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Age and activity-related changes in the respiratory motor system

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Abstract The ability of the respiratory motor system to adapt to various functional demands is very important for maintaining human lifestyles. Age-related changes in the system have been studied in several animals. Furthermore, many kinds of models of altered use have been developed to examine activity-related changes in the system. In this review, the plasticity of the respiratory motor system, including spinal phrenic motoneurons and endplates on muscle fibers are discussed, mainly relating to inactivity and over-activity models in rat diaphragm muscle.
Keywords : diaphragm muscle, phrenic motoneuron, endplate, plasticity

Respiratory muscle

Skeletal muscle is in a constant state of remodeling to adjust to changes in activity and/or load from birth until death. To perform a variety of tasks like walking, running, talking, eating and breathing, muscles have unique structural properties in terms of fiber type composition and neural organization. Respiratory muscles serve to support the primary function of the lungs, which is to provide gas exchange by supplying O_2 and removing CO_2 from the blood. Basically, the structure and function of respiratory muscles do not differ from those of other skeletal muscles.

There are two main types of skeletal muscles involved in respiration: pump muscles and upper airway muscles. The focus in this review is on the diaphragm muscle, which is the major pump muscle, and one that is unique to mammals. The diaphragm muscle is a dome-shaped thin sheet of muscle that is inserted into the lower ribs to form a septum separating the thoracic and abdominal compartments. Diaphragm muscle contraction generates a negative pressure in the thoracic cage leading to inflation of the lungs (inspiration). With relaxation of the diaphragm muscle, the recoil forces of the lung and chest wall induce air movement from the lung (expiration). Therefore, the diaphragm muscle works as an active inspiratory pump for lung ventilation and its contribution to total ventilation volume is estimated to be over 70% in the resting state. In addition to the diaphragm muscle, parasternal intercostal muscles are the other primary inspiratory muscles that are phasically recruited with resting ventilation. The intercostal muscles form two layers, but the ventral portion contains only internal intercostal muscles (parasternal intercostals).

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Motor unit organization and recruitment pattern of the diaphragm

Based on the shortening velocity and fatigue resistance of muscle fibers, motor units consisting of phrenic motoneurons and diaphragm muscle can be classified into the following four groups: 1) slow twitch, fatigue resistant (type S); 2) fast twitch, fatigue resistant (type FR); 3) fast twitch fatigue intermediate (type FI); and 4) fast twitch, fatigable (type FF)[1,2]. With the development of antibodies against specific myosin heavy chain (MyHC) isoforms, muscle fiber type is now classified into the following four types: 1) type I (involved in the S motor unit); 2) type IIa (involved in FR); 3) type IIx (involved in FI); 4) type IIb (involved in FF)[3,4].

Based on the sizes of motoneurons and their intrinsic electrophysiological properties, type S motor units are recruited first, followed in order by FR, FI and FF units (Henneman's size principle)[5]. Size-related recruitment order of motor units is directly related to the mechanical and fatigue properties of the muscle fibers. Similar to other skeletal muscle, several studies have shown orderly recruitment of diaphragm motor units. In a study of cats[6], only about 23% of all phrenic motoneurons are recruited during inspiration, which is the approximate proportion of type S and FR motor units in the diaphragm muscle[2]. Therefore, phrenic motoneurons recruited in resting respiration are composed of type S and FR motor units. Similar findings were previously reported in rats and hamsters[7,8]. In other words, these results suggest that a large number of diaphragm motor units remain fairly inactive during resting ventilation and are recruited only when short duration, high-force efforts are necessary. Although the order of motor unit recruitment may occasionally be determined by specific synaptic input, it seems that motoneuron size and the respiratory motor system motor unit type are important in determining recruitment order.

Age-related changes in the respiratory motor system

Diaphragm muscle. Basically, the mechanical output of muscle during growth is altered by increases in the cross-sectional area (CSA) of each muscle fiber, that is, hypertrophy. In addition to changes in muscle mass, fiber-type composition changes have also been reported. For example, in rat diaphragm muscle, it has been demonstrated that the replacement of neonatal myosin with adult slow and fast myosin occurs rapidly in the prenatal period, and the proportion of slow MyHC increases from 7% at birth to 18% in adults[9]. In human diaphragm muscle, the proportion of slow MyHC also increases from 9% at less than 37 weeks gestational age to 25% at birth, and reaches the adult level (55%) at 2 years old[10]. Furthermore, there is a progressive loss of muscle mass and mechanical output in later stages of life. It is well recognized that selective atrophy of type II fibers with aging leads to slower contraction and relaxation times[11,12].

