ABSTRACT: Decomposition-enhanced spike-triggered averaging (DE-STA) has been developed as a method for obtaining a motor unit number estimate (MUNE). We describe the method and report control data for the first dorsal interosseous/adductor pollicis and thenar muscles and reliability in the thenar muscles. Seventeen subjects (ages 20–50 years) took part in the study. The maximum M potential was elicited with supramaximal stimulation of the ulnar or median nerve at the wrist. Surface and intramuscularly detected electromyographic signals were then collected simultaneously during mild to moderate contractions. Decomposition algorithms were used to detect and sort the individual motor unit potential (MUP) occurrences of several concurrently active motor units in the needle-detected signals. The MUP occurrences were used as triggering sources to estimate their corresponding surface-detected MUPs (S-MUPs) using STA. The mean S-MUP size was calculated and divided into the maximum M-potential size to derive a MUNE. The MUNE values were consistent with those previously reported with other methods, and thenar MUNEs for the two trials were similar (249 $\pm$ 78 and 246 $\pm$ 90), with high test–retest reliability ($r = 0.94$, $P < 0.05$). DE-STA thus appears to be a valid and reliable method to obtain MUNEs.
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MOTOR UNIT NUMBER ESTIMATION BY DECOMPOSITION-ENHANCED SPIKE-TRIGGERED AVERAGING: CONTROL DATA, TEST–RETEST RELIABILITY, AND CONTRACTILE LEVEL EFFECTS

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Motor unit number estimates (MUNEs) provide information related to the number of functioning motor neurons or motor axons in a given muscle or muscle group.[2,6–8,11] This information is useful when evaluating the extent of motor unit (MU) loss associated with motor neuron disease or peripheral neuropathy and when assessing the course and outcome of treatment for these disorders.[3,11,17,19]

The same basic principle is employed in all MUNE techniques. Supramaximal electrical stimulation of the motor nerve to a given muscle group produces a maximum M potential detected with surface electrodes. This M potential represents the sum of the individual surface-detected motor unit potentials (S-MUPs) in the muscle group. A representative sample of S-MUPs is collected and their mean size calculated. A MUNE is then derived by dividing a size-related parameter of the mean S-MUP into the corresponding maximal M-potential value. The various MUNE techniques essentially differ only with regard to the method used to collect a sample of S-MUPs. Each of the currently available methods—which include incremental stimulation, multiple point stimulation (MPS), the statistical method, and spike-triggered averaging (STA)—has a number of positive and negative features that make them more or less applicable in a given clinical situa-

Abbreviations: AP, adductor pollicis; DE-STA, decomposition-enhanced spike-triggered averaging; EMG, electromyography; FDI, first dorsal interosseous; MPS, multiple point stimulation; MU, motor unit; MUNE, motor unit number estimate; MUP, motor unit potential; MVC, maximum voluntary contraction; RMS, root mean square; S-MUP, surface motor unit potential; STA, spike-triggered averaging

Key words: electromyography; motor unit number estimation; motor unit; quantitative EMG; skeletal muscle

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tion.[1,8,21,26,27] If one assumes that all 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.[2,6,8,21]

The STA method utilizes a selective intramuscular electrode and surface electrodes to simultaneously detect electromyographic (EMG) signals during minimal isometric contraction. Needle-detected motor unit potentials (MUPs), collected individually with the aid of a level or window-based discriminator, are used as triggering sources to select specific sections of the surface EMG signal, which are averaged to produce an S-MUP. The mean sizes of the representative S-MUPs are used to derive a MUNE.[8] The STA 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 are associated with the STA technique, including the amount of time required to collect a requisite sample of S-MUPs and the need for considerable patient cooperation. Moreover, based on the size principle, low-level voluntary contractions may not recruit the full range of MUs with differing sizes and physiological properties in a given muscle.[6,8,21,27]

