Published in final edited form as:

Prog Brain Res. 2014 ; 209: 309–329. doi:10.1016/B978-0-444-63274-6.00016-3.

Convergence of pattern generator outputs on a common mechanism of diaphragm motor unit recruitment

Carlos B. Mantilla^{1,2}, Yasin B. Seven², and Gary C. Sieck^{1,2}

¹Department of Anesthesiology, Mayo Clinic College of Medicine, Rochester MN 55905

²Department of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester MN 55905

Abstract

Motor units are the final element of neuromotor control. In manner analogous to the organization of neuromotor control in other skeletal muscles, diaphragm motor units comprise phrenic motoneurons located in the cervical spinal cord that innervate the diaphragm muscle, the main inspiratory muscle in mammals. Diaphragm motor units play a primary role in sustaining ventilation, but are also active in other non-ventilatory behaviors, including coughing, sneezing, vomiting, defecation and parturition. Diaphragm muscle fibers comprise all fiber types. Thus, diaphragm motor units display substantial differences in contractile and fatigue properties, but importantly properties of the motoneuron and muscle fibers within a motor unit are matched. As in other skeletal muscles, diaphragm motor units are recruited in order such that motor units that display greater fatigue resistance are recruited earlier and more often than more fatigable motor units. The properties of the motor unit population are critical determinants of the function of a skeletal muscle across the range of possible motor tasks. Accordingly, fatigue-resistant motor units are sufficient to generate the forces necessary for ventilatory behaviors whereas more fatigable units are only activated during expulsive behaviors important for airway clearance. Neuromotor control of diaphragm motor units may reflect selective inputs from distinct pattern generators distributed according to the motor unit properties necessary to accomplish these different motor tasks. In contrast, widely-distributed inputs to phrenic motoneurons from various pattern generators (e.g., for breathing, coughing or vocalization) would dictate recruitment order based on intrinsic electrophysiological properties.

Keywords

Ventilation; respiratory muscles; inspiration; motor unit; diaphragm muscle

Introduction

Motoneurons and the muscle fibers they innervate together form a *motor unit* and determine the output of skeletal muscles. In mammals, the main inspiratory muscle is the diaphragm and it is innervated by phrenic motoneurons located within the ventral horn (lamina IX) of

cervical spinal cord segments C₃-C₅ in rats (Mantilla et al., 2009, Prakash et al., 2000, Song et al., 2000), C₃-C₆ in mice (Qiu et al., 2010), C₄-C₆ in cats (Webber et al., 1979), C₅-C₇ in ferrets (Yates et al., 1999), and C₃-C₅ in humans (Keswani and Hollinshead, 1955). The phrenic motoneuron pool consists of ~300 motoneurons in rats (Mantilla and Sieck, 2003, Mantilla et al., 2009, Prakash et al., 2000), comprising motor units with considerable heterogeneity in their contractile and fatigue properties (Burke et al., 1973, Fournier and Sieck, 1988). Motor unit properties generally match the histochemical and biochemical properties of the muscle fibers (Butler et al., 1999, Sieck, 1991b, Sieck, 1994, Su et al., 1997), which are essentially homogeneous within a specific motor unit (Enad et al., 1989, Sieck et al., 1989a).

The properties of the motor unit population are critical determinants of the function of a skeletal muscle across the range of possible motor tasks (Clamann, 1993). Ultimately, the range of muscle forces that can be generated by a skeletal muscle depends on the contractile and fatigue properties of recruited motor units. Furthermore, recruitment of motor units provides the functional limits for individual motor tasks by determining the muscle response to the varying mechanical demands that are imposed.

Neuromotor control is executed by recruitment of motor units with diverse functional properties (Fournier and Sieck, 1988, Sieck, 1988) and frequency coding of motor unit activation (Iscoe et al., 1976). All skeletal muscles display generally similar organization of neuromotor control. Motoneurons within a motor pool being innervated by premotor interneurons which distribute motor output across groups of agonist and antagonist muscles in order to achieve coordinated muscle activation in complex motor tasks. This review will focus on the common mechanisms of diaphragm motor unit recruitment and the convergence on motor outputs from various pattern generators on respiratory motor units with a special emphasis on diaphragm motor units.

Central Pattern Generators

Rhythmic behaviors such as breathing have been recognized as being dependent on the existence of specific features of a motor system across many different species. In particular, it is clear that rhythmic behaviors do not require afferent input in order to be generated (Brown, 1914). The neuronal circuitry responsible for generating such rhythmic behaviors is commonly referred to as a “central pattern generator”. There are three main components to the neuronal circuitry of a motor system: 1) the pattern generator; 2) premotor interneurons responsible for “broadcasting” the motor output; and 3) motoneurons responsible for generating the forces necessary for the desired motor behavior. Afferent inputs modulate motor output at any of these components either via direct spinal connections or both direct and indirect supraspinal signals (including volitional input).