In a previous study[13], we examined age-related changes in mechanical output and biochemical properties of the rat diaphragm muscle obtained from three age groups: young (10-week), adult (1-year) and old (2-year) after birth. Although there were no significant differences in specific twitch tension (force/CSA) of the diaphragm muscle among the groups, significant reductions were found in specific tetanic tension in the adult and old groups as compared to the young group. The contraction and half relaxation time of twitch contraction in diaphragm muscle were significantly prolonged with aging. The patterns of age-related changes in Ca^{2+}-ATPase activity in the sarcoplasmic reticulum were similar to that of the half relaxation time of muscle contraction. Furthermore, as compared to young diaphragm muscle, there was a significant increase in the relative composition of the slow MyHC isoform and a concomitant decrease in that of the fast MyHC of old diaphragm muscle. Our findings demonstrated that older diaphragm muscle has slower contraction and relaxation, and these alterations were attributed to changes in the MyHC isoform composition and Ca^{2+}-ATPase activity of the sarcoplasmic reticulum, respectively.

Endplate on diaphragm muscle. Morphological changes in the neuromuscular junction with aging have been reported from half a century ago[14]. Recently, with the development of immunohistochemical staining and confocal microscopy, the three-demensional morphology of the endplate on type-identified muscle fibers was studied in rat diaphragm muscle in young to very old (30-months after birth) groups[15]. In each age group, the planar area and volume of endplates on type–IIx/b muscle fibers were larger than those of type–I and IIa muscle fibers, while the normalized planar areas of the endplate (endplate area/muscle fiber diameter) and the mean thickness of the endplate (volume/endplate area) were identical for all fiber types within the same age group. Reduced endplate density (endplate area/surrounding area) in very old diaphragm muscle indicated fragmentation of the endplate, especially in type-IIx/b fibers (Fig. 1). These morphological changes may lead to functional deficiency and selective denervation of type–IIx/b muscle fibers with aging.

It is possible that the expressions of basal laminar proteins involved in maintaining the integrity of the endplate and its corresponding nerve terminal, e.g., the neural cell adhesion molecules agrin[16] and neuregulin (NRG)[17], are different across fiber types, and thus account for differences in endplate fragmentation. It has been clearly demonstrated that expression of these basal laminar proteins is altered by conditions that affect the integrity of the endplate and nerve terminal[18,19]. These results are in accordance with the recruitment patterns during normal respiration in the rat diaphragm muscle, in which only type I and type IIa motor units are recruited, and type IIx/b motor units are rarely recruited[20]. Furthermore, it is of considerable interest that our results clearly indicate age-related endplate remodeling proceeding from an earlier time compared to muscle fiber remodeling. Although there were no significant age-related differences in the endplate density of type IIx/b muscle fibers, the endplate density of the adult group was 22% smaller than the young group. These results suggest that age-related muscle fiber atrophy may follow endplate deterioration with aging[15].

Phrenic motoneurons. Recently, we investigated age-related morphological changes in rat motoneurons innervating diaphragm muscle (DI-MN) and lumbar longissimus muscle (LL-MN) in which quite different activation

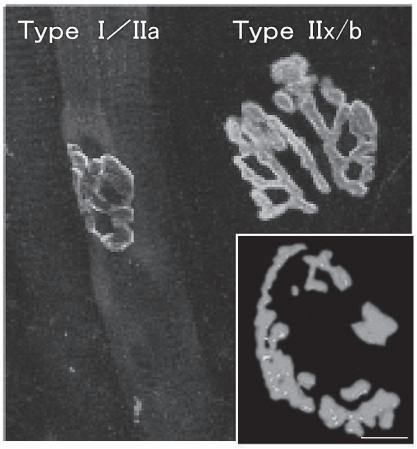

Fig. 1 Two-dimensional images of labeled endplates (red) on type-identified muscle fibers with anti-myosin heavy chain (green) from an old rat (24-month old)[15]. The image in the bottom right corner is a typical three-dimensional endplate on a type II x/b fiber from a very old rat (30-month old). Decreased endplate density (endplate area/surrounding area) in a very old diaphragm indicated fragmentation of the endplate, especially on type IIx/b fibers. White bar indicates 10 μm.