In an effort to improve upon the limitations of the STA technique, we developed a new method for obtaining a MUNE that involves decomposition-enhanced spike-triggered averaging (DE-STA). The DE-STA technique differs from standard STA in that, as opposed to extracting single MU firings, DE-STA is able to extract multiple MUP trains from a given contraction through a series of signal-processing and pattern-recognition algorithms.[12] Thus, in essence, the decomposition algorithms act as intelligent triggers, with the ability to sample multiple MUs from each contraction.[25] The firing times from the MUP trains serve as triggers to extract the corresponding S-MUPs using STA. Previous studies employing the DE-STA technique demonstrated its ability to decompose an EMG signal into its constituent MUP trains and related macro MUPs as well as to provide other important information regarding the physiological properties of MUs.[4,5,25] However, DE-STA has yet to be described as a MUNE method. In this article, MUNEs and electrophysiological data from the first dorsal interosseous (FDI)/adductor pollicis (AP) and thenar muscle groups are presented along with the effect of varying levels of contractile force. The test–retest reliability of DE-STA as applied to the thenar muscles is also examined.

METHODS

Subjects. Seventeen subjects, aged 20–50 years (31 ± 8 years), volunteered to take part in the study. All gave informed consent in accordance with The University of Western Ontario Standing Committee on Human Research. All subjects were healthy, with no evidence of known neurological disease. In total, 10 median-innervated thenar muscles and 21 ulnar-innervated FDI/AP muscles were studied.

Electromyographic Data Collection. The DE-STA method and associated algorithms have been described in detail elsewhere.[12,25] The EMG signals were acquired using custom software on the Neuroscan Comperio system (Neuroscan Medical Systems, El Paso, TX). Intramuscular signals were detected with a commercially available disposable concentric-needle electrode (model no. N53153; Teca Corp., Hawthorne, NY) with a bandpass of 10 Hz to 10 kHz, whereas surface signals were detected with a bandpass of 5Hz to 5kHz using self-adhering electrocardiogram electrodes (Kendall-LTP, Chicopee, MA) cut in strips of 1 cm $\times$ 3 cm. A full-size electrode (3 cm $\times$ 2 cm) placed on the posterior aspect of the distal forearm served as a ground.

Subjects were seated with their arms comfortably supported during data collection. The active surface electrode was positioned over the motor point for each muscle, with the reference surface electrode positioned over the first metacarpophalangeal joint for the thenar muscles and the second metacarpophalangeal joint for the FDI/AP. All the surface-electrode positions were further reinforced with the use of strips of surgical tape to ensure that no movement occurred during the studies, and the second digit (for FDI studies) and first digit (for thenar studies) were immobilized with the other digits in an extended position, to allow resistance during voluntary contraction and to minimize movement artifact.

The maximum M potential was elicited with supramaximal stimulation of either the median or ulnar nerve at the wrist. Markers indicating negative onset, negative peak, negative-peak duration, and positive peak were automatically positioned. Following a visual check of the markers (and manual adjustments if required), the software calculated size-related parameters of the M potential including negative-peak area, negative-peak amplitude, and peak-to-peak amplitude.

Subjects then performed a maximum voluntary contraction (MVC) for 3 s. Visual and auditory feedback were provided in the form of the EMG signal. Following a 5-s rest period, subjects performed a

