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Respiratory responses to stimulation of spinal or medullary locomotor structures in decerebrate cats

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Abstract. Respiratory and locomotor EMG activity was recorded in cats after a precollicular post-mamillary decerebration. Locomotion was induced by stimulating either the dorsolateral funiculus (DLF) in the cervical spinal cord or the medullary locomotor strip (MLS). At the onset of locomotion, both ventilation and blood pressure were enhanced. During locomotion, the activity of external intercostal muscles decreased but that of the internal intercostal muscles increased. The respiratory pattern changed with the onset of stimulation. The locomotor movements were evoked after a delay. The inspiratory-inhibitory Hering-Breuer reflex was attenuated. Stimulation of the MLS and DLF evoked similar respiratory and circulatory effects. Our data resemble the effects observed during stimulation of the subthalamic or mesencephalic locomotor regions. We conclude that respiratory changes are part of an integrated response involved in the onset of exercise and are independent of the neuronal site where stimulation evoked locomotion. In contrast to previous reports, we suggest that the pattern of interaction among respiratory, circulatory, and locomotor systems does not have to be the specialty of supramedullary structures. Coupling between locomotion and breathing during the post-inspiratory phase suggests that this interaction occurs at the medullary level.

Key words: respiration, locomotion, dorsolateral funiculus, electrical stimulation, decerebration

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INTRODUCTION

Since the study of Krogh and Lindhard (1913), it has been known that the onset of exercise is associated with a concurrent increase in ventilation. To explain this fast respiratory response to exercise, the existence of a neural feed-forward mechanism has been suggested to integrate respiration, circulation and locomotion (Eldridge et al. 1981, DiMarco et al. 1983). In support of this hypothesis, experiments were performed on decerebrate cats in which stepping movements were elicited by electrical stimulation of subthalamic (SLR) or mesencephalic (MLR) locomotor regions (Waller 1940, Orlovsky 1969, Orlovsky and Shik 1976). Ventilation and blood pressure were increased concomitantly with locomotion (Eldridge et al. 1981, DiMarco et al. 1983). Furthermore, respiratory muscles were activated during SLR stimulation in a pattern which resembled changes observed during exercise. Specifically, during transition from rest to locomotion, the external intercostal (IC) muscles were inhibited, whereas the internal IC muscles were strongly excited (DiMarco et al. 1983). These changes in muscle activity decrease FRC (functional residual capacity) which is a common occurrence during exercise (Yamashiro and Grodins 1973, Linnarson 1974, Ainsworth et al. 1989). It was also suggested (Romaniuk et al. 1986) that an increase in ventilation at the beginning of locomotion in cats may result partially from an attenuation of the inspiratory-inhibiting Hering-Breuer reflex.

In addition to SLR and MLR, which are supramedullary structures, it has been shown that stimulation in the cervical spinal cord and medulla may induce locomotion (Kazennikov et al. 1983, Selionov and Shik 1984, Kazennikov et al. 1985).

In this study, we examined whether the stimulation of medullary and spinal structures produces respiratory responses similar to those observed during stimulation of supramedullary locomotor structures.

METHODS

Experiments were performed on adult male cats (2.5-4.5 kg, n=7). Animals were anaesthetized by an initial intramuscular injection of 30 mg/kg Ketanest (Parke-Davis and Co.) with subsequent 6 mg/kg doses at 30 min intervals. Additionally, 2% Xylocaine (Astra) was injected at incision sites for local analgesia. The animals had a tracheostomy, a T-shaped cannula was inserted into the lower tracheal stump, and the jugular vein and common carotid artery were cannulated.

Following this preliminary surgical preparation, the animal was positioned in a stereotaxic apparatus above the treadmill. Subsequent surgical preparation consisted of a precollicular post-mammilar decerebration (DiMarco et al. 1983) and laminectomy at the C1-C5 level. After decerebration, general anaesthesia was discontinued. Approximately 1.5 h lapsed between decerebration and the first measurement. Experiments were performed when blood pressure was above 80 mg Hg (usually it was above 100 mm Hg).

