Test–retest reliability of a modified multiple point stimulation technique for motor unit number estimation

Cheryl L. Porter^a, Arodi Alvarez^a, Kelvin E. Jones^{a,c}, and K. Ming Chan^{a,b,*}

^aCentre for Neuroscience, University of Alberta, 513 Heritage Medical Research Centre, Edmonton, AB, Canada T6G 2S2

^bDivision of Physical Medicine and Rehabilitation, University of Alberta, Edmonton, Canada

^cFaculty of Physical Education, University of Alberta, Edmonton, Canada

Abstract

Objective—Multiple point stimulation (MPS) is a widely used technique to estimate the number of motor units in a muscle. Test–retest reliability must be high for the motor unit number estimates to be clinically useful. We hypothesized that the reliability of MPS can be improved by using a long stimulus pulse width (1 ms), in addition to the standard 0.05 ms pulse width.

Methods—Median nerve innervated thenar muscles were examined in 11 young subjects, 27 ± 3 (mean $\pm$ SD) years old and in 5 elderly subjects, 71 ± 11 years old. The experimenter conducted the studies twice on each subject.

Results—Test–retest reliability, using the modified MPS technique, was substantially higher (Cronbach's α of 0.88) than using the standard method (Cronbach's α of 0.80) in the young subjects. In contrast, for the elderly subjects the test–retest reliability of MUNE was high for both the modified technique (Cronbach's α of 0.96) and the standard technique (Cronbach's α of 0.99).

Conclusions—Test–retest reliability of MPS can be significantly improved by using a long stimulus pulse width and the standard stimulus pulse width in younger subjects. However, this is not necessary in elderly subjects.

Significance—The greater reliability of this modified MPS method should enhance its clinical utility.

Keywords

Motor unit; Human; Test–retest reliability

1. Introduction

Motor unit number estimation (MUNE) is a neurophysiological method that can help to define the severity, follow the progression and detect the efficacy of treatment of neuromuscular diseases, in which motor neuronal or axonal loss is a major feature (Doherty

*Corresponding author. Address: Centre for Neuroscience, University of Alberta, 513 Heritage Medical Research Centre, Edmonton, AB, Canada T6G 2S2. Tel.: +1 780 492 1614; fax: +1 780 492 1617. ming.chan@ualberta.ca (K.M. Chan).

and Brown, 1993; Simmons et al., 2001). There are a variety of MUNE techniques, most of which derive the estimate of motor units from the ratio of the maximum compound muscle action potential (CMAP) to the average surface motor unit action potential (SMUP). To be clinically useful, the method must have sufficiently high test–retest reliability with no sampling bias. The number of motor axons within the nerve likely has the biggest impact on MUNE reproducibility. However, the sample size of SMUPs collected may also be an important contributor (McComas, 1991). One assumption of MUNE is that the sample of SMUPs is representative of the whole population. SMUP sizes range considerably within a muscle. Therefore, it is important to collect a large sample size of SMUPs (McComas, 1991). All MUNE techniques share the same challenge of acquiring an adequate sample of SMUPs that accurately represents all the motor units within a muscle (Blok et al., 2005; Bromberg, 2007).

Multiple point stimulation (MPS) is one of the most commonly used MUNE techniques (Doherty et al., 1995; Lomen-Hoerth and Olney, 2000). In standard MPS, SMUPs are collected along the superficial portion of a nerve using only short pulse width. Doherty and Brown (1993) showed that the mean percentage error of the MUNE could be reduced if more than 10 SMUPs were obtained. In a recent study, we demonstrated that a greater increase in the sample size of SMUPs could be achieved by additionally using a long stimulus pulse width (1 ms) (Porter et al., in press). However, whether that would result in higher test–retest reliability has not been investigated. The objective of this study was to test the hypothesis that this modification would substantially increase the within-subject test–retest reliability of MPS.

2. Methods

2.1. Subjects

The experiment was conducted on 11 young, healthy volunteers [6 males; 27 ± 3 years old (mean $\pm$ SD)] and 5 elderly, healthy volunteers (2 males; 71 ± 11) with the approval from the Human Ethics Board at the University of Alberta.