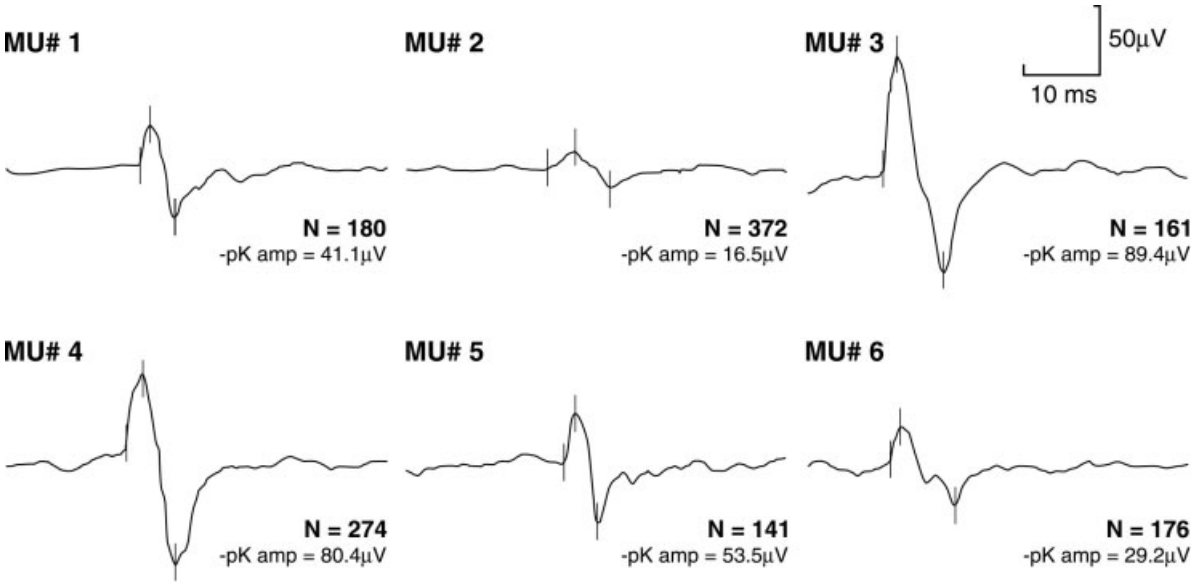

FIGURE 1. Sample of typical FDI/AP S-MUPs used to derive the mean S-MUP. Markers, from left to right, indicate S-MUP onset and negative and positive peak. (Abbreviations: N, number of samples contributing to the average S-MUP; −pk amp, negative peak amplitude.)

second maximal contraction. The maximal root mean square (RMS) value of the EMG signal over a 1-s interval was calculated, and subsequent contractions were described as a percentage of the maximal RMS, allowing an estimate of contractile level during the subsequent submaximal contractions.

The concentric intramuscular electrode was then inserted into the muscle being examined just proximal or distal to the active surface electrode. Subjects were asked to minimally contract the muscle isometrically while the needle position was adjusted to minimize the rise times of the MUPs of the first two to three recruited MUs. With the needle manually maintained in a stable position by the examiner, the subject was instructed to increase the contraction force, as isometrically as possible, to a moderate level such that MUPs from several active MUs were detected. Contraction intensity ranged from mild to moderate, relative to MVC, with each isometric contraction maintained for a period of 30 s. Subjects were instructed to maintain consistent contraction intensities throughout the period and were aided in doing so by auditory and visual feedback from the EMG signal. Four to six contractions were required to obtain 20 or more MUP trains. To decrease the chances of sampling the same MUs from different contractions, the needle position was adjusted between contractions to collect from superficial, intermediate, and deep portions of the muscle. If more than three contractions were required, as was often the case, a second needle insertion site was utilized. After EMG signal decomposition and analysis,[12] the MUP trains and S-MUPs were reviewed with regard to their acceptability.

First, visual checks were made of each MUP train to ensure that it represented a consistent MU firing pattern and displayed physiological firing properties. Second, the interpulse interval histogram was examined to confirm that the distribution was Gaussian in nature. Each train was required to include a minimum of 50 detected potentials that would serve as triggers for STA. Last, the S-MUP waveform was inspected to ensure that the onset and peak markers were accurate (and, if not, they were repositioned manually) and that a signal-to-noise ratio of $\geq$10:1 was present in comparing the peak-to-peak amplitude of the S-MUP with the RMS value of the baseline. Any MUP trains and S-MUPs that failed to meet the inclusion criteria were not included in further data analysis. A computer algorithm then onset-aligned all the accepted S-MUPs (Fig. 1) and calculated a mean S-MUP template based on the data-point by data-point average of all valid S-MUPs.[13] The MUNE was determined by dividing a size-related parameter of the maximum M potential (either the negative-peak amplitude or area) by the corresponding size-related parameter of the mean S-MUP.

