Plasticity in Respiratory Motor Control
Invited Review: Mechanisms underlying motor unit plasticity in the respiratory system

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Mantilla, Carlos B., and Gary C. Sieck. Invited Review: Mechanisms underlying motor unit plasticity in the respiratory system. J Appl Physiol 94: 1230–1241, 2003; 10.1152/japplphysiol.01120.2002.—Neuromotor control of skeletal muscles, including respiratory muscles, is ultimately dependent on the function of the motor unit (comprising an individual motoneuron and the muscle fibers it innervates). Considerable diversity exists across diaphragm motor units, yet remarkable homogeneity is present (and maintained) within motor units. In recent years, the mechanisms underlying the development and adaptability of respiratory motor units have received great attention, leading to significant advances in our understanding of diaphragm motor unit plasticity. For example, following imposed inactivity of the diaphragm muscle, there are changes at phrenic motoneurons, neuromuscular junctions, and muscle fibers that tend to restore the ability of the diaphragm to sustain ventilation. The role of activity, neurotrophins, and other growth factors in modulating this adaptability is discussed.

neurotrophins; diaphragm muscle; inactivity; respiration; phrenic nerve

NEUROMOTOR CONTROL OF THE diaphragm muscle is organized similarly to other skeletal muscles, with the final common output being the motor unit, comprising a phrenic motoneuron and the diaphragm muscle fibers it innervates (28, 129). Properties of the motor unit population are critically important in determining the functional characteristics of the diaphragm during a variety of motor behaviors. In fact, diaphragm motor units exhibit great diversity in terms of their mechanical and fatigue properties (28, 129). Diversity is also evident by structural and functional differences in phrenic motoneurons (3, 14, 54, 61, 71, 106, 143, 149), neuromuscular junctions (69, 105, 107, 109, 110, 112, 135, 137), and muscle fibers (35, 36, 130, 131, 133, 134).

As the major inspiratory muscle in mammals, activation of the diaphragm is very unique in relation to most other skeletal muscles. The daily duty cycle (ratio of active to inactive times) for hindlimb muscles ranges from ~2% for the extensor digitorum longus muscle (predominantly composed of type IIb fibers) to ~14% for the soleus muscle (predominantly composed of type I fibers) (53). In contrast, the duty cycle of the diaphragm muscle in most species is ~45%. Because of its remarkable and unique activation history, the diaphragm may be particularly responsive to disuse, especially inactivity. A variety of other factors may also influence diaphragm muscle remodeling. For example, the diaphragm is not a load-bearing muscle like the soleus and other hindlimb muscles. The diaphragm muscle also differs with regard to afferent input derived from muscle spindles, which are scarcely found in the diaphragm muscle. Premotoneurons providing rhythmic excitatory drive to the phrenic motoneuron pool are more remotely located in the medulla. This stands in contrast to the close proximal location of premotoneurons providing afferent input to limb motoneuron pools. Because of the remote location of premotoneurons and lack of muscle spindle input, upper cervical spinal cord injury completely disrupts rhythmic excitatory drive to phrenic motoneurons, leading to complete paralysis of the diaphragm muscle. In con-
contrast, because premotoneurons providing afferent input to limb motoneuron pools are locally distributed, afferent drive and the potential for locomotor patterns remain largely intact after spinal cord injury. These major differences are very important when considering neuroplasticity in motor control.

CLASSIFICATION OF MOTOR UNIT TYPES

Although motor unit heterogeneity probably reflects a continuum of properties, motor units have been categorized into different types primarily based on the mechanical and fatigue characteristics of the innervated muscle fibers (10–12, 28, 129). Accordingly, motor units are classified into four types: 1) slow-twitch, fatigue-resistant (type S), 2) fast-twitch, fatigue-resistant (type FR), 3) fast-twitch, fatigue-intermediate (type FInt), and 4) fast-twitch, fatigable (type FF) (Fig. 1). Muscle fiber-type classification also generally follows a corresponding scheme, whether based on histochemistry or myosin heavy chain (MHC) isoform expression. Thus muscle fibers are classified into four types: 1) type I (MHCSlow), 2) type IIa (MHC2A), 3) type IIb (MHC2B), and 4) type IIx (MHC2X) (7, 99, 116–119, 129, 136, 138).