Stimulating electrodes were inserted vertically into the medulla or the cervical spinal cord (dorsolateral funiculus) using a micromanipulator (Kazennikov et al. 1983, Kazennikov et al. 1985). Stimulating electrodes were glass micropipettes with a tip diameter of 10-20 μm, filled with Wood's alloy and covered electrolytically with platinum. Stimulus pulses had 20-50 μA intensity and 0.2 ms width, and were delivered continuously at a frequency of 60 Hz.

EMG activities of the diaphragm, intercostal muscles and the limb muscles were recorded with concentric needle electrodes, filtered (band pass 0.1-1.0 kHz), amplified and integrated. Raw and integrated EMG activities were recorded on an 8-channel ink recorder (Mingograf), simultaneously with tracheal and arterial pressure.

In experiments on paralyzed animals, i.e. during fictive locomotion, efferent activities of C5 phrenic (Phr) root and ulnar (U) nerve were recorded with bipolar electrodes, amplified and integrated. The

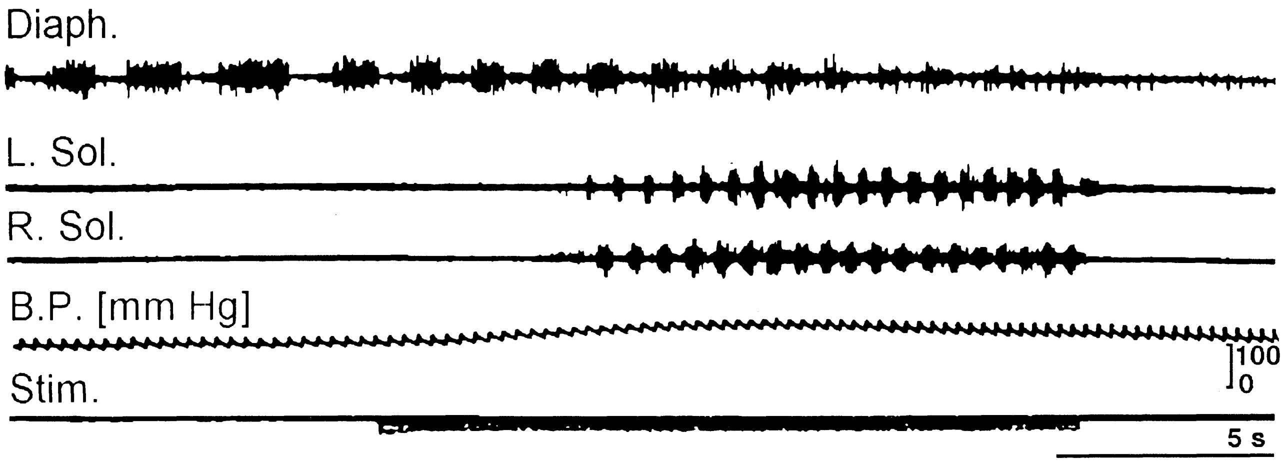

Fig. 1. Effect of electrical stimulation of the dorsolateral funiculus on respiratory and locomotor activity. Stimulation produced an increase in frequency of breathing, evoked phasic EMG activity in soleus muscles and increased blood pressure. Records from top to bottom; Diaph., raw EMG activity of diaphragm: L. Sol. and R. Sol., EMG activities of left and right soleus muscles; BP, blood pressure; Stim., stimulus marker.

animals were paralyzed with gallamine and artificially ventilated. During artificial ventilation end-tidal CO_2 was measured (Godart) as well as arterial blood gasses (ABC-1 analyzer, Radiometer).

Physiological fluids were supplemented during the experiment by administration of dextran or glucose in physiological saline, the animal's temperature was maintained at 37-38^{o}C by applying external radiant heat.

The results presented are those which were consistent in all animals studied. We did not perform statistical analysis or averaging of the data since the results obtained were a function of many parameters (i.e. stimulus intensity or frequency) and could change over a wide range.