Diaphragm motor units contribute to a number of motor tasks including rhythmic behaviors that sustain ventilation as well as to non-ventilatory behaviors that are important for airway clearance, including swallowing, coughing and sneezing (Butler et al., 2001, Mantilla et al., 2010, Mantilla and Sieck, 2011, Milano et al., 1992, Sieck, 1994, Sieck and Fournier, 1989). Similarly, motor units for limb muscles participate in multiple motor tasks including

standing and maintenance of posture as well as locomotive behaviors such as walking, running and jumping (Walmsley et al., 1978). These motor tasks reflect a broad range of behaviors, some of which require co-activation of agonist and antagonist muscles in a concerted, multi-phased maneuver. For instance, near maximal co-activation of the diaphragm and abdominal muscles during coughing and sneezing is necessary to generate the large inspiratory effort and high intra-abdominal pressure that precedes diaphragm elevation and increased intra-thoracic pressure to “clear” the airway (Milano et al., 1992, Rybak et al., 2008, Shannon et al., 1998).

In locomotor systems, appropriate afferent information is mainly proprioceptive, conveying information about the spatial location (position) of joints and limbs, the elongation of agonist and antagonist muscle groups, and the degree of tension generated within each muscle. Thus, muscles involved in locomotion have substantial innervation by intramuscular muscle spindles and specialized musculotendinous Golgi tendon organs (Proske and Gandevia, 2012). These afferents serve to provide detailed information on body movement, position, force and effort, thus shaping the activation of muscle groups across a wide range of motor tasks. In contrast, appropriate afferent information for respiratory muscles (e.g., indicating adequacy of ventilatory efforts) must reflect lung volume and chest wall distension. Accordingly, respiratory muscles such as the diaphragm have minimal innervation by muscle spindles (Duron et al., 1978), and phrenic afferents contribute little to respiratory modulation (Corda et al., 1965, Jammes et al., 2000).

Phrenic motoneurons receive their primary excitatory drive from premotor interneurons located in the medulla (Dobbins and Feldman, 1994, Ellenberger and Feldman, 1988, Feldman et al., 1985). Input to phrenic motoneurons is generally thought to be widely distributed (Cohen et al., 1974, Davies et al., 1985) and mostly synchronous (Davies et al., 1985, von Euler et al., 1973). However, systematic investigation of inputs to phrenic motoneurons across a range of motor tasks has not been conducted. In this sense, distinct pattern generators likely contribute to ventilatory, vocalization and expulsive behaviors with varying degrees of functional overlap (Rybak et al., 2008, Smith et al., 1991). Whether phrenic motoneurons receive distributed vs. selective inputs from these bulbospinal pathways is presently not clear (Dick et al., 1987, Hudson et al., 2011, Jodkowski et al., 1987). Regardless, phrenic motor units are the final element of neuromotor control and execute respiratory motor output across this wide range of motor tasks. Accordingly, it is important to elucidate whether motor unit recruitment reflects intrinsic electrophysiological properties of the motoneurons as a result of graded, distributed inputs to motoneurons across a range of motor tasks. Alternatively, neuromotor control of diaphragm motor units could reflect selective inputs from distinct pattern generators that are matched to the motor unit properties necessary to accomplish these different motor tasks.

Ventilatory and Non-Ventilatory Behaviors

It is important to recognize that the respiratory muscles such as the diaphragm are also activated during non-ventilatory motor behaviors such as sighing, coughing, sneezing, vomiting, defecation, parturition, vocalization and postural control (Butler et al., 2001, Holstege, 2014, Mantilla et al., 2011, Mantilla et al., 2010, Milano et al., 1992, Ramirez,

2014). Respiratory muscles such as the diaphragm and intercostal muscles differ from other skeletal muscles in the level of activity that is sustained throughout the lifespan since activity is necessary to maintain adequate ventilation from birth until death (Bowes et al., 1981). For instance, rat hind limb muscles such as extensor digitorum longus and soleus muscles display duty cycles (i.e., portion of time active) of ~2% and ~14%, respectively (Hensbergen and Kernell, 1997), compared to ~30-40% for the diaphragm (Kong and Berger, 1986, Mantilla et al., 2010, Sieck and Fournier, 1989). Ventilatory behaviors can be accomplished by activating only ~10-25% of the total force-generating capacity of the diaphragm muscle (Mantilla et al., 2010, Mantilla and Sieck, 2011, Sieck, 1991b, Sieck and Fournier, 1989). Thus, there is a large reserve for force generation in the diaphragm and only during expulsive non-ventilatory behaviors responsible for airway clearance is the diaphragm muscle fully-activated (Mantilla et al., 2010, Mantilla and Sieck, 2011, Sieck, 1991b, Sieck and Fournier, 1989). As the major muscle for inspiration in mammals, it is not surprising that the diaphragm muscle exhibits such high levels of activation.

