Motor Unit Number Estimation (MUNE) and Quantitative EMG
(Supplements to Clinical Neurophysiology, Vol. 60)
Editors: M.B. Bromberg

Chapter 11

Decomposition-enhanced spike triggered averaging MUNE: validity, reliability, and impact of contraction force

Timothy J. Doherty[a,*], Daniel W. Stashuk[b] and Shaun G. Boe[c]

[a]*Department of Clinical Neurological Sciences, University Hospital, London Health Sciences Centre, London, ON N6A 5A5, Canada*

[b]*Department of Systems Design Engineering, University of Waterloo, Waterloo, ON N2L 3G1, Canada*

[c]*Heart and Stroke Foundation Centre for Stroke Recovery, Sunnybrook Health Sciences Centre, University Centre, Toronto Rehabilitation Institute, Toronto, ON M5G 2A2, Canada*

1. Introduction

Motor unit number estimation (MUNE) provides the only direct electrophysiological indication of the number of functioning motor neurons or motor axons supplying a given muscle or muscle group (Doherty and Brown, 2002; Shefner and Gooch, 2003). This information is useful when evaluating the extent of motor unit (MU) loss associated with aging, disorders of the motor neuron, or peripheral neuropathies. Furthermore, MUNE can provide valuable data when assessing the natural history and outcome of treatment for these disorders (Shefner, 2001; Doherty and Brown, 2002).

The same basic principles are used for all MUNE techniques. Supramaximal electrical stimulation of the motor nerve to a given muscle group evokes a maximum M-potential that represents the sum of the contributing individual surface-detected motor unit potentials (S-MUPs). A representative sample of S-MUPs is collected and the mean S-MUP size is calculated. Dividing a size-related parameter of the mean S-MUP into the corresponding value for the maximum M-potential then derives a MUNE. The various MUNE techniques differ mainly with regard to the method used to collect a sample of S-MUPs. The most commonly used methods, which include incremental stimulation, multiple point stimulation (MPS) and its variations, the statistical method, and spike triggered averaging (STA), each have a number of positive and negative features that make them more or less applicable in a given clinical situation (Shefner, 2001; Doherty and Brown, 2002). If one assumes that all of the aforementioned MUNE methods are valid, their clinical utility or usefulness as an outcome measure is predominantly dependent on their ease of use and reliability.

*Correspondence to: Dr. Timothy J. Doherty, Department of Clinical Neurological Sciences, University Hospital, London Health Sciences Centre, 339 Windermere Road, London, ON N6A 5A5, Canada.
Tel.: +1 (519) 663-3337; Fax: +1 (519) 663-3328;
E-mail: tim.doherty@lhsc.on.ca

The original STA method utilizes a selective intramuscular electrode and surface electrodes to simultaneously detect electromyographic (EMG) signals during low intensity isometric contraction (Brown et al., 1988). Needle-detected motor unit potentials (MUPs), collected one-by-one with the aid of a level or window-based discriminator are used as triggering sources to select the time-locked sections of the surface EMG signal associated with the triggering MUP. The surface EMG data are subsequently ensemble averaged, usually requiring one hundred or more triggers, to extract the S-MUP. Ten or more different S-MUPs are collected in this fashion in order to derive the mean S-MUP size.

The STA MUNE technique has several advantages, including the elimination of alternation as well as the applicability of the technique to proximal muscles that cannot be readily studied with other methods. Despite these advantages, limitations associated with the STA technique include the amount of time required to collect a requisite sample of S-MUPs, and the need for considerable patient cooperation over the 30–60 min that may be required to complete the study. Additionally, based on the size principle of MU recruitment, low-level voluntary contractions may not recruit the full range of MUs with differing sizes and physiological properties in a given muscle (Henneman, 1957; Milner-Brown et al., 1973; Henneman and Mendell, 1981; Brown et al., 1988).