RESULTS

Electrical stimulation of the dorsolateral funiculus (DLF) evoked responses similar to the effects of the stimulation of SLR and MLR (DiMarco et al. 1983). Figure 1 presents the effect of DLF stimulation on diaphragm and soleus EMG activities and on the blood pressure. With the onset of the stimulus train, breathing frequency increased and both inspiratory and expiratory phases shortened. Respiratory changes induced by stimulation preceded the increase in blood pressure and onset of locomotion. After 4-5 s, stepping movements began and were maintained to the end of the stimulation period. Several breaths or stepping movements (lasting not

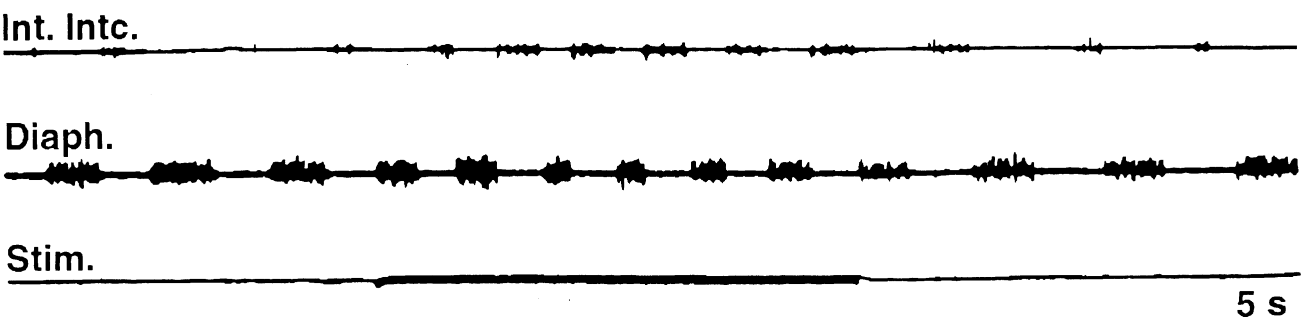

Fig. 2. Effect of electrical stimulation of DLF on internal intercostal and diaphragm EMG activities. Stimulation increased the frequency of breathing and enhanced internal intercostal EMG activity. Records from top to bottom: Int. Intc., internal intercostal EMG activity; Diaph., diaphragm EMG activity; Stim., stimulus marker.

longer than 5 s) were required to achieve a maximal response during stimulation. Also after cessation of stimulation several breaths passed before breathing returned to the control level.

The respiratory effects induced by DLF stimulation were investigated further by recording the pattern of respiratory muscle activation. DLF stimulation shortened expiratory time and enhanced internal intercostal EMG activity (Fig. 2). We also compared the efficacy of the Hering-Breuer reflex at rest and during DLF stimulation (Fig. 3). At rest (Fig. 3A), the tracheal occlusion performed at the end of inspiration prolonged the expiratory phase (T_E) and evoked internal intercostal EMG activity. During DLF stimulation, the