HETEROGENEITY OF MOTOR UNIT PROPERTIES

The morphology of motoneurons within a pool varies considerably (13–15, 106, 143). Heterogeneity in somal morphology and dendritic arborization may contribute to differences in electrophysiological properties across motoneurons. In fact, it has been shown that the intrinsic electrophysiological properties of motoneurons display considerable heterogeneity, which may depend on motoneuron size and motor unit type. For example, it has been reported that motoneurons belonging to type S motor units generally have the highest input resistance, lowest rheobase, and slowest axonal conduction velocities among motoneurons. In contrast, motoneurons belonging to type FF motor units are the largest (lowest input resistance), least excitable (highest rheobase), and display the fastest axonal conduction velocities (10, 155).

The structural and functional properties of neuromuscular junctions also vary considerably across different motor unit types (Fig. 2) (27, 62, 105, 107–112, 128, 132, 135). For instance, neuromuscular junctions at type IIx and IIb diaphragm muscle fibers are larger and have a far more complex structure compared with neuromuscular junctions at type I and IIa fibers. Ul-
Results were reported for motor units from other muscles (18, 114).

Despite the considerable diversity across motor unit types, there is remarkable homogeneity in the properties of muscle fibers comprising an individual motor unit. The mechanical and biochemical properties of innervated muscle fibers are consistent within a motor unit and are precisely matched to the properties of the motoneuron (10, 47, 76, 89, 90, 129, 130). This precise matching reflects neuron-target cell interactions that could be mediated by the activation history of the motor unit or the influence of neurotrophic (or myotrophic) substances.

Although diversity in motor unit phenotype could be determined genetically and established early during embryonic development, multiple lines of evidence indicate that communication between motoneurons and muscle fibers plays a continuing role in maintaining and remodeling motor units (8, 21, 45, 68, 113). This communication can take the form of neural electrical activity (propagated action potentials) or chemical influences derived from either the motoneuron or muscle fiber (8, 41, 97). In addition, a variety of environmental factors such as external load, muscle blood flow, local oxygen tension, and accumulation of metabolites can influence the properties of motor units (compare Refs. 97, 99, 101, 119, 142 for reviews). It is beyond the scope of the present review to explore the multitude of factors that can influence motor unit plasticity. Instead, we will focus on neural activity and neurotrophic influences.

MOTOR UNIT RECRUITMENT

Phenotypic differences in the components of the motor unit are likely to result in altered functional capacity of the diaphragm muscle. Based on the classic model of Sherrington and colleagues (77, 124), force development in skeletal muscle is achieved through the orderly recruitment of motor units. Henneman and colleagues (51, 52) provided a unifying principle for motor unit recruitment based on the size of motoneurons and their intrinsic electrophysiological properties.
(Henneman “size principle”). According to the size principle, smaller motoneurons with smaller axons and slower conduction velocities have lower membrane capacitance, higher input resistance, and lower rheobase and thus are recruited first for a given synaptic input. In support of the size principle, it has been shown that phrenic motoneurons with slower axonal conduction velocities are recruited first during inspiratory efforts (61). Not inconsistent with Henneman’s size principle, specific motor unit types also appear to be an important determinant of motor unit recruitment order (10, 26, 129, 140, 141). It has also been shown that type S and FR motor units [comprising type I (MHC<sub>Slow</sub>) and IIa (MHC<sub>2A</sub>) muscle fibers, respectively] are recruited first and more often, whereas type FInt and FF motor units [comprising type IIx (MHC<sub>2X</sub>) and IIb (MHC<sub>2B</sub>) fibers, respectively] are recruited later and less frequently. Assuming a recruitment order based on motor unit type, the more sustained motor behaviors of the diaphragm muscle can be achieved by the recruitment of only type S and FR motor units (28, 125–127, 130, 134, 146). Recruitment of type FInt and FF motor units would be required only during more forceful and less frequent motor behaviors of the diaphragm muscle (28, 129). Thus it is likely that phrenic motoneurons and diaphragm muscle motor units vary considerably in their activation history.