In an effort to improve upon the limitations of the STA technique, we have linked decomposition analysis of the needle-detected EMG signal, the central component of decomposition-based quantitative EMG (DQEMG) (Stashuk, 1999), to MUNE. So called decomposition-enhanced STA (DE-STA) differs from standard STA mainly with regard to how individual MUPs and their firing times are isolated for STA of the surface EMG signal. As opposed to a level or window discriminator, as used to isolate individual MUPs for standard STA, DE-STA uses a series of signal-processing and pattern-recognition algorithms to extract multiple motor unit potential trains (MUP trains) from a given contraction (Fig. 11.1). Thus, in essence the decomposition algorithm acts as an *intelligent trigger* with the ability to detect and classify the occurrences of MUPs from multiple MUs during each contraction. The firing times from these MUP trains serve as triggers to extract the corresponding S-MUPs using STA. This brief review will describe the methodology in detail, validation of the method, its reliability, impact of contraction intensity on MUNE values, and the application of DE-STA to the study of aging.

2. DE-STA methodology

The specific methodology used to address each of the above areas will be outlined in the corresponding section. The following will describe, in brief, the basic components and steps inherent in the DE-STA MUNE technique (for complete review of methodology see (Stashuk, 1999; Boe et al., 2004).

All electromyographic signals were acquired using the DQEMG and Acquire EMG software on the Neuroscan Comperio (Neuroscan Medical Systems, El Paso, TX). Intramuscular signals were recorded with commercially available, disposable concentric needle electrodes (Model N53153; Teca Corp., Hawthorne, NY) with a bandpass of 10 Hz–10 kHz, while surface signals were recorded with a bandpass of 5 Hz–5 kHz using self-adhering electrodes (Kendall-LTP, Chicopee, MA). In each case, the active surface electrode was positioned over the motor point of the muscle group under study. In this position, the M-potential initial rise time is minimized and the negative peak amplitude maximized. The reference electrode was positioned over the distal tendon or a bony point. The maximum M-potential was obtained in each series of studies by supramaximal stimulation of the corresponding motor nerve. The stimulation site was typically distal (e.g., median nerve at the wrist for thenar muscles). Following collection of the maximum M-potential, subjects were required to

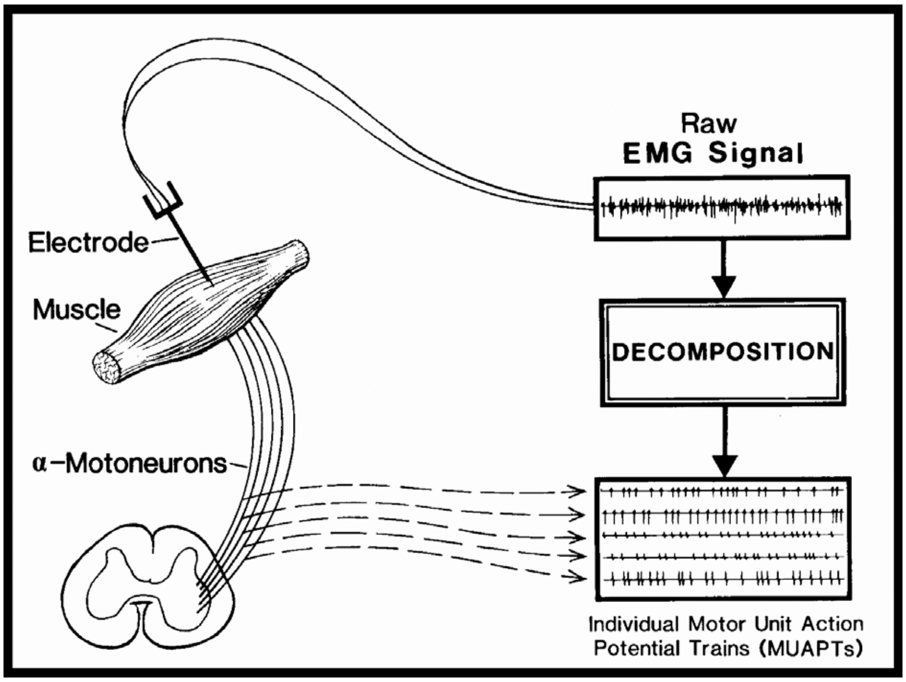

Fig. 11.1 This illustration outlines the basic concepts of decomposition analysis of needle-detected EMG signals. The composite EMG interference pattern is decomposed into its individual motor unit potential (MUP) trains by a series of pattern detection and recognition algorithms. For the purposes of MUNE, the spike occurrences of individual MUPs serve as triggers for spike-triggered averaging of the surface EMG signal.