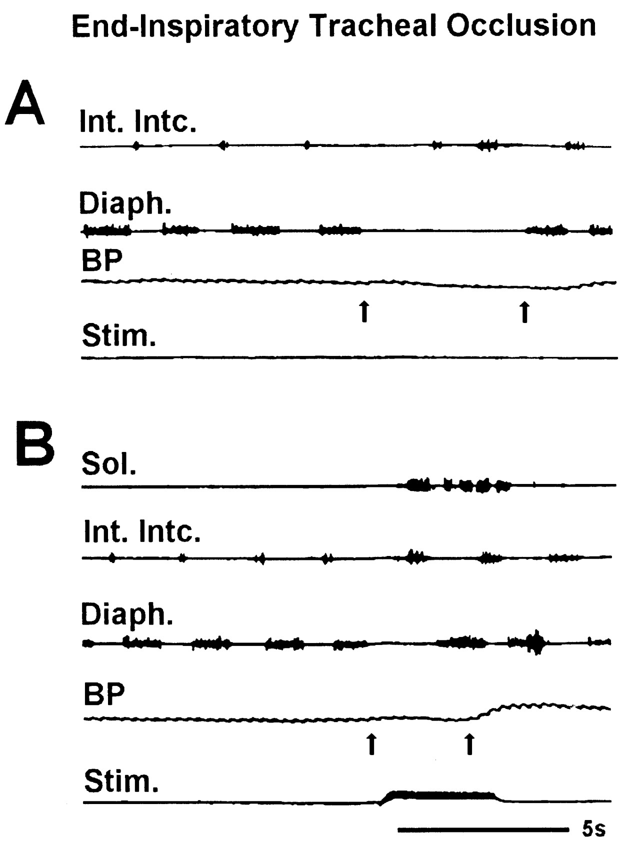

Fig. 3. Effect of electrical stimulation of DLF on prolongation of expiratory phase (T_E) during tracheal occlusion performed at the top of inspiration. A, rest (no stimulation); B, electrical stimulation of DLF as marked. Arrows show duration of tracheal occlusion. During stimulation, prolongation of T_E in response to tracheal occlusion was shorter than at rest. Denotations as in Figs. 1 and 2.

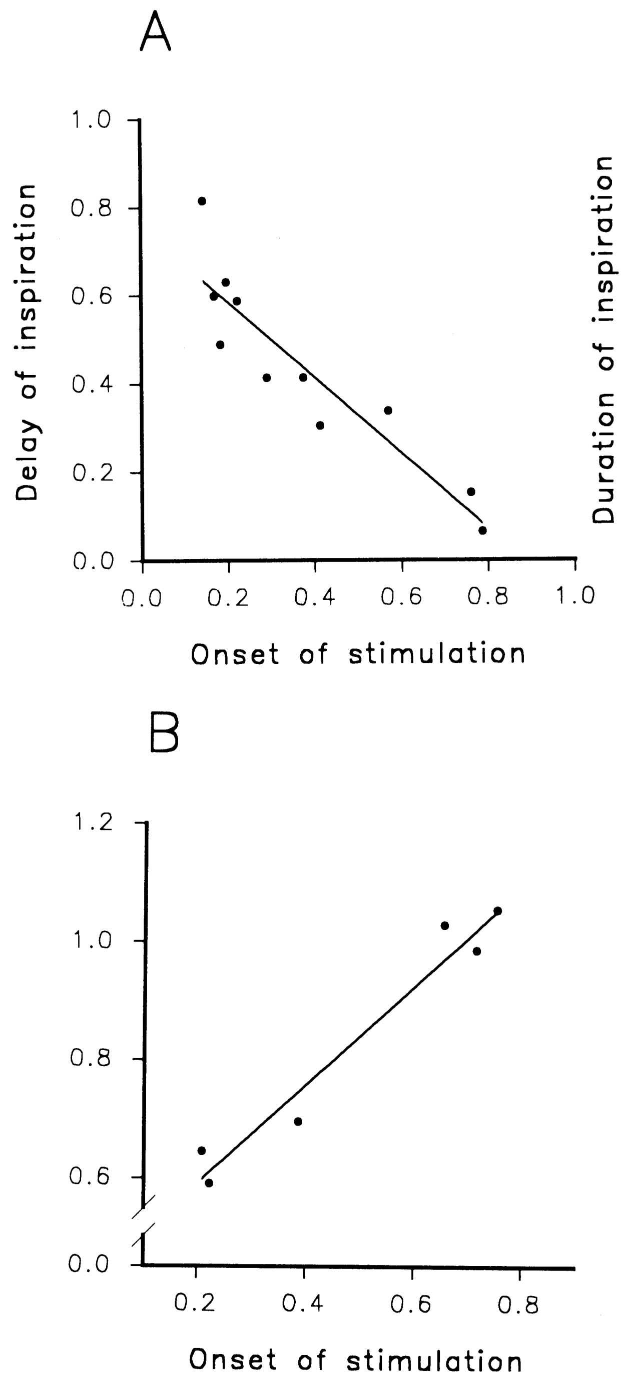

Fig. 4. Time dependence between onset (A) and duration (B) of first inspiration during DLF stimulation and onset of stimulation within expiratory phase. The later during the expiratory phase the stimulus was turned on, the shorter was the delay of the onset of inspiration and the longer was the inspiratory duration of the first breath during stimulation. In A, delay of inspiration and onset of stimulation expressed as a ratio of expiration phase normalized to 1.0; in B, duration of first breath and onset of stimulation expressed as a ratio of inspiratory phase (B) normalized to 1.0.

same manoeuvre produced shorter prolongation of T_E and earlier onset of internal IC EMG activity (Fig. 3B).