2.2. Motor unit number estimation

Modified MPS was performed on the median nerve innervated thenar muscles by the first author, a graduate student with little prior experience in doing MUNE, on separate days. The amplified EMG signal was digitalized using an A-D converter card (National Instruments, San Antonio, Texas) in a PC computer running Lab-VIEW 5.0 custom written program (National Instruments). The signals were band-pass filtered between 5 Hz and 2 kHz and sampled at 5 kHz. The skin was gently abraded and cleansed with rubbing alcohol. Disposable surface (Ag/AgCl) EMG strip electrodes (1×2.5 cm) were used. The active electrode was positioned over the motor point of the median nerve innervated thenar muscles, and the reference electrode over the metacarpophalangeal joint of the thumb with the ground electrode attached to the dorsum of the hand. The thumb was immobilized by a tape in an adducted position. Hand temperature was maintained between 32 and 36 °C by using a heat pack. The course of the median nerve was mapped between the wrist and the distal forearm, and from the antecubital fossa to the axilla, using a 2 cm high, flat low profile

hand-held bipolar stimulator with saline soaked felt tip electrodes. Co-activation of the ulnar nerve was avoided by having patients indicate if any sensation was felt in their 5th digit. The maximum CMAP was acquired by supramaximally stimulating the median nerve just proximal to the distal wrist crease. The superficial course of the median nerve was stimulated at consecutive sites from the distal wrist crease to the distal forearm and then from the antecubital fossa to the axilla, by using finely graded threshold stimulation to activate single motor axons. SMUPs, defined as all-or-none responses that occurred in a reproducible and orderly fashion, evoked with distinct thresholds, were collected at each site. Features in the program that allowed the lowest threshold SMUPs to be reliably identified included: superimposition and editing capability of all stimulated responses and template subtraction of successively higher threshold SMUPs. In most instances, only the first 1 or 2 lowest threshold SMUPs were collected, thereby circumventing the difficulties associated with alternation.

In the first 10 subjects, the lowest threshold SMUPs were first evoked with the conventional pulse width of 0.05 ms, followed by 1 ms. To ascertain whether the order of administration had an impact on the MUNE results, in the remaining 6 subjects the stimulus pulse widths were randomized. This was accomplished by using a random number generator (SPSS 12, Chicago, Illinois). To establish the practicality of applying this modified technique in the clinical setting, the extra time required to capture additional SMUPs with the 1 ms stimulus pulse width was measured with a stop watch.

To avoid duplication, if the same SMUP was activated with both pulse widths, only the one activated with the conventional 0.05 ms pulse width was kept. Position of the stimulator was carefully adjusted to find the optimum orientation to discretely activate SMUPs with distinct stimulus thresholds. To ensure maximal stability of the stimulator over the nerve, it was securely tied down with a Velcro strap looped around the subject's arm. The Velcro strap was so adjusted that it prevented inadvertent displacement of the stimulator, but yet not so tight that it indented the skin or induced ischemia and discomfort. Once the lowest threshold SMUPs had been collected, the stimulator was moved approximately 15 mm along the nerve trunk. This distance was so chosen because, beyond that, the risk of inadvertently activating the same SMUPs is negligible (Doherty and Brown, 1993). After all SMUPs were collected (i.e., no additional SMUPs were observed at either stimulus pulse width), the program averaged the SMUPs in a "data point by data point" manner. The MUNE was calculated by dividing the negative-peak amplitude of the maximum CMAP by that of the average SMUP. MUNE based on the standard MPS method only included SMUPs captured using the conventional 0.05 ms pulse width, while results obtained by the modified technique included all the SMUPs captured by both pulse widths. Test-retest reliability of these two methods was computed for the young subjects and the elderly subjects separately using the intraclass correlation (ICC) method. In comparison with Pearson's correlation analysis, ICC is more vigorous because it takes both random and systemic errors into account. Cronbach's α value of 1 represents perfect correlation. The data were analyzed using SPSS 15 (SPSS, Chicago, Illinois). Descriptive statistics are reported as mean $\pm$ SD. *A priori* the statistical significance was set at $p < 0.05$. To detect whether there was any sampling bias with each of the stimulus pulse width, sizes of the SMUPs obtained using the standard and modified MPS method were compared using paired sample student's *t* test.