perform a 4–5 s maximal voluntary contraction (MVC), during which the maximum surface EMG signal, as represented by the maximum root mean square (RMS) value over a 1 s window, was automatically measured. In some cases, force or torque was also measured during the MVC with a dynamometer. Determination of the MVC RMS, or MVC force, allowed subsequent submaximal voluntary contractions to be performed at specific contraction intensities.

Following determination of the maximum M-potential and MVC, a concentric needle electrode was inserted into the muscle, usually within 2 cm of the active electrode. Subjects were then asked to minimally contract the muscle isometrically while the needle position was adjusted in order to minimize the rise-times of the MUPs of the first 2–3 recruited MUs. With the needle manually maintained in a stable position by the examiner, the subject was instructed to increase the contraction force to the desired percent of MVC or MVC RMS as indicated by visual feedback on an oscilloscope or Acquire EMG. If the needle-detected signal was of poor quality based on visual inspection, the needle was repositioned and the process repeated to ensure adequate signal quality. Each isometric contraction lasted for 30 s. Subjects were instructed to maintain consistent contraction intensities throughout the 30 s contraction period and were aided in doing so by the presence of a target line displayed on the oscilloscope or computer, and audio feedback from the needle signal. Following each contraction, the needle was repositioned in an attempt to sample from different MUs. Contractions were performed until a minimum of 20 MUP trains was obtained. Following needle-detected signal decomposition and analysis, the MUP trains were reviewed with regard to their acceptability based on criteria that has been previously reported.

In brief, the MUP trains were required to have 50 or more detected MUPs, consistent MU firing times and display interdischarge interval histograms that were Gaussian in nature with coefficients of variation of less than 0.3. The S-MUPs extracted by STA for each MUP train were reviewed and the onset, negative and positive peak markers were adjusted as required. All acceptable S-MUPs were then computer aligned based on their onsets and a mean S-MUP template was calculated. Size-related parameters (usually negative peak amplitude) were then divided into the corresponding value for the maximum M-potential to calculate the MUNE.

3. Validity of DE-STA MUNE

The validity of MUNE, given that there is no gold standard for comparison, is usually based on the underlying physiological basis of the technique and comparison of the MUNE results with other previously established methods. The DE-STA method is essentially an extension of the previously established traditional STA method (Brown et al., 1988; Doherty et al., 1993). As outlined in the methods section, for the purposes of MUNE, DQEMG simply provides the triggers for STA, based upon the occurrences of needle-detected MUPs within the MUP train. Thus, given that there has been considerable literature validating STA as a MUNE method, the validity of the decomposition algorithms is the main issue with regard to DE-STA.

In terms of validation of the decomposition algorithms, Stashuk (1999) reported very low error rates and high assignment rates in comparison to manually decomposed “gold standard” data sets. Subsequently, Doherty and Stashuk (2003) demonstrated the ability of DQEMG to successfully decompose needle-detected EMG signals across a number of muscles and varying contraction intensities. The results obtained were similar to those previously reported for other decomposition EMG methods for these muscles. Boe et al. (2004) first described DE-STA as a MUNE method and reported mean thenar MUNE values in healthy controls of 249 +/− 78, in keeping with MUNE results for this muscle group from other methods. Furthermore, the S-MUP size distribution was similar to that previously reported for other methods, including MPS (Doherty and Brown, 1993). From a practical standpoint, it was reported that a MUNE could be obtained in about 10 min following completion of a standard motor nerve conduction study, thereby providing the required maximum M-potential data. Therefore, DE-STA appeared to be a valid and practical MUNE method.