A common method for determining diaphragm muscle force is measuring trandiaphragmatic pressure (Pdi). Measurements of Pdi can be obtained with dual balloon manometry, usually based on catheters spanning the thoracic and abdominal surfaces of the diaphragm muscle. Indeed, a joint position statement by the American Thoracic and the European Respiratory Societies (2002) outlines the recommended technique. Catheters need to be placed in close apposition to the wall of the esophagus or stomach to appropriately record pressures within the thoracic and abdominal cavities. In larger species such as rats (Mantilla et al., 2010) and cats (Sieck and Fournier, 1989) this is easily accomplished using balloons filled with air or fluid. Balloon manometry systems can be compromised when very small-diameter catheters are placed in mice. A recent study validated the use of solid state-pressure transducers, requiring only that catheters be of sufficient size to permit ease of placement in the mouth yet occupy the esophagus and gastric cavity in the bound abdomen (Greising et al., 2013b). No complications were reported with this technique.

In a series of studies, Pdi measurements were used to estimate the forces generated by the diaphragm muscle during ventilatory and non-ventilatory behaviors. Measurements were obtained in multiple species including cats (Fournier and Sieck, 1988, Sieck and Fournier, 1989), hamsters (Sieck, 1991b, Sieck, 1994), rats (Mantilla et al., 2010) and mice (Greising et al., 2013b). For each behavior, Pdi was normalized to maximal Pdi obtained by bilateral supramaximal phrenic nerve stimulation (at 75 Hz; Figure 1). During eupnea in cats and mice, Pdi is between 10-12% of maximum Pdi compared to hamsters and rats where Pdi is between 21-27% of maximum. In humans, eupneic Pdi is estimated at ~10% of maximum (Sieck, 1994). During exposure to hypoxia (10% O₂) –hypercapnia (5% CO₂), Pdi is ~28% in both cats and rats and ~12% in mice. Clearly, exposure to hypoxia-hypercapnia constitutes a robust ventilatory stimulus but forces generated by the diaphragm muscle are not near maximal. Spontaneous deep breaths generate ~60% of maximum Pdi in rats and ~30% of maximum in cats. During airway occlusion, Pdi is ~50% of maximum in cats, ~45% in hamsters, ~65% in rats and ~70% of maximum in mice.

Interspecies differences in Pdi normalized to maximum reveal distinct ranges for force-generating capacity by the diaphragm muscle beyond resting breathing. This reserve

capacity for force generation likely across the various ventilatory and non-ventilatory behaviors reflects the various demands that are imposed on the diaphragm muscle. For instance, larger species may require a broader range of behaviors reflecting high-intensity expulsive behaviors necessary for clearing the airway. In addition, differences in the reserve capacity for force generation by the diaphragm muscle may relate to the mechanical properties of the respiratory system itself. For instance, mice exhibit increased minute ventilation, tidal volume, and duty cycle compared to rat on a weight-adjusted basis (Sieck et al., 2012) as well as reduced lung compliance and increased resistance (Gomes et al., 2000). As a result it would be expected that mice exhibit a reduced reserve capacity for force generation by the diaphragm muscle compared to rats, and consequently, the diaphragm muscle would generate an increased fraction of maximal Pdi during ventilatory behaviors. However, intrinsic end-expiratory pressures resulting from the frequency-dependence of airway impedance in mice likely shift lung volumes to a more compliant portion of the elastance curve, and thus ventilatory behaviors in mice require only a small fraction of maximal Pdi (Greising et al., 2013b). Thus, the motor unit composition of ventilatory muscles such as the diaphragm is closely related to the demands imposed by the respiratory system mechanics such that a sufficiently large reserve capacity for force generation is maintained.

