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Titre : Number and relative size of thenar motor units estimated by an adapted multiple point stimulation method. **Langue du document :** anglais **Auteur, co-auteur :** Wang, François-Charles *[Centre Hospitalier Universitaire de Liège - CHU > > Médecine de l'appareil locomoteur >]* Delwaide, Paul *[Université de Liège - ULg > Services généraux (Faculté de médecine) > Relations académiques et scientifiques (Médecine) >]* **Date de publication :** 1995 **Titre du périodique :** Muscle & nerve **Volume/Tome :** 18 **Fascicule /saison :** 9 **Pagination :** 969-79 **ISSN :** 0148-639X **Pays :** UNITED STATES **Mots-clés : [en]** Action Potentials/physiology ; Adult ; Aged ; Cell Count ; Cell Size ; Electric Stimulation ; Female ; Hand/innervation/physiology ; Humans ; Male ; Median Nerve/physiology ; Middle Aged ; Motor Neurons/cytology/physiology ; Muscles/innervation/physiology ; Neuromuscular Junction/physiology **Résumé : [en]** An adapted multiple point stimulation (AMPS) method is described for estimating the number and relative size of thenar motor units. With this method, the median nerve was stimulated at various sites from the wrist to the elbow. To avoid alternation, only two or three clearly identifiable surface-recorded motor unit action potentials (S-MUAPs) were recruited at each point by incremental stimulation. A total of 10 S-MUAPs, elicited from four to five distinct stimulation points, was used to calculate the average S-MUAP size. By dividing the maximum M-potential size by that value, a motor unit number estimate (MUNE) was derived. In 59 healthy volunteers, from 19 to 87 years old, the mean average S-MUAP size was 87 +/- 27.6 microV.ms and the mean MUNE was 278 +/- 113 motor units. When performed repeatedly, the results were reproducible. The number of motor units declined exponentially with age while average S-MUAP sizes increased only moderately. To assess the validity of the AMPS method, its results were correlated with those obtained using the F-response technique. The correlation coefficient was 0.83 (P < 0.001). **DOI :** 10.1002/mus.880180908

Merci,

François Wang

An adapted multiple point stimulation (AMPS) method is described for estimating the number and relative size of thenar motor units. With this method, the median nerve was stimulated at various sites from the wrist to the elbow. To avoid alternation, only two or three clearly identifiable surface-recorded motor unit action potentials (S-MUAPs) were recruited at each point by incremental stimulation. A total of 10 S-MUAPs, elicited from four to five distinct stimulation points, was used to calculate the average S-MUAP size. By dividing the maximum M-potential size by that value, a motor unit number estimate (MUNE) was derived. In 59 healthy volunteers, from 19 to 87 years old, the mean average S-MUAP size was 87 ± 27.6 μV · ms and the mean MUNE was 278 ± 113 motor units. When performed repeatedly, the results were reproducible. The number of motor units declined exponentially with age while average S-MUAP sizes increased only moderately. To assess the validity of the AMPS method, its results were correlated with those obtained using the F-response technique. The correlation coefficient was 0.83 ($P < 0.001$).

Key words: motor unit number estimate (MUNE) • surface-recorded motor unit action potential (S-MUAP) • thenar muscles • adapted multiple point stimulation method (AMPS)

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NUMBER AND RELATIVE SIZE OF THENAR MOTOR UNITS ESTIMATED BY AN ADAPTED MULTIPLE POINT STIMULATION METHOD

FRANÇOIS-CHARLES WANG, MD, and PAUL J. DELWAIDE, MD, PhD

Estimation of the number and relative size of motor units in a muscle is of clinical interest to quantify peripheral denervation, to monitor the clinical course, and to assess the efficacy of treatments. In addition, such data should permit one to study the structure and function of a muscle in terms of development and age.

Several electrophysiological techniques have been developed for estimating the number and size of motor units. With all these methods, number of motor units was calculated by dividing the maximum M-potential size, obtained after supramaximal stimulation of the motor nerve, by an estimate of the average surface-recorded motor unit action potential (S-MUAP) size.

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The first technique, described by McComas et al.,[17] consisted of applying weak incremental stimuli at a single point over the course of the peroneal nerve and attributing steplike increases of the evoked response from the extensor digitorum brevis (EDB) muscle to recruitment of new single motor units. The stimulus intensity was gradually increased until 11 successive increments of the EDB muscle response were obtained; the average S-MUAP size was then derived and the motor unit number estimated. Later on, that procedure was adapted to the median nerve.[4,18] The main potential error of this technique is due to the so-called alternation phenomenon.[6] Alternation is a result of the various possible configurations of responses from motor units with similar axonal thresholds. For example, if two motor units (motor unit 1 and motor unit 2) have similar activation thresholds, at constant stimulus intensity, three configurations of the motor response are possible (either motor unit 1 is recruited, motor unit 2 is recruited, or motor units 1 and 2 are simultaneously recruited). Thus, when thresholds of n axons overlap at a specific stimulus intensity, a sufficient number of successive stimuli could theoretically evoke $2^n - 1$ steps in the muscle evoked response.[6] Alternation is thus able to cause underestimation of the average S-MUAP size and overestimation of the number of motor units.