Stimulation applied during expiration shortened this phase by provoking a premature inspiratory effort. The delay between the onset of the stimulus and the onset of evoked inspiration decreased when the stimulus was applied later during the expiratory phase. The duration of the first inspiration depended on the timing of stimulation onset during the expiratory phase (Fig. 4B), i.e. the earlier the stimulation was initiated during expiration, the shorter was the next inspiratory phase.

Stimulation of medullary locomotor strip (MLS) produced a similar increase in breathing frequency and a rise in blood pressure. Since experiments with MLS stimulation were performed mostly during "fictive" locomotion, and the animal was artificially ventilated, we did not quantitatively compare these two different groups of data. However, after muscle paralysis, the observed interaction between locomotor and respiratory rhythm generators resulted from their central organization without being affected by peripheral feedback. Different patterns of interaction between locomotion and breathing are presented in Fig. 5. In Figure 5A, it is shown that the MLS stimulation may evoke rapid, phasic activity in the ulnar nerve, augmentation of phrenic nerve activity and shortening of T_E. Furthermore, in Fig. 5B, MLS stimulation elicited phasic activity in the ulnar nerve which was modulated by the rhythm of respiratory discharges. Locomotor activity was locked to the post-inspiratory phase. In one breath, there was a high-amplitude burst of locomotor activity after which both locomotor and inspiratory activities discharged in phase.

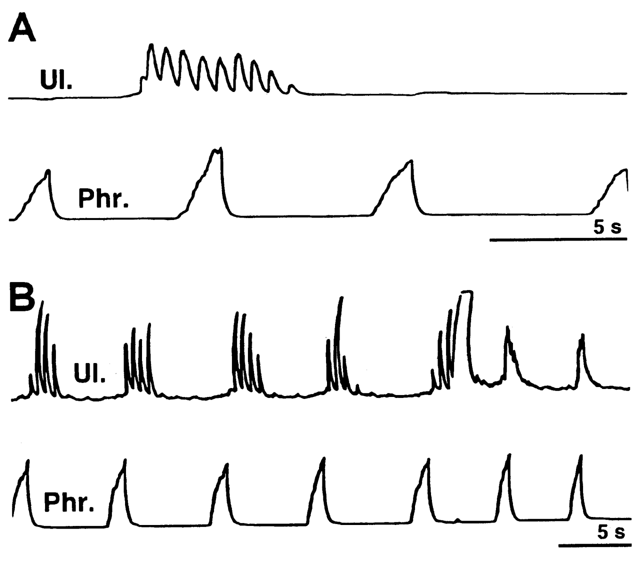

Fig. 5. Examples of interactions between locomotor and respiratory nerve activities during fictive locomotion induced by stimulation of the medullary locomotor strip (MLS). A, MLS stimulation evoked phasic activity in the ulnar nerve (Ul.), augmentation of phrenic nerve (Phr.) activity and a decrease in T_E. In B, the locomotor activity was locked to the post-inspiratory phase and later was shifted to the inspiratory phase.

DISCUSSION

Electrical stimulation of the dorsolateral funiculus (DLF) and medullary locomotor strip evoked locomotor and respiratory responses similar to those obtained during stimulation of supramedullary SLR and MLR structures. It has been suggested (Eldridge et al. 1981, DiMarco et al. 1983) that subthalamic and mesencephalic locomotor regions are the source of the neurally mediated increase in ventilation at the onset of exercise (Eldridge et al. 1981, DiMarco et al. 1983). Since both these regions are localized hierarchically above the respiratory and circulatory control networks, it was assumed that they send a "central command" which provides the primary drive for changes of respiration and circulation during exercise (Eldridge et al. 1981).