Diaphragm Motor Unit Recruitment

Recruitment of motor units within a motor pool is orderly (Butler et al., 1999, Kernell, 2006, Sieck and Fournier, 1989). Henneman and colleagues obtained motor unit recordings from ventral root filaments and showed that motor units which are recruited first have smaller amplitudes and slower conduction velocities than units recruited later (Henneman, 1957, Henneman and Olson, 1965, Henneman et al., 1965, McPhedran et al., 1965). Based on previous observations by Gasser showing that motoneuron size underlies the relationship between action potential amplitude, conduction velocity and axon diameter (Gasser and Grundfest, 1939), Henneman formulated the “size principle”. According to the size principle motor units are recruited in an orderly fashion according to size-dependent, intrinsic electrophysiological properties. Consistent with the size principle, the order of motor unit recruitment was shown to relate to axonal conduction velocity, with lower threshold units that were recruited first displaying slower axonal conduction velocities than higher threshold motor units that were recruited later (Henneman and Olson, 1965, Henneman et al., 1965, McPhedran et al., 1965). The order of motor unit recruitment is consistent with the size principle in a variety of muscles (Gordon et al., 2004, Mendell, 2005), including the diaphragm (Dick et al., 1987). Indeed, motor unit recruitment order matches the contractile and fatigue properties of motor units (Burke et al., 1973, Mendell, 2005, Sypert and Munson, 1981).

Classification of Motor Unit Types

Motor units are commonly classified based on twitch contraction time: slow-twitch (type S) vs. fast-twitch (type F) units. In addition, motor units are classified based on fatigue resistance: whereas type S motor units are fatigue resistant, type F units display varied fatigue resistance from fatigue-resistant (type FR) to fatigue-intermediate (type FInt) to

fatigable (type FF) units. Importantly, the contractile and fatigue properties of motor units correspond with the expression of myosin heavy chain (MyHC) isoforms by the muscle fibers they comprise. Although there is considerable diversity in motor unit properties, all muscle fibers within an individual motor unit are homogeneous in their type composition (Fournier and Sieck, 1988, Hamm et al., 1988, Nemeth et al., 1986). Accordingly, diaphragm muscle fibers within a motor unit are of the same fiber type (Enad et al., 1989, Johnson et al., 1994, Sieck et al., 1989a, Sieck et al., 1996).

Type S motor units comprise type I muscle fibers expressing MyHC_{slow}. Type I fibers have smaller cross-sectional areas (Lewis and Sieck, 1990, Miyata et al., 1995, Prakash et al., 2000, Sieck et al., 1989b, Zhan et al., 1997), higher mitochondrial volume densities and higher capacities for oxidative phosphorylation (Enad et al., 1989, Sieck et al., 1996). Single fiber studies show that type I muscle fibers have slower maximum shortening velocities than type II fibers, consistent with slower cross-bridge cycling kinetics (Sieck and Prakash, 1997). Type I fibers also generate low levels of specific force (force per cross-sectional area) (Geiger et al., 2002, Geiger et al., 2001, Geiger et al., 2000, Geiger et al., 1999).

Type FR motor units comprise type IIa fibers expressing MyHC_{2A}. Type IIa fibers resemble type I fibers in that they display smaller cross-sectional areas (Lewis and Sieck, 1990, Miyata et al., 1995, Prakash et al., 2000, Sieck et al., 1989b, Zhan et al., 1997), higher mitochondrial volume densities and higher oxidative capacities compared to other type II fibers (Enad et al., 1989, Sieck et al., 1996). In addition, type IIa muscle fibers show faster maximum shortening velocities, consistent with faster cross-bridge cycling kinetics compared to type I fibers (Sieck and Prakash, 1997). Specific force is comparable to type I fibers (Geiger et al., 2002, Geiger et al., 2001, Geiger et al., 2000, Geiger et al., 1999).

More fatigable type FInt and type FF motor units display a continuum of fatigue resistance and comprise type IIx and IIb fibers which commonly co-express varying proportions of MyHC_{2X} and MyHC_{2B} isoforms (Sieck et al., 1996). Type IIx and IIb diaphragm fibers have larger cross-sectional area (Greising et al., 2013a, Lewis and Sieck, 1990, Miyata et al., 1995, Prakash et al., 2000, Sieck et al., 2012, Sieck et al., 1989b, Zhan et al., 1997), lower mitochondrial volume densities and oxidative capacities than type I and IIa fibers (Enad et al., 1989, Sieck et al., 1996). Type IIx and IIb fibers display the fastest maximum shortening velocities and generate the greatest specific forces of all fiber types (Geiger et al., 2002, Geiger et al., 2001, Geiger et al., 2000, Geiger et al., 1999).