Doherty and Brown[11] recently devised the multiple point stimulation method (MPS) to avoid alternation. This technique consists of stimulating the median nerve at multiple sites between the distal forearm and thenar motor point and between the elbow and axilla to collect 10 or more S-MUAPs. At each stimulus site, only one S-MUAP is selected. Currently, investigators are proposing other methods for avoiding alternation: spike-triggered averaging (STA),[3,7,11] an F-response technique,[5] and statistical estimates.[10]

We tried some of these methods but, in our hands, none was completely satisfactory. They were time consuming, invasive, or too sophisticated for routine clinical use. Moreover, none of these techniques avoid a possible selection bias toward motor units of a particular size.

Therefore, we attempted to elaborate an alternative technique free of both alternation and motor unit selection bias. This technique was initially reported by Kadrie et al. but was not further developed by them.[14] We are reporting preliminary results obtained in a population of normal volunteers.

MATERIALS AND METHODS

Subjects. Data were collected from 59 volunteers (25 males and 34 females), after having obtained their informed consent; age ranged from 19 to 87 years (mean 47 ± 20 SD). In each subject, the medical history was reviewed and a standardized neurological examination was administered. None of our subjects reported symptoms or had neurological abnormalities on careful clinical examination suggestive of a peripheral nerve disease. None had symptoms or signs of a metabolic disorder such as diabetes or renal failure. We excluded carpal tunnel entrapment neuropathy on the basis of clinical examination and electrophysiological tests (motor conduction velocity with terminal latency index and palmar stimulation of sensory fibers). No one presented with hypo- or hyperreflexia. Subjects with any health problems were eliminated from the test group.

Only 1 subject was left-handed. The left hand of each volunteer was examined. In 26 subjects, both hands were studied for comparison.

Electrode Placement. The median innervated thenar muscles were studied in each subject. The stigmatic and reference electrodes consisted of silver foil, 6 cm long and 0.8 cm wide. The stigmatic electrode was placed in as close proximity as possible to the endplate zone of the thenar muscles. The reference electrode was attached over the proximal phalanx of the thumb. The ground electrode (silver foil) was fixed over the dorsum of the wrist.

The stimulating electrodes (Medelec, Model LBS 53051; interelectrode distance 2.5 cm) were moved over the median nerve from the wrist to the elbow with the cathode distal.

Stimulating and Recording Systems. All studies were performed on a Nicolet Viking I EMG machine (Nicolet Instrument Corp.). S-MUAPs and compound motor responses were recorded with a gain of 50–100 μV/division and were evoked by 0.05-ms constant current square waves repeated at 1 Hz. Maximum M-potentials were recorded with a gain of 1–5 mV/division and were evoked by 0.2-ms stimuli at 1 Hz, stimulating electrodes being 7 cm proximal from the stigmatic electrode. Surface recordings were filtered with a bandpass from 20 Hz (in order to obtain a stable baseline) to 5 kHz. Increments of stimulus intensity were about 0.1–0.5 mA.

The hand temperature was maintained above 30°C.

Description of the Adapted Multiple Point Stimulation (AMPS) Method. AMPS is a procedure combining elements of the original McComas technique[17] and MPS.[11] Any originality consists in taking into consideration only two or three S-MUAPs obtained in response to incremental stimulations at different stimulation points.

The median nerve was stimulated at different points from the wrist to the elbow. At each stimulation point, the stimulus intensity was increased by increments to the level at which the first and then the second and sometimes the third subsequent S-MUAPs were elicited in an all-or-nothing manner. To avoid alternation, motor axons had to be recruited with distinct thresholds, in an orderly and reproducible manner without any fractionation of the compound motor response to successive stimuli. At each stimulus site, only combinations of two or three S-MUAPs were accepted into the motor unit sample for the following reason: S-MUAPs consist of negative as well as positive components but the various phases do not necessarily occur at the same delay because of differences in axonal conduction velocities between individual S-MUAPs. Moreover, the shapes of

S-MUAPs differ from one another. Thus, the summated maximum M-potential cannot represent the arithmetic sum of the different S-MUAPs; we wanted to take this concept, the so-called cancellation phenomenon,[12] into account in the motor unit sample.