Concomitantly with the studies of Eldridge et al. (1981) and DiMarco et al. (1983), new data were published documenting that locomotor movements may be induced by stimulation at a lower level: the medulla and spinal cord (Kazennikov et al. 1983, Selionov and Shik 1984). These structures are at the same level or below the respiratory neuronal network. In the present study, we recorded respiratory activity during medullary (MLS) and spinal (DLF) electrical stimulation that was sufficient to evoke locomotion in decerebrate cats. We demonstrated that this stimulation increased the breathing frequency and blood pressure. This increase in ventilation was usually the first response noted at the onset of stimulation. Similar effects were described for SLR and MLR stimulation, and it was thus concluded that the threshold for eliciting locomotion is above the threshold for eliciting respiratory changes (Eldridge et al. 1981, DiMarco et al. 1983). The effects of stimulation increased with the duration of continuous stimulation and slowly decreased after the stimulus had been removed. Similar temporal summation during electrical stimulation was described earlier (Karczewski et al. 1976) for respiration during peripheral and mesencephalic stimulation.

It is interesting that such an integrated response such as the increased internal intercostal EMG activities (Ainsworth et al. 1989) was also observed during MLS and DLF stimulation. This response was observed during locomotor movements induced by stimulation of SLR and MLR, and it was attributed to a decrease in FRC at the onset of exercise (DiMarco et al. 1983). The attenuation of the inspiratory-inhibiting Hering-Breuer reflex observed during SLR and MLR (Romaniuk et al. 1986) stimulation was also observed during stimulation of MLS and DLF.

The present results show no qualitative differences in respiratory effects induced by electrical stimulation of supramedullary, medullary or spinal locomotor neuronal structures. It is difficult, at this point, to speculate about specific neuronal connections involved in the integration of respiratory and locomotor responses. However, there are neuronal substrates well recognized at the level of the medulla (Selionov and Shik 1984, Euler 1986, Richter et al. 1987) and the spinal cord (Aoki et al. 1984, Kazennikov et al. 1985, Budzinska and Romaniuk 1986, Lipski and Duffin 1986, Kubin and Romaniuk 1988) interconnected with the supramedullary locomotor regions (e.g.: Sinnamon 1984, Garcia-Rill 1986, Kasicki et al. 1991) which may contribute to such integration. Present results obtained during fictive locomotion (Fig. 5) suggest that the activity of post-inspiratory neurones (shown by Richter et al. 1987) may be involved in the central coupling between respiration and locomotion (Viala and Freton 1983, Kawahara et al. 1989) according to the three-phase model of respiratory rhythmogenesis (Richter et al. 1987, Dick et al. 1993). The synchronization of breathing activity and movements of the limb was also observed in cats and rats after deafferentation of hind limbs (Kunstman and Orbeli 1924, Hnik personal communication). Hnik attributed this phenomenon to the fact that the increased excitability of motoneurones after dorsal root section is disclosing a subthreshold excitability status between respiration and locomotor mechanisms. A paradigm of fictive locomotion used in our experiment is to some extent close to the acute effect of deafferentation. However, the interaction between respiration and locomotion may take place at all mentioned above levels: supramedullary, medullary and spinal. The specific paradigm of interaction would thus depend on actual respiratory drive and behavioural (motor) status.

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REFERENCES

Ainsworth D.M., Smith C.A., Eicker S.W., Henderson K.S., Dempsey J.A. (1989) The effects of locomotion on respiratory muscle activity in the awake dog. Res. Physiol. 78: 145-162.

Aoki M., Kasaba T., Kurosawa Y., Ohtsuka K., Satomi H. (1984) The projection of cervical respiratory neurons to the phrenic nucleus in the cat. Neurosci. Lett. (Suppl.) 13: 59.

Budzińska K., Romaniuk J.R. (1986) Phrenic reflexes in the decerebrate and spinal rabbit. Bull. Eur. Physiopath. Respir. 22: 65.

Dick T.E., Oku Y., Romaniuk J.R., Cherniack N.S. (1993) Interaction between central pattern generators for breathing and swallowing in the cat. J. Physiol. (London) 465: 715-730.

DiMarco A.F., Euler C. von, Romaniuk J.R., Yamamoto Y. (1983) Changes in respiratory activity associated with locomotion. J. Physiol. 343: 1-16.

Eldridge F.L., Millhorn D.E., Waldrop T.G. (1981) Exercise hyperpnea and locomotion: parallel activation from the hypothalamus. Science 211: 844-846.

Euler C. von. (1986) Brain stem mechanism for generation and control of breathing pattern. In: Handbook of physiology - the respiratory system. Vol. 2. Control of breathing

(Eds. N. Cherniack and J. Widdicombe) Chapter 1. Amer. Physiol. Soc., Washington, D.C.