patterns exist[21]. In young and old rats, the motoneurons innervating both muscles were retrogradely labeled by intramuscular injection of a cholera toxin B subunit into the muscles, followed by immunohistochemical staining and observation with a confocal microscope (Fig. 2). Compared to the soma volume in young rats, significantly smaller values were found in old rats in both motoneurons; and the degrees of decline were 16.1% in DI-MN and 20.3% in LL-MN. In addition, significant decreases in the thickness of primary dendrites were found in both motoneurons, and the degrees of decline were 17.5% in DI-MN and 22.3% in LL-MN. Smaller changes were found in DI-MN than in LL-MN, indicating the possibility that increased activation by central drives can attenuate age-related morphological changes in the motor system in the spinal cord.

From another point of view, the decreases in soma volume and dendrite surface area may be a compensatory adaptation to obtain enough excitatory post-synaptic potential, as motoneuron excitability is determined by intrinsic electrophysiological properties and by extrinsic factors such as synaptic input. For a given synaptic input, smaller surface areas of soma and dendrites imply greater excitability because of the higher input resistance and lower rheobase of motoneurons.

It has been reported that active behavior decreases with aging in daily life, so activity in the central nervous system also decreases[22]. It is speculated that the decrease in central drive with aging is much more evident in LL-MN than in DI-MN. The greater decrease in central drive may induce greater compensatory adaptation in motoneuron morphology.

Inactivity-related changes in diaphragm muscle

Many kinds of inactivity models have been developed to determine inactivity-related effects on structural and functional properties of the diaphragm muscle: 1) unilateral denervation (DNV), where neurotrophic influence on muscle fibers was completely removed; 2) tetrodotoxin (TTX) blockade of nerve action potential propagation, where DI-MN activity actually increased while the diaphragm muscle was paralyzed, causing a mismatch between motoneuron and muscle activity that may disrupt normal myoneural interactions; and 3) cervical spinal cord hemisection at C_2 (SPH), where ipsilateral descending excitatory drive to DI-MN from medullary premotor neurons was removed, causing both DI-MN and diaphragm muscle to become inactive. In our previous studies[23], after 2 weeks of unilateral diaphragm muscle paralysis induced by SPH, there was only a small decrease in muscle-specific force, and no change in the maximum unloaded shortening velocity. In contrast, after DNV or TTX blockade, there was a marked decrease in specific force and a substantial slowing of maximum unloaded shortening velocity in the diaphragm muscle. Furthermore, the diaphragm muscle of the SPH model resulted in much smaller changes in succinate dehydrogenase (SDH) activity of type I and IIa fibers, and a lower reduction in SDH variability among fibers (Fig. 3)[24]. Based on these results, we concluded that the effects of inactivity on diaphragm muscle properties are dependent on a match between DI-MN and muscle fiber activities, leading to intact and coherent myoneural interactions. These results suggest that disruption of neurotrophic influence has a greater impact on muscle fiber functional and metabolic properties than inactivity per se.

There are many candidate neurotrophic factors, e.g., agrin, NRG, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), Neurotrophin-4 (NT-4), Insulin-like growth factor I (IGF-I), hepatocyte growth factor (HGF) and ciliary neurotrophic factor (CNTF), which mediate myoneural interaction. One of these potential nerve-derived trophic factors is NRG, which contributes to the regulation of muscle fiber growth by increasing protein synthesis. NRG contains an epidermal growth factor (EGF)-like domain that signals by stimulating the ErbB (erythroblastic leukemia viral oncogene homolog) family of receptor tyrosine kinases expressed in motoneurons and skeletal muscle fibers[25,26]. Its signal transduction in muscle requires the phosphorylation of receptors, followed by activation of downstream