4. Reliability of DE-STA MUNE

The reliability of DE-STA MUNE has been examined for the thenar, first dorsal interosseous and biceps brachii muscles. Boe et al. (2004) examined the intra-tester reliability by comparing the test–retest values for thenar MUNEs in a group of healthy controls. The mean values for the two trials were similar (249 +/− 78 and 246 +/− 90), with high test–retest reliability ($r = 0.94$, $P < 0.05$). In a second study, Boe et al. (2006) examined the within-subject intra-tester reliability for both first dorsal interosseous and biceps brachii MUNEs using a test–retest design. There were no significant differences for the test–retest values for either muscle group and the correlations for test–retest were high in both cases (0.72 first dorsal interosseous, 0.97 biceps). Ninety-five percent confidence intervals were calculated to establish the range of expected MUNE variability for re-test; these were ± 41 MUs for both muscles.

5. Effect of contraction intensity on DE-STA MUNE

The addition of decomposition analysis to the STA technique allows for the inclusion of MUP trains produced by, at least, moderate intensity contractions. This is in contrast to the standard STA technique where the use of simple level or window triggering necessitates the use of very low intensity contractions (Brown et al., 1988). While the use of higher intensity contractions is helpful because it increases the number of MUs collected per contraction and allows for the inclusion of higher threshold MUs in the sample of S-MUPs, it was clear, based on initial studies, that it would necessitate controlling for contraction intensity when performing a MUNE (Boe et al., 2004). This is due to the well-known relationship between contraction intensity and MU size evident during voluntary contractions, the so-called "size principle" of MU recruitment (Henneman and Mendell, 1981; Ertas et al., 1995; Doherty et al., 2002).

To establish the potential effect of the size principle on S-MUP size and MUNE, we examined the impact of increasing levels of voluntary contraction force for both the first dorsal interosseous and tibialis anterior muscles (Boe et al., 2005b). These muscles were chosen because their voluntary force can be reasonably measured in a single vector by a dynamometer without significant contributions from other muscles; however, this is arguably truer for the first dorsal interosseous than tibialis anterior muscles.

The relationship between increasing levels of contraction force and both needle MUP and S-MUP size for the first dorsal interosseous muscle is illustrated in Fig. 11.2. It is important to recall that the decomposition analysis is only performed on the needle-detected EMG signal, which then provides triggers for STA to extract the S-MUPs from the surface EMG. It is clear that there is an obvious relationship between

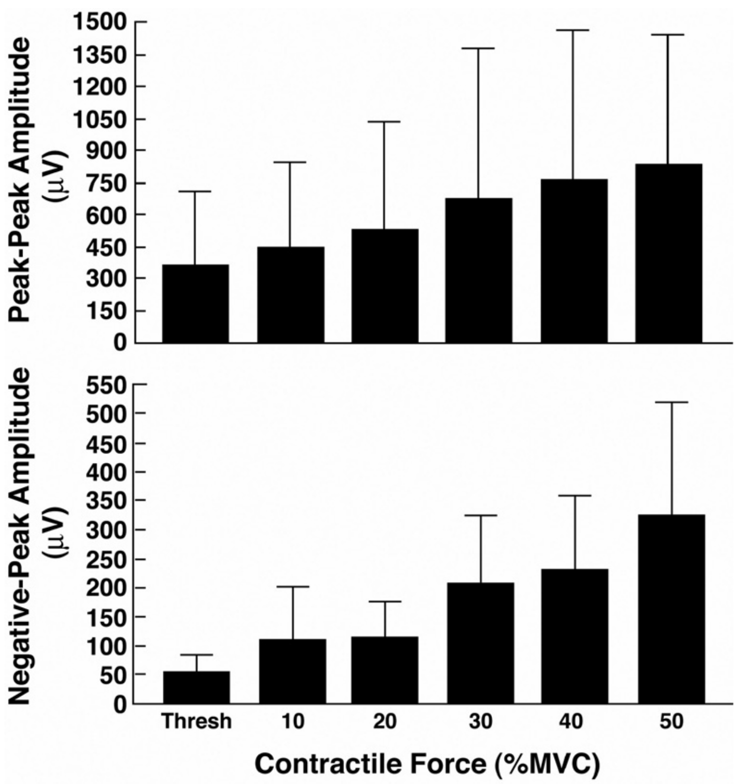

Fig. 11.2 This figure illustrates the needle- (upper graph) and surface-detected (lower graph) MUP size at specific percentages of the maximum voluntary contractile force (μV = microvolt, MVC = maximal voluntary contraction) for the first dorsal interosseous muscle.