The number of muscle fibers that is innervated by a motoneuron (i.e., innervation ratio) varies across muscles and motor unit type. For instance, in the diaphragm muscle, type FInt and FF motor units exhibit greater innervation ratios than type S or FR units (Sieck, 1988). The larger fiber cross-sectional areas, greater innervation ratios and greater specific forces generated by type FInt and FF diaphragm motor units results in substantially greater levels of overall force being contributed by type FInt and FF compared to type S and FR units (Figure 2)(Fournier and Sieck, 1988, Mantilla et al., 2010, Mantilla and Sieck, 2011, Sieck and Fournier, 1989). The force contributed by a motor unit strongly predicts its recruitment order (Zajac and Faden, 1985), with type S and FR recruited before and more often than type FInt and FF units. Indeed, during inspiratory efforts, diaphragm motor units with

slower conduction velocities (i.e., type S or FR) are recruited preferentially (Jodkowski et al., 1987, 1988, Seven et al., 2013).

There is considerable heterogeneity in motoneuron morphology, even within a single motor pool such. Morphological differences across motoneurons (viz. differences in dendritic arborization and soma dimensions) contribute to their varied intrinsic electrophysiological properties (Cushing et al., 2005, Su et al., 1997, van Lunteren and Dick, 1992). Morphological differences in phrenic motoneurons comprising diaphragm motor units are considerable (Burke et al., 1992, Cameron et al., 1985, Cameron and Fang, 1989, Issa et al., 2010, Mantilla et al., 2009, Prakash et al., 2000, Qiu et al., 2010, Torikai et al., 1996). Notably, motoneuron properties may depend on motor unit type. Motoneurons comprising type S motor units exhibit the higher input resistance, lower rheobase and slower axonal conduction velocities than motoneurons comprising type FF motor units (Burke, 1981, Zengel et al., 1985), consistent with a smaller size and greater excitability. According to intrinsic electrophysiological properties, a given synaptic input would result in recruitment of smaller, more excitable motoneurons comprising type S and FR units before recruitment of larger motoneurons comprising type FInt and FF units. This recruitment order thus matches the mechanical and fatigue properties of motor units and likely determines the gradation of force development across motor behaviors.

Modeling Diaphragm Motor Unit Recruitment across Motor Tasks

Sieck and Fournier originally developed a model of motor unit recruitment in the cat diaphragm muscle based on the sequential, orderly recruitment of type S, FR, FInt and FF units and the relative force contributed by motor units of each type (Sieck and Fournier, 1989). This model was subsequently developed for hamsters (Sieck, 1995) and rats (Figure 3) (Mantilla et al., 2010, Mantilla and Sieck, 2011, Sieck, 1995). In these models, the mechanical properties of diaphragm motor units were estimated based on measurements of specific force generated by single muscle fibers (Geiger et al., 2002, Geiger et al., 2001, Geiger et al., 2000, Geiger et al., 1999, Sieck, 1988, Sieck and Fournier, 1989, Walmsley et al., 1978) and cross-sectional areas of type-identified muscle fibers (Lewis and Sieck, 1990, Miyata et al., 1995, Prakash et al., 2000, Sieck et al., 1989b, Zhan et al., 1997), as well as the proportion of different fiber types in the diaphragm muscle (Enad et al., 1989, Fournier and Sieck, 1988, Sieck, 1988, Sieck et al., 1989a, Sieck et al., 1996). The gradation of force developed by additional diaphragm motor units (Figure 2; type FF > FInt > FR > S) results in differences in the slope of force development as motor units of each type are progressively recruited (Figure 3).

Forces generated by the diaphragm muscle were approximated by Pdi measurement across a range of ventilatory and non-ventilatory behaviors and maximum Pdi was determined by bilateral phrenic nerve stimulation (see section on *Ventilatory and Non-Ventilatory Behaviors* above). Importantly, diaphragm muscle forces generated during rhythmic ventilatory behaviors (viz. eupnea and hypoxia-hypercapnia) are accomplished by recruitment of only type S and FR motor units (Mantilla et al., 2010, Sieck, 1991b, Sieck, 1994, Sieck and Fournier, 1989). In agreement, estimates of the number of phrenic motoneurons recruited during inspiratory efforts in cats (Jodkowski et al., 1987) are

consistent with the expected proportion of motoneurons comprising type S and FR diaphragm motor units (Fournier and Sieck, 1988, Sieck et al., 1989a). During sustained airway occlusion, it is necessary to recruit all type S, FR and a portion of type FInt units to accomplish this level of force. During coughing (Sieck and Fournier, 1989) or sneezing (Mantilla et al., 2010), the level of force generation was near maximal necessitating full recruitment of all motor unit types in the diaphragm muscle. Of note, a large portion of the motor unit pool in the cat medial gastrocnemius would only need to be recruited during high-intensity behaviors such as jumping (Sieck, 1991a, Walmsley et al., 1978).