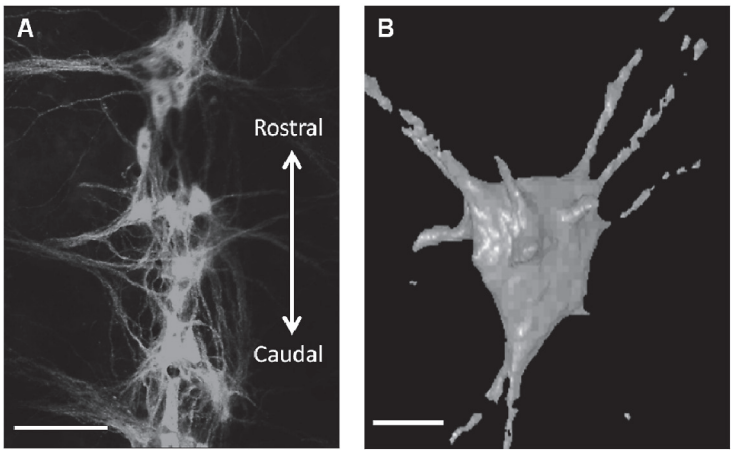

Fig. 2 Labeled phrenic motoneuron pool in the spinal cord (A) and a three-dimensional reconstructed image of a labeled phrenic motoneuron (B) in the rat[21]. Cell clustering and rostrocaudal orientation of dendritic projections were observed in the phrenic motoneuron pool. White bars indicate 300 μm and 10 μm in A and B, respectively.

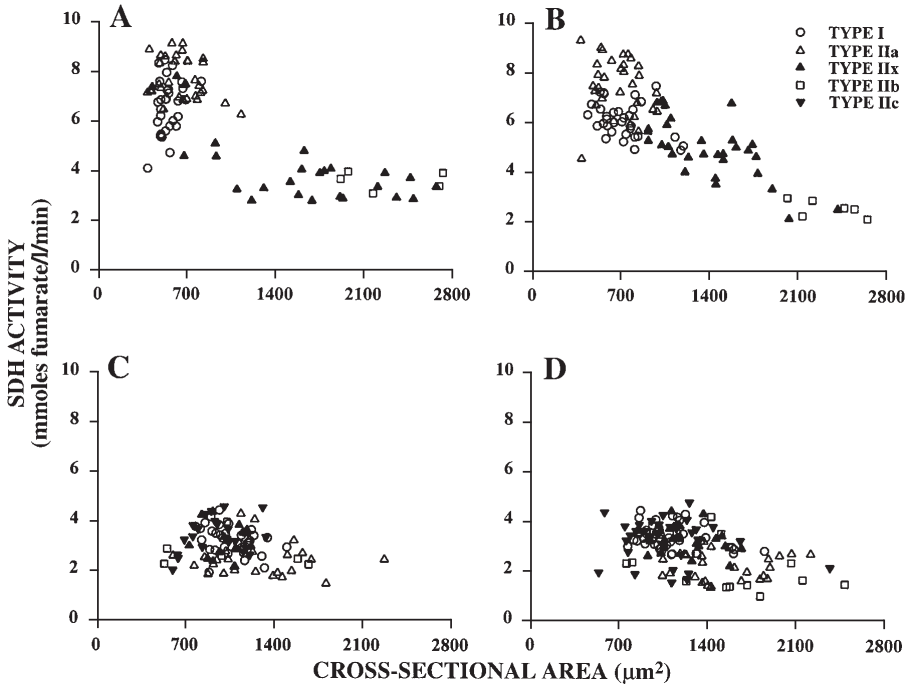

Fig. 3 Inverse relationships between cross-sectional area and succinate dehydrogenase (SDH) activity of type-identified diaphragm muscle fibers in each experimental group[24]. Although smaller type I and IIa fibers had higher SDH activities than larger type IIx and IIb fibers in control (A) and spinal hemi-section at C2 (B) groups, the correlations disappeared in tetrodotoxin treatment (C) and denervation (D) phrenic nerve groups.

pathways including the PI3K/Akt and MAPK/Erk pathways[27]. Neurotrophic factors such as BDNF and GDNF trigger the release of pre-synaptic NRG from nerve terminals[28,29]. Importantly, it is also reported that BDNF and GDNF are stored in vesicles and released with activity, and that NRG controls acetylcholine receptor synthesis at neuromuscular junctions[30,31].

Compensatory over activation-related changes

Diaphragm muscle. In all the hemidiaphragm muscle paralysis models mentioned above, the intact diaphragm muscle in the contralateral side was compensatory activated (CAC model). In our previous study[32], as compared to pre-surgery values, the mean value of the duty cycle and root mean square of the spontaneous inspiratory-related EMG activity of the CAC diaphragm muscle were increased by 7 and 41%, respectively, immediately after surgery (Fig. 4). In total, EMG activity in the CAC diaphragm muscle was 51% higher than the value before surgery, and the increased activity continued for at least 30 min. In the CAC models, we examined functional, morphological and metabolic adaptations of the diaphragm muscle to 4-week CAC induced by contralateral DNV surgery in young, adult and old rats[33]. Although there were no clear improvements in contraction properties between CAC and CTL in all age groups, metabolic enzyme activities, especially SDH and β-Hydroxyacyl-CoA dehydrogenase, were increased in the CAC groups.