Electrical stimulation was applied at enough points—usually between 4 and 10 points—to obtain 10 distinct S-MUAPs from the thenar muscles. For any compound motor response evoked at each stimulus site, the negative peak area was automatically selected and measured. If necessary, adjustments were made manually by moving the cursors. Measurements of negative peak area (from the onset of the first negative peak to the first crossing of the baseline) have been preferred to peak-to-peak amplitude or negative peak amplitude measurements, because measurements of negative peak area minimized the cancellation error.[12] The maximum M-potential was elicited by supramaximal stimulation of the median nerve at the wrist, 7 cm from the stigmatic electrode, and its negative area automatically measured. Supramaximal intensity was fixed at 150% of the intensity at which the M-potential area no longer increased.

The number of motor units was estimated by dividing the area of the maximum M-potential by the S-MUAP area averaged from the 10 distinct S-MUAPs.

Except for the first S-MUAP evoked at a stimulation point, the precise morphology of successive S-MUAPs is not visualized, the overall potential representing, as it does, two or more units. It is theoretically possible to record the same S-MUAP twice, from two different stimulation points, in any one trial because their morphology may not be recognizable. To make sure that selected S-MUAPs are different, the morphology of individual S-MUAPs may be reconstructed by subtracting from each compound motor response the preceding one obtained from the same stimulus site with a lower intensity. This reconstruction takes place automatically when point-by-point differences are taken between successive digitized traces (Fig. 2B).

In 54 subjects, AMPS and F-response methods were compared. The F-response technique was previously described by Brown and coworkers.[5] Successive stimuli, with intensities which evoke 5–30% of the maximum M-potential amplitude, were delivered to the median nerve at the wrist and the resultant F-responses were compared. F-responses identical in shape, size, and latency, on two or more occasions, were assumed to represent the activity of a single motor unit. Ten different F-responses were used to estimate the average S-MUAP size.

Statistical Analysis. Mean values have been expressed with their standard deviations. The significance of differences between means was determined by Student's *t*-tests for dependent samples. When the variable under study evolved exponentially, a logarithmic transform of the values preceded Student's *t*-tests. Correlations between associated variables have been calculated with regression analyses. The best curve-fit coefficients between linear, exponential, and polynomial regressions were chosen. Reproducibility of AMPS was quantified as the coefficient of variation between data collected on 2 successive days.

RESULTS

Number of Motor Units: an Illustrative Example. The results of an experiment in a 55-year-old control subject are shown in Figures 1 and 2. As shown in Figure 1, stimulus intensity at the first selected point at the elbow was gradually increased from 0 to 11 mA, in increments of about 0.5 mA. At 11 mA, the first S-MUAP (MU1) was elicited in an all-or-nothing manner. At the same stimulus site, with an increment of 0.5 mA (11.5 mA), MU1 was recruited following each stimulation. Then, stimulus intensity was increased further to 13 mA at which time a second S-MUAP (MU2) appeared in an all-or-nothing manner. At 13.5 mA, MU1 and MU2 were evoked by each stimulus and there was no fractionation of the compound motor response (it was identical in each trial). Stimulus intensity was increased again to 15 mA and a third S-MUAP (MU3) was elicited in an all-or-nothing manner. At 15.5 mA, MU1, MU2, and MU3 were evoked by each stimulus without fractionation of the compound motor response. Since motor units were recruited with distinct thresholds, in an all-or-nothing manner, and as there was no fractionation of the compound motor response with successive stimuli, we assume that alternation was eliminated. But, when the stimulus intensity was further increased, alternation clearly appeared. The same sequence was repeated at the second stimulus site (2, Fig. 2A), 6.5 cm from the distal crease of the wrist, from which two S-MUAPs were successively generated by incremental stimulation. At the third (3, Fig. 2A) (5 cm from the distal crease at the wrist) and fourth stimulus sites (4, Fig. 2A) (4 cm from the distal crease at the wrist), respectively, three and two S-MUAPs were successively selected by incremental stimulation. By subtracting from