3. Results

3.1. Sample size and potential sampling bias

Compared with the standard MPS method, there was a substantial increase in the number of SMUPs collected, from 18 ± 3 to 23 ± 4 , in the young subjects using the modified MPS technique (Table 1). In the elderly subjects, there was an increase from 16 ± 3 to 20 ± 3 motor units using the modified MPS technique.

To address the question of whether there was any bias in the sizes of the SMUPs obtained using the two different pulse widths, we tabulated the SMUP negative-peak amplitudes for all subjects using the modified and standard MPS methods (Table 2). No significant difference between SMUPs activated by using the standard or modified method was found. At test 1 for the young subjects, the SMUP negative-peak amplitude for the modified method was $42 \pm 12 \mu\text{V}$ and for the standard method was $46 \pm 14 \mu\text{V}$. At test 2, it was 40 ± 9 for the modified method and $41 \pm 7 \mu\text{V}$ for the standard method. As for the elderly subjects, the SMUP negative-peak amplitude was $45 \pm 11 \mu\text{V}$ for the modified technique and $43 \pm 7 \mu\text{V}$ for the standard technique. At test 2, the SMUP amplitude was 45 ± 5 for the modified technique and $46 \pm 5 \mu\text{V}$ for the standard technique.

3.2. MUNE results and test–retest reliability

In the younger subjects, the MUNE results obtained using the modified technique for test 1 and test 2 were 335 ± 98 and 345 ± 90 motor units, respectively. As for the standard MUNE method, it was 302 ± 86 for test 1 and 332 ± 57 for test 2. Test–retest reliability of the MUNE results obtained using the modified MPS technique (Cronbach's $\alpha = 0.88$) was substantially higher than that obtained using the standard MPS technique (Cronbach's $\alpha = 0.80$; Fig. 1). To test whether the increased test–retest reliability seen with the modified technique was due to a difference in the sample size of SMUPs obtained, we also analyzed the percentage errors of the MUNE when the same number of SMUPs was collected by the two methods. As described by Doherty and Brown (1993), the percent error represents the percentage difference between the motor unit number estimate derived with increasing numbers of SMUPs used to calculate the average SMUP size and the MUNE derived using 11 SMUPs. Eleven, the maximum number of SMUPs, was deliberately chosen so that we could combine results from all the studies done in this paper for the analysis (Table 1). As shown in Fig. 2, the mean percentage errors of MUNE at the same number of SMUPs are virtually identical, regardless of which technique was used. This buttresses our argument that the increase in test–retest reliability with the modified MPS technique was due to the larger sample of SMUPs obtained. To determine whether the number of stimulation sites used might also have an effect on the test–retest reliability, we also compared the results of the standard MPS technique when all the sites were included to that when 28% fewer sites were included. The latter percentage was so chosen because that corresponded with the difference in the number of SMUPs collected by using the standard and modified MPS techniques. With 28% fewer sites and consequently fewer SMUPs included, Cronbach's α decreased to 0.74.

In comparison, in the elderly subjects, test–retest reliability of the MUNE results with both techniques was very high. Cronbach's α was 0.96 with the modified technique and 0.99 with the standard MPS technique. For the modified MPS method, the MUNE results were 226 ± 85 motor units for test 1 and 220 ± 73 motor units for test 2. For the standard MPS method, the MUNE results for test 1 and test 2 were 215 ± 47 and 204 ± 40 motor units, respectively.

The order of pulse width administration did not appear to affect the reliability of results. The MUNE test retest reliability in the last 6 subjects, in whom the pulse widths were randomized, was the same as in the first 10 subjects, where the short pulse width was administered first (Cronbach's $\alpha = 0.88$ in both cases).

The additional use of 1 ms with the modified method took an extra 23 ± 6 min, which represented a 28% increase in the amount of time required.

