effects of medullary midline lesions on cough and other airway reflexes in anaesthetized cats. *Physiol Res* **47**: 203-213, 1998,

KASÉ Y: Antitussive agents and their sites of action. *Trends Pharmacol Sci* **1:** 237-239, 1980.

LALLEY PM, BEŇAČKA R, BISCHOFF AM, RICHTER DW: Nucleus raphe obscurus evokes 5-HT-1A receptor-mediated modulation of respiratory neurons. *Brain Res* **747:** 156-159, 1997.

MILLER AD, RUGGIERO DA: Emetic reflex arc revealed by expression of the immediate-early gene c-fos in the cat. *J Neurosci* **14:** 871-888, 1994.

MILLER AD, NONAKA S, JAKUŠ J, YATES BJ: Modulation of vomiting by the medullary midline. *Brain Res* **737:** 51-58, 1996.

OKU Y, TANAKA I, EZURE K: Activity of bulbar respiratory neurons during fictive coughing and swallowing in the decerebrate cat. *J Physiol Lond* **480:** 309-324, 1994.

PAVLÁSEK J, PETROVICKÝ P: *The Reticular Formation and Reticulospinal System.* Veda, Bratislava, 1994, 224 p.

PETROVICKÝ P: *Reticular Formation and Its Raphe System I. Cytoarchitectonics with Comparative Aspects.* Acta Univ. Carolinae Med. Monographia XLIX, Prague, 1980, 117 p.

RICHTER DW, BALLANTYNE D: A three phase theory about the basic respiratory pattern generator. In: *Central Neurone Environment.* ME SCHLÄFKE, HP KOEPCHEN, WR SEE (eds), Springer, Berlin, 1983, pp 164-174.

SCHEIBEL AB: The brain stem reticular core and sensory function. In: *Handbook of Physiology. The Nervous System, Sensory Processes.* Bethesda, MD. American Physiological Society, 1984, sect. I, vol III, part I, Chapt. 6, pp 213-256.

SHANNON R, BAEKEY DM, MORRIS KF, LINDSEY BG: Brainstem respiratory networks and cough. *Pulm Pharmacol* **9:** 343-347, 1996.

SHANNON R, BOLSER DC, LINDSEY BG: Neural control of coughing and sneezing. In: *Neural Control of the Respiratory Muscles.* AD MILLER, AL BIANCHI, BP BISHOP (eds) CRC, Boca Raton, FL, 1997, pp 213-222.

SHANNON R, BAEKEY DM, MORRIS KF, LINDSEY BG: Ventrolateral medulla respiratory network and a model of cough motor pattern generation. *J Appl Physiol* **84:** 2020-2035, 1998.

ST. JOHN WM: Neurogenesis, control and functional significance of gasping. *J Appl. Physiol* **68:** 1305-1315, 1990.

ST. JOHN WM: Medullary regions for neurogenesis of gasping: noeud vital or noeuds vitals. *J Appl Physiol* **81:** 1865-1877, 1996.

WALLOIS F, GROS F, MASMOUDI K, LARNICOL N: C-Fos like immunoreactivity in the cat brainstem evoked by sneeze-inducing air puff stimulation of the nasal mucosa. *Brain Res* **687:** 143-154, 1995.

XU F, FRAZIER DT, ZHANG Z, BAEKEY DM, SHANNON R: Cerebellar modulation of cough motor pattern in cats. *J Appl Physiol* **83:** 391-397, 1997.

YATES BJ, BALABAN CD, MILLER AD, ENDO K, YAMAGUCHI Y: Vestibular inputs to the lateral tegmental field of the cat: potential role in autonomic control. *Brain Res* **689:** 197-206, 1995.

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