Frequency Coding of Motor Unit Recruitment

Neuromotor control of force generation by a skeletal muscle also is executed via coding of motor unit discharge frequencies. Indeed, frequency coding contributes to force generation by the diaphragm muscle (Iscoe et al., 1976, Kong and Berger, 1986, Lee and Fuller, 2011, Lee et al., 2009). In a recent study, changes in motoneuron discharge frequencies were estimated using quantitative analyses of the diaphragm EMG signal across a range of motor tasks (Seven *et al.*, 2013). In limb muscles, frequency-domain analyses of EMG signals show a shift toward higher frequencies as force generation increases under various conditions (Arendt-Nielsen and Mills, 1985, Solomonow et al., 1990). Models of the EMG power spectrum that consider the varying muscle fiber sizes and numbers of active fibers at different activation levels indicate that the EMG power spectral density (PSD) depends primarily on the action potential conduction velocity of activated muscle fibers, and thus on fiber diameter (Lindstrom and Magnusson, 1977). In support of this interpretation, the proportion and type of motor units that are recruited across a range of motor tasks influence EMG PSD (Gerdle et al., 1991, Gerdle et al., 2000, Kupa et al., 1995). Simply stated, a shift toward higher frequencies in the EMG PSD is evident when large-diameter, fast-conducting muscle fibers are recruited. Since rat diaphragm muscle fibers at type S and FR motor units have smaller cross sectional areas than fibers at type FInt and FF units (Aravamudan et al., 2006, Geiger et al., 2000, Lewis et al., 1986, Mantilla et al., 2008, Prakash et al., 1993, Sieck and Fournier, 1989, Verheul et al., 2004), the shift in diaphragm muscle EMG PSD to higher frequencies (e.g., increased centroid frequency) during high-intensity behaviors such as sneezing supports activation of larger muscle fibers comprising more fatigable fast-twitch motor units. Inspiratory resistive loading of the diaphragm muscle was also shown to increase the centroid frequency of the EMG PSD in pigs (Hussain et al., 1991) and rabbits (Cairns and Road, 1998).

Motor unit recruitment order can be modeled assuming submaximal levels of activation of motor units in order to accommodate varying discharge rates. For instance, Figure 3 shows the progressive increase in force generated by the diaphragm muscle as motor units are progressively recruited at 50% of their maximal discharge rate. However, motor unit discharge frequencies change during the inspiratory burst (Butler et al., 1999, Kong and Berger, 1986). Furthermore, onset and offset times for different motor units within an inspiratory burst also varies (Iscoe et al., 1976, Milano et al., 1992). At present, there is a dearth of information on motor unit discharge rates across behaviors, especially for behaviors that require high levels of force generation (Lee and Fuller, 2011). As is to be

expected, single motor unit recordings during motor tasks associated with high demand are technically exceedingly difficult.

In a recent unpublished study, single motor unit firing frequencies were recorded from the diaphragm muscle across a range of motor behaviors. Of note, firing rates at the onset of the inspiratory burst were comparable during rhythmic ventilatory behaviors that included eupnea and hypoxia-hypercapnia as well as during deep breaths. During sustained airway occlusion, onset firing frequencies were significantly higher. The higher firing frequencies reflect increased drive at the onset of the behavior, consistent with the steeper rise in Pdi evident in Figure 1. Indeed, the strong, early increase in drive suggests ballistic motor activation (Desmedt and Godaux, 1978, Milano et al., 1992). Motor units consistently increased discharge rates during the burst. Information regarding the rates of motor unit discharge across a range of motor behaviors will be important in developing more detailed models of force generation.

It is important to realize that interspecies differences in overall force generation (i.e., Pdi expressed as a fraction of maximum Pdi) during different motor tasks reflects the proportion of fatigue-resistant (type S and FR units) vs. more fatigable motor units (type FInt and FF units). For example, in the rat and hamster diaphragm muscle, ~65% and ~54% of motor units are type S or FR, respectively, compared to ~34% in the cat (Sieck, 1991b, Sieck and Fournier, 1989). This proportion of fatigue-resistant vs. more fatigable motor units is in general agreement with the dissimilar demand imposed by rhythmic breathing during eupnea (~27% of maximum Pdi in hamsters and rats vs. ~10% in cats). Of note, relatively infrequent, yet large spontaneous “sigh” breaths were detected in the rat during eupnea that generated forces ~60% of maximum Pdi (Mantilla et al., 2010). Thus, it is possible that recruitment of some type FInt motor units would occur during spontaneous ventilatory behaviors. The recruitment of fatigable motor units during sighs or behaviors associated with airway clearance (e.g., coughing or sneezing) would restrict the frequency and duration of such non-rhythmic behaviors in order for muscle fatigue to be avoided. These motor tasks also usually require coordinated activation of other muscle groups and thus may reflect activation of distinct pattern generators which may only partially involve pattern generators associated with rhythmic, ventilatory behaviors.