contraction intensity and the size of the needle-detected potentials, and their associated S-MUPs. Given the increasing mean S-MUP size with higher contraction forces, the MUNEs were substantially lower with the samples drawn from higher force contractions (e.g., MUNE 282 at threshold; 53 at 50% MVC). This same relationship, as expected, held true for the tibialis anterior muscle. In this study (McNeil et al., 2005a), an ensemble mean S-MUP was calculated from over 100 MUs from all contraction intensities which was then extrapolated to a force of about 25% (Fig. 11.3).

The underlying cause of the increasing S-MUP size with increasing contraction intensity is, based on our observations, a result of both physiological and technical factors. Figure 11.4 illustrates the needle- and surface-detected MUPs collected at various contraction intensities for two representative subjects. It is clear that with increasing contraction intensity, increasing numbers of larger MUPs and their S-MUPs are contributing to the sample of S-MUPs. This likely reflects the recruitment of increased numbers of large S-MUPs based on the size principle. However, it is also evident that at 30, 40, and 50% MVC there are fewer small MUPs detected. This is a direct result of the increasing complexity of the EMG interference pattern and the inability of DQEMG

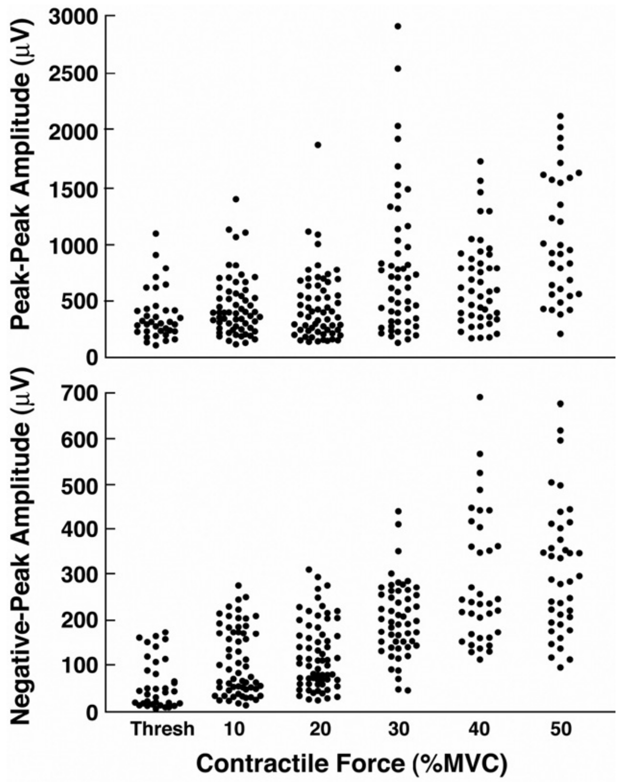

Fig. 11.4 This figure illustrates the individual needle-detected (top panel) and surface-detected (bottom panel) motor unit potentials for two individual subjects (see text for details).