One of the authors (S. B.) acted as examiner for all tests and performed subsequent review of the MUP trains and S-MUPs. For the test–retest portion of the study, the repeat study was either performed on a second day (two subjects) or on the same day

Table 1. The M-potential, mean macro, and MUNE values for FDI/AP.

	M-potential negative peak		Mean macro negative peak		Negative peak MUNE	
	Amplitude (mV)	Area (mVms)	Amplitude (μV)	Area (μVms)	Amplitude	Area
Minimum	5.4	11.3	25.9	48.3	67	59
Maximum	20.2	57.1	220.3	606.3	434	296
Mean	11.9	29.2	84.4	219.9	177	167
SD	4.4	10.9	46.8	143.2	98	82

following the removal and reapplication of new surface electrodes. The initial active electrode position was not marked, and data analysis was completed only following collection of both test and retest data.

Statistics. Mean values along with their standard deviations are presented throughout. Test–retest reliability was calculated using the intraclass correlation coefficient. All other correlations were examined with the Pearson product moment statistic.

RESULTS

Maximum M potentials were generally biphasic for both muscle groups examined, but an initial small positive deflection was often detected for the FDI/AP. The FDI/AP M-potential size (based on negative-peak amplitude) ranged from 5.4 to 20.2 mV (11.96 ± 4.36 mV; Table 1) and thenar M potentials ranged from 9.2 to 15.8 mV (12.74 ± 1.59 mV; Table 2). We sampled 769 MUs from the FDI from a total of 97 contractions, with an average of 8 MUs/contraction. Intensity of the voluntary contractions ranged from 2 to 21% MVC RMS (10 ± 5% MVC RMS; Table 3), with a mean MUP identification rate of 66 ± 7%. Similarly, 581 MUs were sampled from the thenar muscles from a total of 103 contractions, with an average of 5 MUs/contraction. Intensity of the voluntary contractions ranged from 3 to 11% MVC RMS (7 ± 2% MVC RMS; Table 4), with a mean MUP identification rate of 66 ± 6%. Mean MUNE data for the FDI/AP based on negative-peak amplitude was 177 ± 98 and 269 ± 104 for the thenar muscles (Tables 1 and 2). The relationship between percent MVC RMS and the size of the detected S-MUPs was also examined. A weak but significant correlation was found between the size of the S-MUPs originating in the thenar muscle group and the percent MVC RMS ($r = 0.151$, $P < 0.05$), whereas a nonsignificant correlation was observed for the FDI S-MUPs and percent MVC RMS ($r = 0.017$; $P > 0.05$). Similar weak correlations were found between the FDI/AP needle-detected MUPs and S-MUPs ($r = 0.377$; $P < 0.01$) and between thenar needle-detected MUPs and S-MUPs ($r = 0.297$; $P < 0.01$). The frequency distributions of S-MUP sizes collected from the thenar muscle group are illustrated in Figure 2. The size distributions for the FDI/AP S-MUPs were similar, with larger numbers of small S-MUPs. The size distributions are similar to those reported in previous studies examining intrinsic hand muscles (thenar muscles) and employing various MUNE techniques, including MPS.[11]

Test–retest reliability was examined by comparing subjects' mean negative-peak area thenar MUNE values between tests. The mean test–retest MUNE values were similar (249 ± 78 and 246 ± 90; Table 2) and were highly correlated ($r = 0.94$; $P < 0.05$).

DISCUSSION

This study demonstrates that DE-STA is a valid, reliable, and efficient method for obtaining a MUNE.

Table 2. The M-potential, mean macro, and MUNE values for thenar muscles.

	M-potential negative peak				Mean macro negative peak				Negative peak MUNE			
	Amplitude (mV)		Area (mVms)		Amplitude (μV)		Area (μVms)		Amplitude		Area	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Minimum	9.2	10.7	36.9	36.0	21.1	27.2	121.2	111.9	155	108	122	102
Maximum	15.8	14.6	61.1	57.5	56.1	105.9	269.5	491.4	555	393	394	381
Mean	12.7	12.8	44.5	46.8	44.9	57.1	198.8	220.7	289	248	249	246
SD	2.0	1.1	7.6	6.9	12.3	23.2	70.4	112.6	115	93	78	90

Table 3. The S-MUP and contraction values for FDI/AP.