Convergence of Pattern Generator Outputs and Motor Unit Recruitment Order

Diaphragm motor units contribute to a number of motor tasks (Butler et al., 2001, Mantilla et al., 2010, Mantilla and Sieck, 2011, Milano et al., 1992, Sieck, 1994, Sieck and Fournier, 1989). Rhythmic behaviors that sustain ventilation likely reflect drive imposed by central pattern generator elements. Infrequent, perhaps ballistic, non-ventilatory behaviors that are important for overcoming airway obstruction or airway clearance may reflect selective inputs from distinct pattern generators distributed according to the motor unit properties necessary to accomplish these different motor tasks. Inputs to phrenic motoneurons could involve functional overlap between these different pattern generators. For instance, behaviors requiring co-activation of various muscles groups (e.g., diaphragm and abdominal muscles) across different phases of a maneuver (e.g., cough or sneeze) could “broadcast” the

pattern using the central pattern generator involved in rhythmic behaviors. In this case, it is expected that such inputs to phrenic motoneurons would effect recruitment order based on intrinsic electrophysiological properties.

The period of motor unit recruitment in a burst may become evident within the EMG signal by evaluating stationarity (Mantilla et al., 2011, Mantilla et al., 2010, Seven et al., 2013). Although frequently ignored in power spectral analyses, the EMG signal should be at least wide-sense or weakly stationary in the measurement window (Bilodeau et al., 1997, Papoulis, 1984). Stationarity is commonly established by using the mean square value of the EMG signal for the specific window length (Duchene and Goubel, 1993) followed by a reverse arrangement test that evaluates stochastic variables using non-parametric statistics (Bendat and Piersol, 2010, Bilodeau et al., 1997). Indeed, as the maximum number of motor units activated during a motor behavior is achieved, the EMG signal becomes stationary. Conversely, the period of non-stationarity at the onset of the EMG burst can be used to evaluate motor unit recruitment across a range of behaviors (Mantilla et al., 2011, Mantilla et al., 2010, Seven et al., 2013). In the rat diaphragm muscle, the period of non-stationarity shortened from eupnea to hypoxia-hypercapnia and airway occlusion, consistent with increasing drive. Earlier recruitment of diaphragm motor units was reported during strong muscle contractions such as fictive coughing and fictive vomiting in decerebrate cats (Milano et al., 1992) and during hypercapnia in paralyzed, vagotomized and mechanically ventilated rats and cats (Kong and Berger, 1986, St John and Bartlett, 1979). Studies examining single motor unit activity have not consistently evaluated multiple motor behaviors in neurally intact animals (Arita and Bishop, 1983, Bishop et al., 1981, Jodkowski et al., 1987, Kong and Berger, 1986, Milano et al., 1992, St John and Bartlett, 1979). In this sense, evaluating the stationarity of the EMG signal can be used to obtain global information about recruitment of motoneurons within a motor pool (Figure 4). Indeed, most diaphragm motor units start firing during the period of non-stationarity across both ventilatory and non-ventilatory behaviors.

Analysis of motor unit pairs can be used to compare the relative timing of discharge onset (i.e., recruitment). In a recent, unpublished study, discharge onset for single diaphragm motor units was calculated by averaging across multiple bursts. Overall, motor units that were newly recruited during hypoxia-hypercapnia or airway occlusion compared to eupnea exhibited longer recruitment delays, consistent with higher-recruitment thresholds. In addition, the frequency with which pairs of motor units displayed occasional reversal of their recruitment order (i.e., when the predominant order of recruitment across multiple bursts became reversed) was calculated. Few motor units with large differences in the timing of recruitment displayed reversals. With increasing drive, reversals became more frequent, consistent with intrinsic electrophysiological properties being the primary determinant of motor unit recruitment order across both rhythmic ventilatory behaviors (viz. eupnea, hypoxia-hypercapnia) and non-ventilatory behaviors (sustained airway occlusion).