4. Discussion

In this study, we assessed the within-subject test–retest reliability of MUNE obtained using a modified protocol of MPS. Standard MPS only uses a pulse width of 0.05 ms to obtain SMUPs, while this modified MPS used the combination of two pulse widths (0.05 ms and 1 ms). In the younger subjects, the test–retest reliability of the MUNE was substantially improved by using the modified method, resulting in an increase of the Cronbach's α from 0.80 to 0.88. In contrast, the modified technique did not result in an improvement in the reliability of MUNE in the elderly subjects. However, this is perhaps not surprising since the test–retest reliability of the standard technique was already very high (Cronbach's $\alpha = 0.99$) in the elderly subjects; it was impossible to increase the reliability any further. A likely reason for the substantially higher test–retest reliability seen in the elderly subjects is that the SMUPs sampled as a percentage of the motor unit number in those individuals ($10 \pm 3\%$) were significantly higher than in the younger subjects ($7 \pm 3\%$) ($p < 0.05$). The average SMUP thus derived in the elderly subjects was more likely to be representative of the total motor unit population. This suggestion is in keeping with the results obtained by other investigators (Felice, 1995; Slawnych et al., 1997).

The impact of sample size on the reliability of MUNE was also investigated by Doherty and Brown (1993). In that study, they showed that the MUNE percentage error declined substantially with a sample size of 10–15 SMUPs: a trend that we also observed with our dataset. However, if we only included the first 15 SMUPs obtained from each test session in this study, the test retest reliability would be substantially reduced, with Cronbach's α of only 0.62. This supports the notion that an even larger SMUP sample size can indeed further increase the test–retest reliability of MUNE.

In this study, the median nerve innervated thenar MUNE of 335 ± 98 for the test, and 345 ± 90 for the retest, in the young subjects, and 226 ± 85 for test 1 and 220 ± 73 motor units in the elderly subjects, using the modified MPS method, were consistent with past studies using similar techniques conducted on the thenar muscles: 288 ± 95 (Doherty and Brown, 1993) and 278 ± 113 motor units (Wang and Delwaide, 1995). Since there is considerable

variation in MUNE between healthy individuals, these minor differences are not unexpected (Simmons et al., 2001).

Equally important when considering the clinical utility of this modified technique is that there was no apparent selection bias using the long duration stimulus pulse. Amplitudes of both the SMUPs and the MUNE obtained using the two pulse widths were similar. What might the explanation be for the lack of sampling bias? A likely reason is that with the MPS technique, to collect the SMUPs, the median nerve is stimulated at a large number of accessible sites throughout the wrist and the upper arm. Therefore, the cable properties of the motor axons are likely not to be the only factors that determine which SMUPs are activated. Factors that might be more important are the amount and density of interposed tissues and the relative depth of the motor axons in the fascicle at each location. Using a stimulation model, it was shown that preferential activation of large diameter motor axons is unlikely in MPS (Major and Jones, 2005). Additional SMUPs were selectively recruited when altering the stimulus pulse width, most likely because myelinated nerve fibers of different sizes have different thresholds (Grill and Mortimer, 1996).

Based on our findings, the additional time needed for carrying out the modified MPS technique is worthwhile for younger subjects because it would substantially improve the reliability of the test. Although this took an additional 28% of time by the experimenter of this study who had little prior experience with MUNE, the amount of extra time required by an experienced operator to do this is much less. In contrast, it is unnecessary to use the modified MPS technique in the elderly subjects who have reduced number of motor units.

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References

- Blok JH, Van Dijk JP, Zwarts MJ, Stegeman DF. Motor unit action potential topography and its use in motor unit number estimation. *Muscle Nerve*. 2005; 32:280–91. [PubMed: 15937880]
- Bromberg MB. Updating motor unit number estimation (MUNE). *Clin Neurophysiol*. 2007; 118:1–8. [PubMed: 16996797]
- Doherty T, Simmons Z, O'Connell B, Felice KJ, Conwit R, Chan KM, et al. Methods for estimating the numbers of motor units in human muscles. *J Clin Neurophysiol*. 1995; 12:565–84. [PubMed: 8600172]
- Doherty TJ, Brown WF. The estimated numbers and relative sizes of thenar motor units as selected by multiple point stimulation in young and older adults. *Muscle Nerve*. 1993; 16:355–66. [PubMed: 8455648]
- Felice KJ. Thenar motor unit number estimates using the multiple point stimulation technique: reproducibility studies in ALS patients and normal subjects. *Muscle Nerve*. 1995; 18:1412–6. [PubMed: 7477064]
- Grill WM Jr, Mortimer JT. The effect of stimulus pulse duration on selectivity of neural stimulation. *IEEE Trans Biomed Eng*. 1996; 43:161–6. [PubMed: 8682527]
- Lomen-Hoerth C, Olney RK. Comparison of multiple point and statistical motor unit number estimation. *Muscle Nerve*. 2000; 23:1525–33. [PubMed: 11003787]
- Major LA, Jones KE. Simulations of motor unit number estimation techniques. *J Neural Eng*. 2005; 2:17–34. [PubMed: 15928409]