**MOTONEURON-TARGET CELL INTERACTIONS**

Since the seminal papers of Buller and colleagues (8, 9), considerable evidence has accumulated to support the concept that motoneurons exert a predominant influence on the contractile and metabolic properties of the muscle fibers they innervate. In addition to the cross-innervation studies of Buller et al., support derives from motor unit studies where homogeneous fiber-type composition has been consistently reported (11, 12, 22, 47, 89, 90). For example, diaphragm muscle motor units comprise muscle fibers with remarkably similar enzymatic and contractile protein properties, e.g., succinate dehydrogenase activity and MHC isoform composition (129, 130). Together, these results reflect the importance of neuron-target cell interactions in providing a match between the properties of motoneurons that dictate recruitment order and the mechanical and energetic properties of muscle fibers that sustain motor behaviors. However, these results do not shed light on whether this match depends on activation history or the influence of neurotrophic factors.

**MOTOR UNIT PLASTICITY**

Motor unit plasticity can occur at each of its components, i.e., motoneuron, neuromuscular junctions, and/or muscle fibers. Adaptation of diaphragm muscle motor units to altered use may underlie, at least in part, the etiology of certain diseases. For example, in chronic obstructive pulmonary diseases, diaphragm muscle activity increases, but, to be effective, this increase in diaphragm muscle activity must be met while avoiding fatigue. Therefore, diaphragm muscle neuromotor control must adapt to the increased mechanical loads imposed by respiratory diseases. Conversely, in situations requiring maintenance of patients on mechanical ventilation, the diaphragm muscle is unloaded. Altered use of the diaphragm muscle, whether it is an increase or decrease in activity, may cause adaptations at each level of the motor unit. For example, there may be hypertrophy or atrophy of muscle fibers, altered expression of contractile proteins, and changes in fiber mechanical properties. At neuromuscular junctions, there may be remodeling of pre- and postsynaptic elements and changes in synaptic efficacy. At phrenic motoneurons, there may be changes in somal surface area or dendritic branching. Such adaptations may affect motoneuron recruitment, synaptic transmission, and/or the ability of the diaphragm muscle to generate sufficient force for ventilation while resisting fatigue. In considering plasticity in neuromotor control, it is important to recognize that each diaphragm muscle motor unit type can adapt to altered use in different ways depending on mechanical loads, innervation patterns, activation history, and fiber-type composition. Although the activation history and functional requirements of diaphragm muscle motor units are unique, the innervation patterns and fiber-type composition of the diaphragm muscle are similar to other skeletal muscles.

**MUSCLE FIBER PHENOTYPE TRANSITION**

Role of innervation. Classic studies demonstrated that the metabolic and mechanical properties of skeletal muscles are altered in response to changes in innervation (8, 21, 45, 68, 113). These studies clearly established that adult muscle fiber type is not fixed but mutable when provided with an appropriate stimulus related to innervation. However, this stimulus could be either the pattern of activity, which varies across motor unit types, or due to specific motoneuron-derived trophic influences.

The effects of denervation on muscle fiber properties also demonstrate the predominant role of innervation. As with cross-innervation studies, the effects of denervation do not distinguish between the removal or modification of a neurotrophic influence or inactivity. However, results from denervation studies have shed some light on muscle fiber phenotype transitions. Most importantly, it has been reported that postdenervation changes are inconsistent with predenervation muscle fiber phenotype. For example, denervation of the adult rat soleus muscle results in the transition of only ∼50% of the predenervation slow fibers to fast fibers (1, 60). Thus it is possible that the postdenervation muscle fiber phenotype may depend on the embryological lineage of muscle fibers, reflecting genetically determined patterns of motor unit differentiation.

Role of activity patterns. A number of studies have demonstrated muscle fiber phenotype transitions with altered patterns of electrical stimulation (see Refs. 97, 99, 142 for reviews). For example, fibers in the rat
soleus muscle, which predominantly express MHC_{slow}, can be converted to a faster muscle phenotype by imposing a higher rate of electrical stimulation. Conversely, muscles such as the extensor digitorum longus, which comprise fibers predominantly expressing MHC_{2x} and MHC_{2b} isoforms, can be converted to a slower muscle phenotype by imposing a slower rate of electrical stimulation (98, 99, 102).

Phenotypic transitions in muscle fibers exposed to altered patterns of electrical stimulation (and including conditions of concurrent denervation) involve not only transitions in the expression of MHC isoforms but also the expression of other myofibrillar proteins (e.g., myosin light chains, troponin subunits, tropomyosin, and α-actinin) and Ca^{2+}-regulatory and sarcoplasmic reticulum proteins (e.g., Ca^{2+}-ATPase, calsequestrin, and phospholamban). Furthermore, transitions in the expression of enzymes associated with glycolytic and oxidative pathways have also been reported following electrical stimulation (see Refs. 99, 100 for reviews). Thus it is widely accepted that the pattern of motor unit activity can influence muscle fiber phenotype.