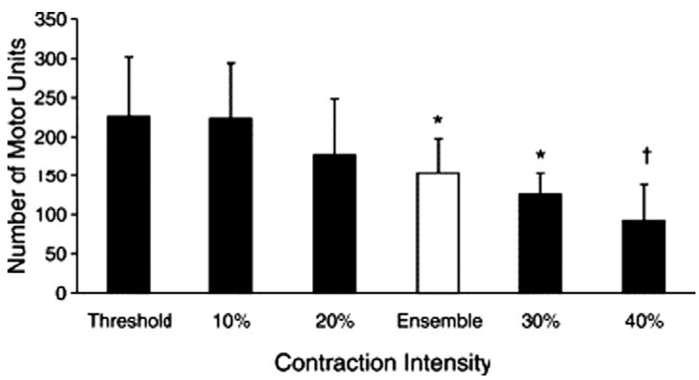

Fig. 11.3 This figure illustrates the impact of increasing voluntary contractile force as a percentage of maximum for the mean MUNE values from the tibialis anterior muscle in young controls. The ensemble MUNE from over 100 S-MUPs from each subject corresponded to about 25% of the maximal voluntary contraction force.

to detect small MUPs based on their signal-to-noise ratio. This, in turn, reduces the number of small S-MUPs at the higher contraction intensities. Further, contributing to the reduced numbers of small S-MUPs are the reduced numbers of triggers available for STA at higher contraction levels due to reduced MUP detection rates. This will impact the smaller S-MUPs to a greater extent due to their lower signal-to-noise ratio and the need for increased numbers of triggers for averaging to extract their S-MUP. As a result of these observations, we have subsequently collected DE-STA MUNEs at a moderate contraction intensity of about 20–30% MVC. Whether this is the most valid approach is the focus of ongoing study. We have recently considered that it may be most appropriate to control for the overall complexity of the needle EMG interference pattern, as opposed to contraction level, particularly when completing longitudinal studies.

6. Investigation of age-related loss of MUs with DE-STA

MUNE has been applied to the study of age-related MU loss almost since its inception (McComas et al., 1971; Brown, 1973; Doherty and Brown, 1993; Doherty et al., 1993). Aging provides a very useful physiological model to validate a given technique's ability to detect changes in the numbers and sizes of MUs. To this end, we applied the DE-STA technique to examine the numbers of MUs in the TA of a group of healthy, active very old (mean age 82 ± 4 years), older (66 ± 3 years) and younger men (27 ± 3 years) (McNeil et al., 2005b). The MUNEs were compared with MVC strength, maximal twitch tensions and twitch contractile properties. The MVC values were similar for the young and older groups, but significantly lower for the very old group (30%). The twitch tensions were lower in the old and very old, but owing to the variability of the data, this did not reach statistical significance. The maximum M-potentials, similar to the MVCs, were reduced by about 30% in the very old men in comparison to the old and younger groups. Conversely, the mean S-MUP size was significantly larger in the very old (82 ± 26 μV), and older subjects (78 ± 19 μV), in comparison to the young (49 ± 16 μV). This then resulted in substantially reduced MUNEs in the very old (59 ± 15) in comparison to the older (91 ± 22) and younger (150 ± 15) subjects. Given these overall results, it would appear that strength, and presumably muscle mass (all subjects were able to maximally activate based on results of twitch interpolation), was reasonably well maintained in the older subjects despite MU loss, likely as a result of collateral reinnervation. However, in the very old, where the MU losses were greater than 50% of the younger subjects, collateral reinnervation does not appear to have been as effective in maintaining muscle mass. These findings are of considerable interest in explaining the rapid decline in muscle mass and strength that occur in the very old (beyond 75 years), and the resultant limitations in mobility and functional independence (Doherty, 2003).

7. Discussion

The DE-STA technique provides a very attractive method of obtaining a MUNE from both distal and proximal muscles. From a methodological standpoint, DE-STA is essentially an extension of the original STA technique with MU decomposition allowing for the collection of multiple MUs from a single contraction, thus substantially decreasing the time required to obtain the required number of MUs to derive a MUNE. The decomposition analysis, furthermore, allows for the sampling of MUs from at least moderately strong contractions, thus leading to less bias in the sample of S-MUPs from which the mean S-MUP size is drawn. Additionally, from a practical standpoint, following placement of surface electrodes to complete a standard motor nerve conduction study, the required MUP trains to derive a MUNE can typically be collected in about 10 min. The biggest drawback of this method is the requirement of needle EMG, which is obviously invasive and moderately uncomfortable. Alternatively, DE-STA also provides standard quantitative EMG data pertaining to MUP size, duration, and complexity.