- McComas AJ. Motor unit estimation: methods, results, and present status. *Muscle Nerve*. 1991; 14:585–97. [PubMed: 1922165]
- Porter CL, Alvarez A, Jones KE, Major LA, Chan KM. Improving the accuracy of the multiple point stimulation technique. *Clin Neurophysiol*. in press.
- Simmons Z, Epstein DK, Borg B, Mauger DT, Kothari MJ, Shefner JM. Reproducibility of motor unit number estimation in individual subjects. *Muscle Nerve*. 2001; 24:467–73. [PubMed: 11268017]
- Slawnych M, Laszlo C, Hershler C. Motor unit number estimation: sample size considerations. *Muscle Nerve*. 1997; 20:22–8. [PubMed: 8995579]
- Wang FC, Delwaide PJ. Number and relative size of thenar motor units estimated by an adapted multiple point stimulation method. *Muscle Nerve*. 1995; 18:969–79. [PubMed: 7643877]

Fig. 1.

Reproducibility of the motor unit number estimation (MUNE), derived from the standard and modified multiple point stimulation (MPS) techniques, for each young subject. The black inverted triangles represent the standard MUNE technique and the open circles represent the modified MUNE technique. The dashed and solid lines represent the lines of regression for the standard and modified MUNE, respectively.

Fig. 2.

Percentage errors of motor unit number estimation (MUNE) obtained when progressively smaller numbers of surface motor unit action potentials (SMUPs) were used for the estimates. The percentage error was calculated by using the following equation: $|(\text{MUNE}^{1\dots 11} - \text{MUNE}^{11}) / \text{MUNE}^{11} \times 100$. Vertical bars denote the mean while the error bars denote standard error. At the same number of SMUPs, the percentage error was almost identical between the two methods.

Table 1

Comparisons of the number of surface motor unit action potentials (SMUPs) collected with the standard and modified MPS techniques during test and retest conducted on separate days

Subject	<u>Modified MPS # of SMUPs</u>		<u>Standard MPS # of SMUPs</u>	
	Test	Retest	Test	Retest
1	23	21	16	17
2	24	19	20	17
3	30	21	24	16
4	25	27	14	20
5	16	26	11	22
6	25	25	21	22
7	18	20	15	19
8	16	16	13	15
9	24	26	21	19
10	22	25	16	19
11	26	23	22	17
12	19	15	15	11
13	19	23	16	18
14	17	22	13	14
15	21	23	17	19
16	22	21	17	18

Subjects 1–11 are young subjects and 12–16 are elderly subjects.

Table 2

Comparisons of the mean surface motor unit action potential (SMUP) negative-peak amplitude, and motor unit number estimates (MUNE) during test and retest conducted on separate days

Subject	Modified MPS SMUP amplitude (µV)		Standard MPS SMUP amplitude (µV)		Modified MPS MUNE		Standard MPS MUNE	
	Test	Retest	Test	Retest	Test	Retest	Test	Retest
1	38	32	44	33	433	443	365	425
2	64	50	72	53	190	288	168	280
3	31	31	33	36	427	403	389	342
4	38	37	45	42	330	352	270	310
5	48	43	56	49	344	343	291	296
6	59	61	69	42	171	181	147	266
7	36	36	40	38	463	430	400	410
8	26	32	25	28	365	275	385	327
9	38	41	40	45	400	388	348	377
10	48	45	44	42	249	234	281	257
11	34	35	38	40	315	455	279	361
12	40	47	36	41	138	130	160	153
13	63	52	54	54	152	176	173	172
14	34	39	38	42	342	292	268	248
15	48	44	45	45	225	204	226	216
16	40	43	42	46	271	298	250	233

Subjects 1–11 are young subjects and 12–16 are elderly subjects.