	S-MUP negative peak				
	Amplitude (μV)	Area (μVms)	Number of contractions	Number of MUs	Percent MVC RMS
Minimum	29.9	56.9	4	23	2.6
Maximum	231.2	628.3	7	49	20.9
Mean	96.6	242.7	5	36	9.7
SD	46.9	141.8	1	6	4.6

The MUNE values of 177 for the FDI/AP and 269 for the thenar muscles (based on negative-peak amplitude) compare favorably to both anatomical estimates[14] and results of previous studies that applied various MUNE techniques, including manual incremental stimulation and MPS in the thenar muscle group.[10,20] Test–retest data show the method to have high reliability, similar to reports for MPS and the statistical technique.[16] In addition to the MUNE data, DE-STA provides other clinically useful data in the form of standard quantitative MUP analysis of concentric- or monopolar-needle EMG (MUP size, duration, numbers of turns, and phases), data related to MUP stability (jitter and jiggle analysis),[12,25] and MU firing pattern information.

The DE-STA method has a number of advantages over other MUNE methods, including standard STA. These include the elimination of alternation and the confirmation that each S-MUP acquired represents a single MU. The method is also applicable to both distal and proximal muscles, the latter usually being inaccessible with other techniques. Multiple S-MUPs are obtained from each contraction with DE-STA, and therefore fewer contractions are required than with conventional STA, and the consistency and accuracy of the MUPs used as triggers for each S-MUP can be reviewed. Less operator manual skill is required when compared to certain other MUNE techniques, such as MPS or incremental stimulation. Unlike MPS and incremental stimulation, which require a high degree of manual skill and decision making by the operator during data collection, DE-STA requires only the ability to perform standard-needle EMG to collect the data. However, DE-STA does require operator involvement to eliminate MUP trains and S-MUPs that do not meet objective inclusion criteria. In addition, the level of contraction during EMG signal acquisition can be greater than for STA, and therefore higher recruitment threshold MUs can be studied. Following a motor nerve conduction study using standard electrode placements, the time required to complete a MUNE using DE-STA is less than 10 min, making it a practical clinical tool and one that could reasonably be included as an outcome measure in clinical trials.

Disadvantages of DE-STA include the fact that the specialized algorithms required for EMG signal decomposition are not widely available, and, because of the use of an intramuscular electrode, the method is necessarily invasive. In addition, poorly defined S-MUPs occasionally require a degree of operator involvement for determination of landmarks (onset, end, and negative and positive peaks), and not all MUP trains result in an acceptable S-MUP, because of a poor signal-to-noise ratio. The latter issue is potentially most apparent for smaller S-MUPs or those S-MUPs generated by trains with low identification rates and thus fewer triggers for subsequent STA. From the standpoint of MUNE, this is problematic only if such trains are biased toward MUs at one end of the physiological spectrum. In our experience, sparsely detected trains are produced by MUs whose MUPs frequently superimpose with the MUPs of other MUs having similar firing patterns. Such MUPs do not occur frequently enough in isolation to permit detection of sufficient numbers of triggers to extract their associated S-MUP. Alternatively, MUs that were variably recruited produce sparse MUP trains and generate few triggers. These are often the highest-threshold MUs in a given contraction.

From the standpoint of obtaining a sample of S-MUPs from which to derive a MUNE, the principal concern is with respect to potential bias in the sam-

Table 4. The S-MUP and contraction values for thenar muscles.