In terms of validity, the mean control MUNE values for the DE-STA technique from the thenar muscles (269 ± 104) based on negative peak amplitude are in keeping with MUNE results for this muscle group from other methods (Shefner, 2001; Doherty and Brown, 2002; Doherty, 2003; Boe et al., 2004). Perhaps most specifically, they are similar to the MUNE values from traditional STA (Doherty and Brown, 1993). The similar thenar MUNE values, based on standard STA and DE-STA, may relate to the fact that the MUs from intrinsic hand muscles tend to be recruited at low force with further force gradation based on rate coding (Kukulka and Clamann, 1981). Alternatively, control biceps brachii/brachialis MUNEs from the DE-STA technique were lower (271 ± 125) than MUNEs from the traditional STA technique (357 ± 97) from an earlier study (Doherty et al., 1993). This may, in part, be related to the broader range of MU sizes examined with the DE-STA method and this muscle's tendency to recruit new MUs, with presumably larger S-MUPs, throughout its recruitment range (Kukulka and Clamann, 1981). In general, we would recommend that control MUNE values based on DE-STA be collected for comparative purposes as they may differ from traditional STA.

The intra-tester reliability of DE-STA MUNE is in keeping with the results from other methods. Inter-tester reliability has not yet been reported but is the topic of a current study in our laboratory. There is very little operator intervention required for the collection of the needle-detected MUP trains, other than the ability to perform routine needle EMG. The contraction intensity, as outlined previously, can also impact inter- or intra-rater reliability (see later discussion). Perhaps the area that requires the greatest operator input is in selecting acceptable MUP trains. However, we have developed consistent criteria for this process that should reduce intra-tester reliability (see Boe et al., 2005). Additionally, future versions of the DQEMG software will likely include the ability to automatically exclude from further analysis any trains that do not meet empirically developed criteria.

The impact of contraction intensity on DE-STA MUNEs, as indicated previously, is significant. This is expected given the well-known relationship between MU size and recruitment. Based on our experience, this impact may be greatest for larger proximal muscles where new MUs are recruited at intensities as high as 80% of MVC force. It is, therefore, necessary to control for contraction intensity when collecting the MUP trains with DE-STA. In some of our recent work this has been accomplished with the use of force dynamometers that allow the contractions to be performed at a specific percentage of MVC. This, however, is not practical in day-to-day practice or for some muscle groups. Therefore, we have also examined whether the surface EMG signal, as measured by the RMS value, could be used as a surrogate of contractile force. We have recently reported acceptable correlations between the RMS of the surface EMG signal and force for both healthy controls and patients with reduced MU numbers (hereditary neuropathy and ALS) for both the first dorsal interosseous and biceps brachii muscles, thus supporting the use of surface EMG as a reasonable indication of contraction intensity (Boe et al., 2005a). We have also considered controlling for the intensity of the contraction based on the overall complexity of the aggregate needle EMG signal; however, the usefulness of this approach has yet to be examined systematically.

As outlined above we have used the DE-STA technique to examine age-related changes in the numbers of MUs in the tibialis anterior of very old and older men (McNeil et al., 2005b). This study was the first to establish that substantive MU losses in the very old (over 80) may be a significant contributing factor to accelerated losses of muscle mass and associated frailty. As outlined elsewhere in this volume, we have also used the DE-STA MUNE technique to examine MU numbers and electrophysiological properties in patients with ALS and hereditary neuropathy.

In conclusion, the DE-STA technique appears to be a valid, reliable means of acquiring a MUNE, with the added value of standard quantitative EMG data from the intramuscular signal. Future software development will be focused on greater automation of MUP train selection and editing of the S-MUP data.

References

Boe, S.G., Stashuk, D.W. and Doherty, T.J. (2004) Motor unit number estimation by decomposition-enhanced spike-triggered averaging: control data, test–retest reliability, and contractile level effects. *Muscle Nerve*, 29: 693–699.