In previous studies, we examined the role of innervation and activity patterns on diaphragm muscle fiber phenotype by comparing adaptations induced by unilateral denervation, tetrodotoxin-induced nerve blockade and C2 spinal cord hemisection (34, 85, 156–158) (Fig. 3). In each of these experimental models, the right side of the diaphragm muscle was paralyzed. However, with unilateral denervation, communication between phrenic motoneurons and diaphragm muscle fibers was completely disrupted; with unilateral tetrodotoxin-induced nerve blockade, communication between phrenic motoneurons and diaphragm muscle fibers was conserved; and, with C2 spinal hemisection, communication between phrenic motoneurons and diaphragm muscle fibers remained intact, but motoneurons were inactive. We found marked differences in the plasticity of diaphragm muscle motor units induced by these three experimental models. For example, our laboratory (156–158) found that both denervation and tetrodotoxin-induced diaphragm muscle paralysis caused selective atrophy of type IIX and IIB diaphragm muscle fibers. Our laboratory also found that denervation (34) and tetrodotoxin-induced nerve blockade (unpublished observations) caused a reduction in MHC content per half sarcomere and reduced specific force of type IIX and IIB fibers. In contrast, diaphragm muscle paralysis induced by C2 hemisection caused little if any change in fiber size, MHC content, or mechanical properties (85). Based on these results, we concluded that muscle inactivity per se is not the major determinant of diaphragm muscle motor unit plasticity. With both C2 hemisection and tetrodotoxin-induced nerve blockade, communication between phrenic motoneurons and diaphragm muscle fibers remains intact; however, after C2 hemisection, phrenic motoneurons are inactive, whereas after tetrodotoxin-induced nerve blockade, phrenic motoneuron activity increases by ~50% (156–158). Therefore, it is possible that a direct trophic influence on diaphragm muscle fibers results from and is affected by motoneuron activity, independent of actual inactivation of muscle contraction. Furthermore, because the effects of denervation and tetrodotoxin-induced nerve blockade on diaphragm muscle fibers were similar, it would appear that a mismatch between phrenic motoneuron activity and presynaptic inactivity, as exists with tetrodotoxin-induced nerve blockade, has the same effect as the complete disruption of communication between motoneurons and muscle fibers. With both unilateral diaphragm muscle denervation and tetrodotoxin-induced phrenic nerve block, it appears that a positive trophic influence is removed, whereas with C2 hemisection this trophic influence is preserved.

A fundamental unresolved question is whether the intrinsic ability of skeletal muscle to adapt to changes in innervation and/or activity is governed by molecular imprinting or activation patterns established during myogenesis. In other words, although extrinsic factors (e.g., activity patterns, gravity, hormonal influences) can modify muscle fiber phenotype at all stages of adult life, the overall effect of these various influences may be constrained by genetic programming appearing as

Fig. 3. Experimental models used for the study of activity-dependent plasticity of diaphragm muscle motor units. After unilateral denervation, tetrodotoxin-induced nerve blockade, and C2 hemisection, the diaphragm muscle is paralyzed. However, differences in communication between the phrenic motoneuron and muscle fibers and in the activity of phrenic motoneurons across these models exist.
early as the myotube stage, i.e., well before formation of distinct fiber types. Other unresolved questions regarding muscle fiber phenotype transitions include the role of intracellular Ca\(^{2+}\) or Ca\(^{2+}\)-dependent intracellular signaling cascades, neurotrophic factors, and activation of transcription factors.

**Role of intracellular Ca\(^{2+}\) and calcineurin-related signaling.** Recent studies suggest that the molecular mechanism(s) responsible for activity-dependent adaptation of muscle fibers may involve changes in Ca\(^{2+}\) handling and calcineurin-related pathways (4, 17, 96).

Calcineurin is a Ca\(^{2+}\)/calmodulin-dependent serine/threonine protein phosphatase, which by dephosphorylating cytosolic members of the NF-AT family (nuclear factors of activated T cells) causes their nuclear translocation (72). Nuclear NF-ATs then bind to specific nucleotide sequences in promoter/enhancer regions and stimulate the transcription of slow fiber genes (17, 122).