Garcia-Rill E. (1986) The basal ganglia and the locomotor regions. Brain Res. Rev. 11: 47-63.

Karczewski W.A., Budzińska K., Gromysz H., Herczynski R., Romaniuk J.R. (1976) Some responses of the respiratory complex to stimulation of its vagal and mesencephalic inputs. INSERM Symposium Respiratory Centers and Afferent System, Amines, (Ed. B. Duron) 4-6 March. Vol. 59: 107.

Kasicki S., Korczyński R., Romaniuk J.R., Sławińska U. (1991) Two locomotor strips in the diencephalon of thalamic cats. Acta Neurobiol. Exp. 51: 135-139.

Kawahara K., Kumagai S., Nakazono Y., Miyamoto Y. (1989) Coupling between respiratory and stepping rhythms during locomotion in decerebrate cats. J. Appl. Physiol. 67(1): 110-115.

Kazennikov O.V., Shik M.L., Yakovleva G.V. (1983) Responses of neurons of upper cervical segments in cat to stimulation of brain stem locomotor region with different frequencies. Neurophysiology (Kiev) 15: 355-361.

Kazennikov O.V., Shik M.L., Yakovleva G.V. (1985) Synaptic responses of propriospinal neurons to stimulation of the stepping strip in the cat dorsolateral funiculus. Neurophysiology (Kiev) 17: 270-278.

Krogh A., Lindhard J. (1913) The regulation of respiration and circulation during the initial stages of muscular work. J. Physiol. 47: 112-136.

Kubin L., Romaniuk J.R. (1988) Propriospinal inspiratory neurons in the upper cervical spinal cord of the rabbit; location and efferent spinal projections. In: Control of breathing during sleep and anesthesia (Eds. W.A. Karczewski, P. Grieb, J. Kulesza and G. Bonsignore). Plenum Press, New York, p. 197-201.

Kunstman K.I., Orbeli L.A. (1924) Izv. Nautch. Inst. im. P.F. Lesgafta 9: 187 (Cited after Vinokurov V.A. (1945) (In Russian) Fiziol. Zh. SSSR 31: 283.)

Linnarson D. (1974) Dynamics of pulmonary gas exchange and heart rate changes at start and end of exercise. Acta. Physiol. Scand. (Suppl). 415: 1-68.

Lipski J., Duffin J. (1986) An electrophysiological investigation of propriospinal inspiratory neurons in the upper cervical cord of the cat. Exp. Brain Res. 61: 625-637.

Orlovsky G.N. (1969) Spontaneous and induced locomotion of the thalamic cat. Biofizika 14: 1154-1162. (Eng. transl.)

Orlovsky G.N., Shik M.L. (1976) Control of locomotion: a neurophysiological analysis of the cat locomotor system. In: Int. Rev. Physiol. Neurophysiology II Vol. 10 (Ed. R. Porter). University Park Press, Baltimore, p. 281-317.

Richter D.W., Ballantyne D., Remmers J.E. (1987) The differential organization of medullary post-inspiratory activities. Pflugers Arch. 410: 420-427.

Romaniuk J.R., Kasicki S., Borecka U. (1986) Breuer-Hering reflex at rest and during electrically induced locomotion. Acta. Neurobiol. Exp. 46: 141-151.

Selionov V.A., Shik M.L. (1984) Medullary locomotor strip and column in the cat. Neuroscience 13(4): 1267-1278.

Sinnamon H.M. (1984) Forelimb and hindlimb stepping by the anesthetized rat elicited by electrical stimulation in the diencephalon and mesencephalon. Physiol. Behav. 33: 191-201.

Viala D., Freton E. (1983) Evidence for respiratory and locomotor pattern generators in the rabbit cervicothoracic cord and for their interactions. Exp. Brain Res. 49: 247-256.

Waller W.H. (1940) Progression movements elicited by subthalamic stimulation. J. Neurophysiol. 3: 300-307.

Yamashiro S.M., Grodins F.S. (1973) Respiratory cycle optimization in exercise. J. Appl. Physiol. 35: 522-525.

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