Boe, S.G., Rice, C.L. and Doherty, T.J. (2005a) The relationship between force and the surface-electromyogram. *Can. J. Appl. Physiol.*, 30: S10.

Boe, S.G., Stashuk, D.W., Brown, W.F. and Doherty, T.J. (2005b) Decomposition-based quantitative electromyography: effect of force on motor unit potentials and motor unit number estimates. *Muscle Nerve*, 31: 365–373.

Boe, S.G., Stashuk, D.W. and Doherty, T.J. (2006) Within-subject reliability of motor unit number estimates and quantitative motor unit analysis in a distal and proximal upper limb muscle. *Clin. Neurophysiol.*, 117: 596–603.

Brown, W.F. (1973) Functional compensation of human motor units in health and disease. *J. Neurol. Sci.*, 20: 199.

Brown, W.F., Strong, M.J. and Snow, R. (1988) Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. *Muscle Nerve*, 11: 423–432.

Doherty, T.J. (2003) Invited review: aging and sarcopenia. *J. Appl. Physiol.*, 95: 1717–1727.

Doherty, T.J. and Brown, W.F. (1993) The estimated numbers and relative sizes of thenar motor units as selected by multiple point stimulation in young and older adults. *Muscle Nerve*, 16: 355–366.

Doherty, T.J., Vandervoort, A.A., Taylor, A.W. and Brown, W.F. (1993) Effects of motor unit losses on strength in older men and women. *J. Appl. Physiol.*, 74: 868–874.

Doherty, T.J., Chan, K.M. and Brown, W.F. (2002) Motor neurons, motor units, and motor unit recruitment. In: W.F. Brown, C.F. Bolton and M.J. Aminoff (Eds.), *Neuromuscular Function and Disease: Basic, Clinical, and Electrodiagnostic Aspects.* Saunders, Philadelphia, PA, pp. 247–273.

Ertas, M., Stålberg, E. and Falck, B. (1995) Can the size principle be detected in conventional EMG recordings? *Muscle Nerve*, 18: 435–439.

Henneman, E. (1957) Relation between size of neurons and their susceptibility to discharge. *Science*, 126: 1345–1346.

Henneman, E. and Mendell, L.M. (1981) Functional organization of motoneuron pool and its inputs. In: V.B. Brooks (Ed.), *Handbook of Physiology, Vol 2*, American Physiological Society, Bethesda, MD, pp. 423–507.

Kukulka, C.G. and Clamann, H.P. (1981) Comparison of the recruitment and discharge properties of motor units in human brachial biceps and adductor pollicis during isometric contractions. *Brain Res.*, 219: 45–55.

McComas, A.J., Sica, R.E., Campbell, M.J. and Upton, A.R. (1971) Functional compensation in partially denervated muscles. *J. Neurol. Neurosurg. Psychiatry*, 34: 453–460.

McNeil, C.J., Doherty, T.J., Stashuk, D.W. and Rice, C.L. (2005a) The effect of contraction intensity on motor unit number estimates of the tibialis anterior. *Clin. Neurophysiol*, 116: 1342–1347.

McNeil, C.J., Doherty, T.J., Stashuk, D.W. and Rice, C.L. (2005b) Motor unit number estimates in the tibialis anterior muscle of young, old, and very old men. *Muscle Nerve*, 31: 461–467.

Milner-Brown, H.S., Stein, R.B. and Yemm, R. (1973) The orderly recruitment of human motor units during voluntary isometric contraction. *J. Physiol. (Lond.)*, 230: 371–390.

Shefner, J.M. (2001) Motor unit number estimation in human neurological diseases and animal models. *Clin. Neurophysiol.*, 112: 955–964.

Shefner, J.M. and Gooch, C.L. (2003) Motor unit number estimation. *Phys. Med. Rehab. Clin. N. Am.*, 14: 243–260.

Stashuk, D.W. (1999) Decomposition and quantitative analysis of clinical electromyographic signals. *Med. Eng. Phys.*, 21: 389–404.