	S-MUP negative peak				Number of contractions		Number of MUs		Percent MVC RMS	
	Amplitude (μV)		Area (μVms)							
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Minimum	36.9	34.9	125.2	117.1	3	3	25	25	4.0	3.0
Maximum	126.1	165.6	432.9	530.5	7	8	37	38	11.5	11.2
Mean	64.3	72.6	227.9	253.6	5	5	28	30	7.1	7.1
SD	25.1	37.0	87.4	123.3	2	2	40	3	2.5	2.6

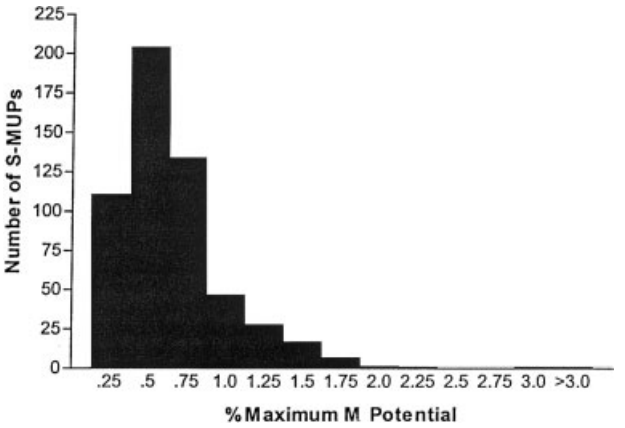

FIGURE 2. Frequency distribution of S-MUPs from the thenar muscles. The negative-peak areas of the S-MUPs have been normalized to the maximum M-potential negative-peak area for each subject.

ple. With DE-STA, it is possible to collect data from moderately high contraction levels (up to 20% of MVC RMS for the FDI/AP muscles), which increase the numbers of active MUs, thus increasing the numbers of MUs sampled per contraction. Such higher-level contractions have increased signal intensity related to a fuller interference pattern, which potentially makes it more difficult to detect smaller needle-detected MUPs. This results from the use of a relative detection threshold (currently 1.5 times the signal RMS). Although this may introduce some bias toward larger needle-detected MUPs, considering that the MUNEs reported here are similar to those reported for standard STA and other methods in the thenar muscles, there is no apparent effect on the distribution of the sample of S-MUPs collected or their mean sizes. This may be explained by the weak relationship reported here between needle-detected MUP size and S-MUP size. That is, based on the current data, larger needle-MUP sizes were not necessarily indicative of larger MUs or S-MUPs. This finding likely relates to the significantly different recording characteristics of the needle and surface electrodes. The amplitude of the concentric-needle MUP is highly dependent on the relative position of the needle detection surface to the muscle fibers of a given MU, and reflects only the contribution of a small number of muscle fibers close to the needle tip.[18] The larger detection area of the surface electrode increases the probability that larger numbers of muscle fibers are equidistant from the electrode. Therefore, the S-MUP size may better reflect MU size.[23,24]

Of particular interest in this study is the relationship between the contractile level, as estimated by the percent MVC-RMS, and the S-MUP sizes. Based on the size principle, one might expect that larger S-MUP sizes would be observed with higher levels of contraction.[15] However, this was not the case, as the correlations between MVC RMS and S-MUP size were weak. The weak correlations may be related to a number of factors including: (1) S-MUP size is not tightly correlated with MU size because of the effect of MU location and depth relative to the active electrode; (2) intrinsic hand muscles may depend predominantly on rate coding to moderate contractile force, and thus most MUs are recruited at low levels of contraction; and (3) neither S-MUP size nor MVC RMS is linearly related to contractile force.[9,22] Conversely, Conwit et al.,[5] using DE-STA, reported a positive relationship between contractile force and S-MUP size for the vastus medialis muscle. This may be because the authors used a larger proximal muscle, relying more heavily on recruitment as opposed to rate coding, and higher contractile levels (up to 80% MVC) in their study. Nevertheless, the current data do not suggest a requirement to control for contractile force or recruitment level when collecting S-MUPs to derive a MUNE in the intrinsic hand muscles. To more thoroughly examine this, we are currently studying the effect of contractile force on S-MUP size using DE-STA.

Overall, it appears that DE-STA is a valid, reliable, and practical tool for obtaining a MUNE. Furthermore, DE-STA has improved upon previously existing MUNE techniques, increasing its applicability while decreasing the time required to collect a representative sample of MUs. Future studies will be directed at examining the applicability of DE-STA in disease populations.

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