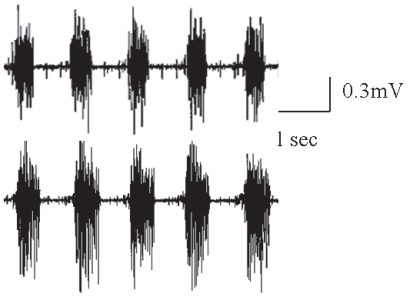

Fig. 4 Electromyographic (EMG) activity taken from the hemi-diaphragm muscle of a rat subjected to contralateral denervation of the phrenic nerve[32]. Spontaneous inspiratory-related EMG activity increased after denervation (lower trace), as compared to pre surgery (upper trace). In hemi-diaphragm paralysis models, the intact diaphragm in the contralateral side was compensatory activated (CAC model).

Endplate on the diaphragm. In addition to the adaptation of diaphragm muscle to CAC as described above, improvements in neuromuscular transmission by CAC were observed in all age groups (Fig. 5)[34]. In young rats, the training effects at neuromuscular junctions were introduced previously, such as an increased safety factor due to increased neurotransmitter release when given active training stimuli[35], nerve terminal enlargement[36], and increased acetylcholinesterase activity[37,38]. From these observations, it was thought that nerve terminal enlargement, increased acetylcholine release, and increased acetylcholinesterase activity were attributable to increased

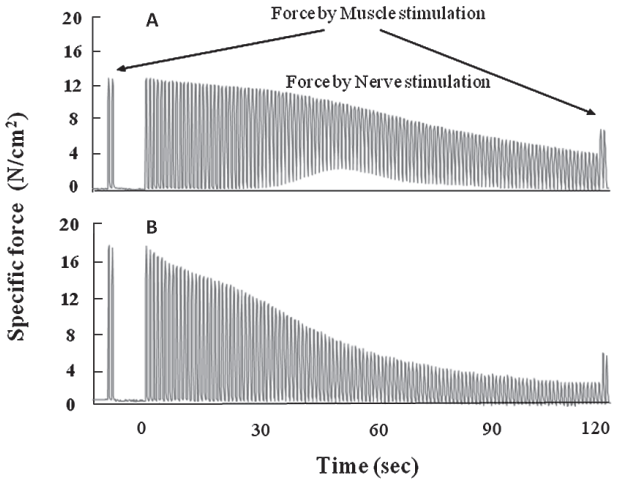

Fig. 5 In-vitro force generation (N/cm^2) of diaphragm muscle by repetitive stimulation of nerve or muscle[34]. Before and after nerve stimulation for 2 min, two direct stimulations of diaphragm muscle were performed. Basically, the difference between the force generated by the last nerve stimulation and the following muscle stimulation represents transmission failure in the neuromuscular junction. The contribution of neuromuscular transmission failure to muscle fatigue was significantly decreased in the CAC diaphragm (A) compared to control diaphragm (B) muscles.