In addition, the peroxisome-proliferator-activated receptor-\(\gamma\) coactivator 1 (PGC-1\(\alpha\)) has been shown to serve as a transcriptional coactivator for slow fiber genes by acting in cooperation with the myogenic regulatory factor Me2 and as a target of calcineurin signaling (78). However, it is possible that multiple pathways are involved in the motor unit type-specific adaptations of muscle fibers.

For example, Ras signaling through the mitogen-activated protein kinase (MAPK) pathway mimics the effect of slow-type electrical muscle stimulation on myosin gene expression (87).

**Myogenic regulatory factors.** Myogenic regulatory factors were initially implicated as possible mediators of the transition of muscle fiber phenotype in response to altered motor unit activity because of their demonstrated role in muscle differentiation (58, 59, 144). Accordingly, a fiber-type-specific effect of the myogenic regulatory factors, MyoD, myogenin, MRF-4, on muscle phenotype has been recently suggested (123, 147).

However, changes in MyoD and myogenin expression after chronic low-frequency stimulation of type FF motor units are only modest and are not associated with a shift in the ratio of MyoD to myogenin (74, 91). Thus the actual role of myogenic regulatory factors in muscle fiber phenotypic transitions in response to changes in neuromuscular activity remains to be determined.

**PLASTICITY AT THE NEUROMUSCULAR JUNCTION**

Maintenance of synaptic efficacy may be the driving force behind neuromuscular junction remodeling (145, 151). In previous studies, our laboratory (85, 108, 109) found that the changes in diaphragm muscle neuromuscular junction morphology and function induced by C\(_2\) hemisection and tetrodotoxin-induced nerve blockade were quite different. After 2 wk of C\(_2\) hemisection, there was an expansion of neuromuscular junction size at type IIa and IIb diaphragm muscle fibers and a marked improvement in neuromuscular transmission.

After 2 wk of tetrodotoxin-induced nerve blockade, there was some evidence of nerve terminal sprouting, but otherwise there was no overt change in neuromuscular junction morphology. However, tetrodotoxin-induced nerve blockade resulted in a marked increase in neuromuscular transmission failure.

In addition to the remodeling of diaphragm muscle neuromuscular junctions evident after altered activity, neuromuscular junction plasticity has been examined in a variety of other conditions. For example, motor unit-type-specific differences in neuromuscular junction morphology and function have been reported during embryogenesis (86), aging (44, 70, 110), hypothyroidism (105), and chronic testosterone (5) or corticosteroid (20, 137) treatment.

**Role of neurotrophins.** Recent evidence, primarily from neuronal culture systems, indicates that neurotrophins participate in activity-induced modification of synaptic transmission (19, 103, 139). These studies have shown that neurotrophin synthesis and release are regulated by neuronal activity and that neurotrophins can also directly modulate synaptic efficacy (19, 66, 67, 121). There are a number of potential neurotrophins that might affect nerve-muscle interactions (Fig. 4). Specifically, brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) have been shown to affect neuromuscular transmission (6, 80).

In a series of studies, Poo and colleagues (80) demonstrated that BDNF rapidly potentiates both spontaneous and evoked synaptic activity of developing neuromuscular junctions of *Xenopus laevis* studied in culture. Furthermore, they found that this effect was presynaptic in origin and was mediated by TrkB recep-

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**Fig. 4.** The family of neurotrophins includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). The effects of neurotrophins are mediated by a family of tyrosine kinase receptors (TrkA, TrkB, and TrkC), which show the following preferences: TrkA for NGF, TrkB for BDNF and NT-4/5, and TrkC for NT-3.
tors. More recently, they demonstrated that BDNF-induced potentiation of synaptic efficacy at developing Xenopus laevis neuromuscular junctions was greatly facilitated by presynaptic depolarization (6). These investigators also demonstrated that the effect of BDNF on synaptic transmission in cultured hippocampal neurons depended on specific properties of the target cells innervated. Exogenous BDNF induced a rapid and persistent potentiation of evoked glutamate release when the target cell was also glutamatergic but not when the target cell was GABAergic (γ-aminobutyric acid). These results suggest that individual nerve terminals can be independently modified by BDNF, depending on the specific target (120).