Conclusions and Future Directions

The diaphragm muscle is the main inspiratory muscle in mammals, and consequently, recruitment of diaphragm motor units is particularly important for neuromotor control of

respiration. Diaphragm motor units contribute to a number of motor tasks including rhythmic behaviors that sustain ventilation as well as to non-ventilatory behaviors important for airway clearance (e.g., swallowing, coughing and sneezing). In several species, measurements of diaphragm muscle force show that ventilatory behaviors require only a fraction (e.g., 12-27% during eupnea) of the maximal force generating capacity of the diaphragm. Estimates of motor unit recruitment during these motor behaviors consistently suggest that the levels of motor activity required for rhythmic ventilatory behaviors can be accomplished by recruiting only fatigue-resistant (type S and FR) motor units. This orderly recruitment of motor units is consistent with order being dictated by intrinsic electrophysiological properties of motoneurons during rhythmic behaviors driven by a central pattern generator. Only infrequent, short-duration behaviors such as coughing or sneezing would require recruitment of all diaphragm motor unit types. Diaphragm EMG activity constitutes a useful surrogate measure for diaphragm muscle force given the high degree of correlation between relative diaphragm root-mean-square EMG and Pdi (Mantilla *et al.*, 2010). In addition, power spectral analyses and assessment of stationarity of the EMG signal provide indirect measures of motor unit recruitment. Collectively, the findings reported herein provide converging support for an orderly recruitment of diaphragm motor units dictated by motoneuron properties. Thus, rhythmic ventilatory behaviors (generated by a central pattern generator) and non-ventilatory behaviors which involve distinct pattern generators for coordinated activation of multiple muscle groups likely converge. The exact neuroanatomical structures responsible for generating these patterns, such as the parabrachial nuclei, lateral solitary nucleus, and the medullary ventrolateral tegmental field including the nucleus retroambiguus (Holstege, 1989, 2014, Holstege and Kuypers, 1982), and the site(s) of signal convergence remain the subject of active investigation. Importantly, when considering neuromotor control of the diaphragm muscle, the large reserve capacity for force generation by the diaphragm likely will conceal disease progression and compromised ventilatory capacity if multiple motor behaviors are not examined.

Acknowledgments

Supported by NIH grants HL096750, AG044615 and the Mayo Clinic.

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Figure 1.

Transdiaphragmatic pressure (Pdi) measurements across various motor tasks. Measurements were obtained in an adult male rat across the following ventilatory and non-ventilatory behaviors: eupnea, hypoxia (10% O₂)/hypercapnia (5% CO₂), deep breaths (“sighs”) and sustained airway occlusion. Values are expressed as percent of maximum Pdi obtained by bilateral supramaximal phrenic nerve stimulation (75 Hz). The Pdi generated during deep breaths and airway occlusion is significantly different from that generated during eupnea or hypoxia-hypercapnia (Mantilla et al., 2010).

Figure 2.

Force elicited by maximal activation of diaphragm motor units in rats according to motor unit type (Mantilla and Sieck, 2011). Estimates were based on measurements of maximum specific force (force per cross-sectional area) in single type-identified fibers, cross-sectional area and proportion of different fiber types and previous reports of motor unit innervation ratios (Geiger et al., 2002, Geiger et al., 2001, Geiger et al., 2000, Geiger et al., 1999, Lewis and Sieck, 1990, Miyata et al., 1995, Prakash et al., 2000, Sieck, 1988, Sieck et al., 1989b, Zhan et al., 1997). The width of each bar represents the proportion of the motor unit pool represented by each motor unit type.

Figure 3.

Model of motor unit recruitment for the diaphragm muscle across ventilatory and non-ventilatory behaviors. Model shown is for the adult rat, modified from (Mantilla et al., 2010). Similar models are available for cats and hamsters (Sieck, 1991b, Sieck, 1994, Sieck and Fournier, 1989). The model is based on complete activation of all motor units of each type in the following recruitment order: type S followed by type FR, type FInt and type FF. Forces generated during the different behaviors were obtained from Pdi measurements and expressed as percent of maximum Pdi obtained by bilateral phrenic nerve stimulation. (shown in shaded areas as mean $\pm$ SE). The slope of force development represents additional motor units being recruited at maximal discharge frequency (black line) and at 50% maximal frequency (gray line).

Figure 4.

Distribution of motor unit recruitment in the rat diaphragm muscle across various motor tasks. Average period of non-stationarity in the diaphragm EMG signal was 150 ms during eupnea, 100 ms during exposure to hypoxia-hypercapnia and 80 ms during airway occlusion. In all three behaviors, most diaphragm motor units start firing within the period of non-stationarity, which may be used as an index of motor unit recruitment. Data from (Seven et al., 2013).