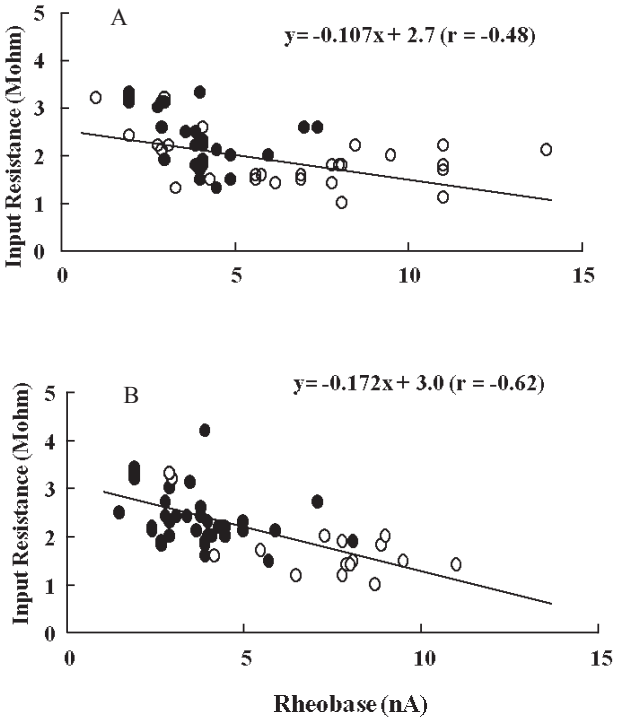

Fig. 6 Relationships between rheobase and input resistance of phrenic motoneurons in the CTL (A) and CAC (B) groups[43]. Motoneurons with spikes and without spikes during the depolarizing shift were defined as recruited (○) and non-recruited (●), respectively. The mean value of the rheobase in non-recruited motoneurons was significantly higher than that in recruited motoneurons in both groups. The mean values of rheobase and input resistance for each motoneuron type were identical between the CTL and CAC groups.

activity levels. These factors contributed to an increased safety factor and influenced neurotransmission.

However, there are some studies equivocal to our speculation with regard to acetylcholinesterase in muscle groups chronically active by nature. Washio et al.[39] observed age-related higher acetylcholinesterase activity in the extensor digitorum longus muscle, but not in the diaphragm or soleus muscles, which usually have higher activity. Although the mechanisms of an activity-induced increased expression of acetylcholine receptor and acetylcholinesterase activity are still unclear, many studies indicate that the plasticity in motoneurons and muscle fibers is influenced by motoneuron or muscle- derived trophic factors. For example, Keller-Peck et al.[40] has reported that excessive expression of GDNF induced multiple neural innervations to skeletal muscle fiber. Alteration in NT-4 expression that occurred with changing activity levels has been reported[41]. It is possible that changes in the activity level trigger alterations in neuron or muscle-derived trophic factors, with a concomitant improvement in neuromuscular junction, leading to improvement in neuromuscular transmission. In fact, Mantilla et al.[42] demonstrated direct evidence that BDNF and NT-4 could improve transmission function in adult rat diaphragm muscle.

Phrenic motoneurons. Furthermore, in the CAC models, electrophysiological membrane properties (resting potential [mV], rheobase [nA] and input resistance [Mohm]) of the DI-MN were measured in adult rats (Fig. 6)[43]. In both groups of intact CTL and CAC DI-MN, there were significant inverse relationships between the rheobase and input resistance of motoneurons. The mean value of the rheobase in non-recruited motoneurons (putatively F type) was significantly higher than that in recruited motoneurons (putatively S type) in both groups. Most of the motoneurons with low rheobase (<5nA) were recruited in the CAC, but not in the CTL. As a result, the incidence of recruited motoneurons in the CAC (69%) was higher than that in the CTL motoneurons (48%). The mean values of rheobase and input resistance of each motoneuron type were identical between the CTL and CAC groups. We concluded that no clear changes in electrophysiological properties of DI-MN membranes were induced by 4-week CAC.

In a previous study, Beaumont & Gardiner[44] examined the electrophysiological properties of the motoneurons innervating the hindlimb via the tibial nerve in rats housed in wheel running cages. The study demonstrated that voluntary running rats had slow motoneurons with significantly deeper resting potentials than CTL rats. In a human study[45], motor units had lower initial firing rates and smaller discharge variability in the muscles of the dominant hands than non-dominant hands. These results suggest that increased chronic activity may cause the mo-

toneuron membrane properties to become the slow type. The implications for these changes are considered to be a delay in the onset of membrane accommodation and late adaptation that occur with repetitive firing[44]. Although an extreme over-activation of DI-MN occurred due to CAC, the mean value of resting membrane potentials was non-significantly different in both the recruited and non-recruited motoneurons between the CAC and CTL groups. The adaptation toward slow-type motoneurons may have already occurred in the DI-MN. Indeed, the rheobase values of recruited and non-recruited motoneurons in our CTL data were slightly lower than those of slow and fast type motoneurons in the CTL data from a previous study on rats[44].

Summary

The respiratory motor system, including diaphragm muscle and phrenic motoneurons, has great adaptability to altered demand. Although the underling mechanism of the adaptation is not clearly understood, trophic interaction between muscle and motoneurons should be studied intensively. The target of these trophic factors on pathways related to protein synthesis and degradation require study in terms of the respiratory motor system, as well as other skeletal muscles, in the near future.

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