The tyrosine kinase receptor for BDNF and NT-4/5 (TrkB) is found at diaphragm muscle neuromuscular junctions (both at presynaptic and postsynaptic sites) (23, 40), suggesting that BDNF and NT-4/5 might influence neuromuscular transmission at diaphragm muscle motor units. It is possible that BDNF and/or NT-4/5 plays an important role in regulating synaptic transmission at neuromuscular junctions under normal conditions. Pre- and/or postsynaptic release of neurotrophins may tightly regulate neurotransmitter release and thus synaptic efficacy (Fig. 5). For example, NT-4/5 has been shown to potentiate presynaptic acetylcholine release in Xenopus nerve-muscle cocultures (148). In addition, BDNF may stimulate synapsin I phosphorylation in a MAPK-dependent manner (64, 65) and therefore regulate neurotransmitter release (55). TrkB activation within the presynaptic terminal may thus exert immediate effects (mediated by MAPK or other pathway activation) in addition to longer-term effects (mediated by transcriptional regulation following retrograde transport of the activated neurotrophin-receptor complex) (121). Accordingly, it is possible that BDNF and/or NT-4/5 may play an important role in the remodeling of diaphragm muscle neuromuscular junctions that occurs following C2 hemisection or tetrodotoxin-induced inactivation of presynaptic terminals. However, these possibilities remain to be explored.

Other growth factors may also regulate the number and/or function of synaptic connections between a motoneuron and its target muscle fibers. Glial-derived neurotrophic factor (GDNF) is a potent survival factor for motoneurons that is synthesized by muscle fibers and Schwann cells (50, 57, 153, 154). Production of GDNF by limb skeletal muscle was reported to be activity dependent (150), and overexpression of GDNF, but not NT-4/5 or NT-3, in developing limb muscles leads to hyperinnervation of neuromuscular junctions (92). It is certainly possible that different neurotrophic factors may have motor unit pool-specific effects, which could be mediated by selective expression of factors, their receptors, or individual signaling pathway components.

**MOTONEURON PLASTICITY**

An important model of functional reorganization of respiratory motor output has been advanced by Mitchell and colleagues (30) and has been termed “long-term facilitation.” Long-term facilitation refers to the prolonged augmentation of respiratory motor output reported following repeated carotid sinus nerve stimulation (29, 48, 84) or repeated episodic hypoxia (2). In fact, a robust effect on respiratory output has been shown in both the phrenic and hypoglossal nerves, even lasting upward of 1 h, and has been observed in awake rats (95). Importantly, long-term facilitation has been reported to be serotonin dependent (30, 32, 79). However, a specific range of hypoxia may exist for the induction of long-term facilitation (83), and this may explain reports with conflicting success in inducing long-term facilitation.

Phrenic motoneurons have also been shown to exhibit an enhancement of long-term facilitation in response to cervical deafferentation (71). A significant increase in the number and density of serotonin immunoreactive terminals in the vicinity of phrenic motoneuron soma and dendrites was reported. However, phrenic motoneuron somal surface areas were found to increase, suggesting a decrease in motoneuron excitability. A similar phenomenon was observed in respiratory motoneurons following C2 hemisection or tetrodotoxin-induced inactivation of respiratory motor neurons. In addition, repeated carotid sinus nerve stimulation (29, 48, 84) or repeated episodic hypoxia (2) may explain reports with conflicting success in inducing long-term facilitation.

**Fig. 5.** Schematic demonstrating the possible pre- and postsynaptic effects of the neurotrophins BDNF and NT-4/5 on synaptic efficacy. Neurotrophins released from pre- or postsynaptic sites may activate presynaptic TrkB receptors, leading to synapsin phosphorylation via a mitogen-activated protein kinase (MAPK) pathway and thus have short-term effects on neurotransmitter (ACh) release. In addition, retrograde transport of the activated neurotrophin-Trk receptor complex may have longer term transcriptional effects within motoneurons.
ability (71). These findings suggest that motor unit plasticity may result from a complex interplay of structural (morphological) changes and synaptic inputs, which contribute to the functional output of phrenic motoneurons. Chronic cervical deafferentation via bilateral cervical dorsal rhizotomy (C3–C6) has also been shown to enhance the recovery of ipsilateral phrenic motor function (crossed-phrenic phenomenon, see below) following a C2 spinal hemisection, although serotonin receptor activation was not necessary for this effect (31). It is possible that the synaptic plasticity of descending serotonergic innervation and the morphological alterations of phrenic motoneurons following chronic deafferentation mediate the functional reorganization of respiratory motor output.

Functional adaptations of phrenic motoneurons to inactivity have also been shown to involve potentiation of the cross-phrenic phenomenon and include partial recovery of motor function under increased ventilatory drive (42, 43, 88, 93). The so-called “crossed-phrenic phenomenon” refers to the restoration of ipsilateral phrenic muscle activity after C2 spinal hemisection when the contralateral diaphragm is paralyzed by denervation (104). Several studies by Goshgarian and colleagues (43, 46) have demonstrated significant ultrastructural changes in the cervical spinal cord after C2 spinal hemisection. For example, within hours after C2 spinal hemisection, there is a significant increase in the number of “double synapses” and the length of dendro-dendritic appositions. Similar changes were also reported after cold-induced blockade of descending cervical drive (16). It has been suggested that retraction of glial processes facilitates these ultrastructural changes (43), unmasking previously ineffective synaptic connections within the spinal cord, and thus serve as substrate for the crossed-phrenic phenomenon. In fact, a time-dependent restoration of diaphragm muscle function after C2 spinal hemisection has been reported (38, 39). Our laboratory has also found that contralateral denervation 4 wk following unilateral C2 spinal hemisection results in reactivation of the ipsilateral paralyzed hemidiaphragm, especially when a hypoxic stimulus is applied (W. Zhan, C. Mantilla, G. Sieck, unpublished observations).

In a recent study (81), our laboratory examined C2 hemisection and tetrodotoxin-induced changes in the morphology of phrenic motoneurons. Phrenic motoneuron inactivation associated with C2 hemisection resulted in an overall decrease in motoneuron size, primarily affecting somal dimensions rather than dendrites. In contrast, tetrodotoxin-induced diaphragm muscle paralysis without concomitant inactivity of phrenic motoneurons resulted in an overall increase in motoneuron size, again primarily restricted to somal dimensions. It is possible that changes in phrenic motoneuron size are motor unit specific, with larger motoneurons being disproportionately affected after C2 hemisection and smaller phrenic motoneurons being disproportionately affected after tetrodotoxin-induced nerve blockade.

**Role of neurotrophins.** BDNF and NT-4/5 have been shown to be produced by motoneurons (33, 49, 73, 115). In fact, after 7 days of cervical dorsal rhizotomy, an increase in BDNF and NT-3 expression in the cervical spinal cord was reported (63). The authors reported that immunohistochemistry localized BDNF and NT-3 to motoneurons and interneurons of the ventral spinal cord. We have documented BDNF and NT-4/5 immunoreactivity in retrogradely labeled rat phrenic motoneurons (unpublished observations), suggesting a potential role of these neurotrophins in phrenic motor unit plasticity. After C2 spinal hemisection, a rapid (3 day) increase in BDNF and NT-4/5 mRNA in the ventral cervical spinal cord and a gradual return to prehemisection levels by 14 days posthemisection were shown (82). It is possible that, in addition to effects of motoneuron-derived neurotrophins, retrograde transport of the activated neurotrophin-Trk receptor complex from presynaptic terminals may affect transcriptional regulation within motoneurons. These retrograde signals may serve to regulate the matching of motoneuron and presynaptic terminal activity and thus contribute to the different morphological adaptations of phrenic motoneurons following diaphragm muscle paralysis induced by C2 hemisection vs. tetrodotoxin nerve blockade. Although neurotrophins likely contribute to spinal cord plasticity, there is currently a dearth of knowledge regarding specific neurotrophin-mediated effects on motoneuron structural, synaptic, and functional adaptations to altered activity.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Although much is now known about the plasticity of respiratory motor units in response to altered activity, many questions remain unanswered. Adaptations in motor units may occur at any and all of its components: the motoneuron, the neuromuscular junction, and/or the target muscle fibers. Motor unit-type-specific adaptations are critical when determining the final output of motor pools and the resulting motor behaviors. Further studies are needed to evaluate the relative contribution of specific neurotrophic factors, signaling pathways, and transcriptional activators to the plasticity of motor units in response to altered activity and other environmental factors.

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