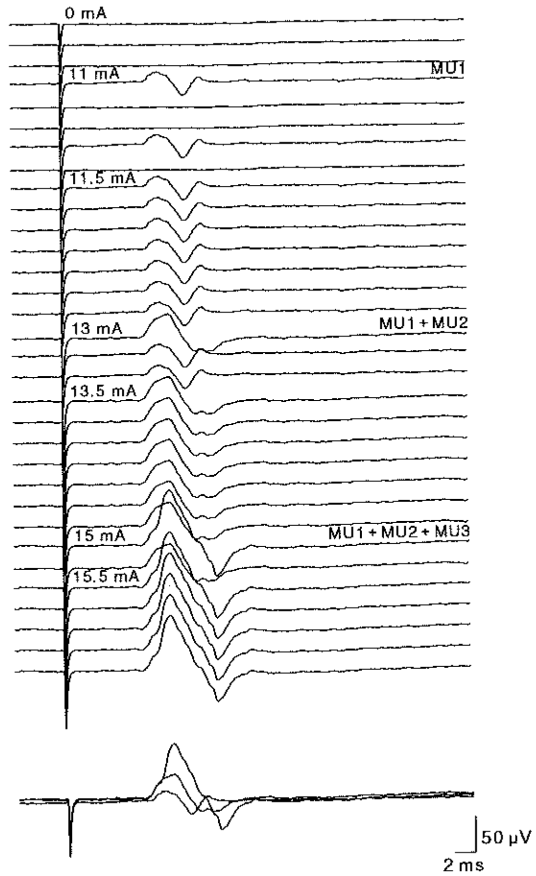

FIGURE 1. The adapted multiple point stimulation (AMPS) method. Results obtained from the left median innervated thenar muscles by stimulation at the first point in a 55-year-old subject. In this example, the first surface-recorded motor unit action potential (S-MUAP) (MU1), the second S-MUAP (MU2), and the third S-MUAP (MU3) were activated at three different thresholds by incremental stimuli at the elbow. However, responses which were not constant at 11 mA, 13 mA, and 15 mA, respectively, were regularly elicited after each stimulation at 11.5 mA, 13.5 mA, and 15.5 mA. They appeared in an orderly manner, without any fractionation of the compound motor responses to successive stimuli, so alternation was not suspected. Bottom: superimposed traces of the three steps MU1, MU1 + MU2, and MU1 + MU2 + MU3.

each compound motor response the preceding one, which had been obtained at the same stimulus site, the morphology of each S-MUAP was reconstructed to make sure that the same motor unit was not recruited twice at two different stimulation sites (Fig. 2B). Other stimulus sites were tested but morphology of the compound motor responses changed following successive stimuli in spite of the fact that stimulus intensity was kept identical. Therefore, alternation was suspected from the recruitment of the first or second motor unit. In that case, the cathode was moved to an alternative distal or proximal site. In general, longitudinal rather than transverse shifts over the median nerve were preferred for the selection of new S-MUAPs. The average S-MUAP size, calculated from the addition of the four compound motor responses divided by 10 (the number of evoked S-MUAPs), was 125 μV · ms (Fig. 2A). The maximum M-potential size obtained by supramaximal stimulation of the median nerve 7 cm from the stigmatic electrode was 18,140 μV · ms. The number of thenar motor units, estimated by dividing the maximum M-potential size by the average S-MUAP size, was thus 145 (Fig. 2A). This MUNE was derived in 17 min. In the same subject, average S-MUAP size and MUNE obtained by the F-response method were 150 μV · ms and 121 motor units.

Reproducibility. In 10 controls, the thenar MUNE and average S-MUAP size were repeated using AMPS by the same examiner on 2 successive days. Results of MUNEs from the two trials were significantly correlated ($r = 0.95$, $P < 0.001$) and the calculated coefficient of variation was 10.4% (Fig. 3A). Results of average S-MUAP sizes from the two trials were also correlated ($r = 0.83$, $P < 0.001$) and the calculated coefficient of variation was 9.5% (Fig. 3B).

Number of Motor Units in Normal Subjects. The mean thenar MUNE in volunteers was 278 ± 113 motor units (Table 1).

It can be seen in Figure 4A that there was a negative correlation between the number of motor units and the age of the subjects ($P < 0.001$), reflecting a progressive loss of motor units throughout life. From a statistical point of view, the curve-fit was better with an exponential ($r = -0.67$) than a linear regression ($r = -0.61$). The calculated mean loss of motor units was 1.3% per year.

Since there is a decline of motor units with age, to minimize the effect of the large age range on the standard deviation, we considered the results in terms of age using 24 subjects from 19 to 39 years old, 18 subjects from 41 to 58, and 17 subjects from 60 to 87. The mean thenar MUNEs are 353 ± 120 motor units, 258 ± 64 motor units, and 194 ± 74 motor units, respectively.

In 26 subjects, both hands were examined. MUNEs from the two hands were analyzed by linear regression and proved to be significantly correlated ($r = 0.74$, $P < 0.001$) (Fig. 5A). Average S-MUAP sizes in both hands were also correlated ($r = 0.59$, $P < 0.001$) (Fig. 5B). Paired Student's *t*-tests were carried out to compare the different

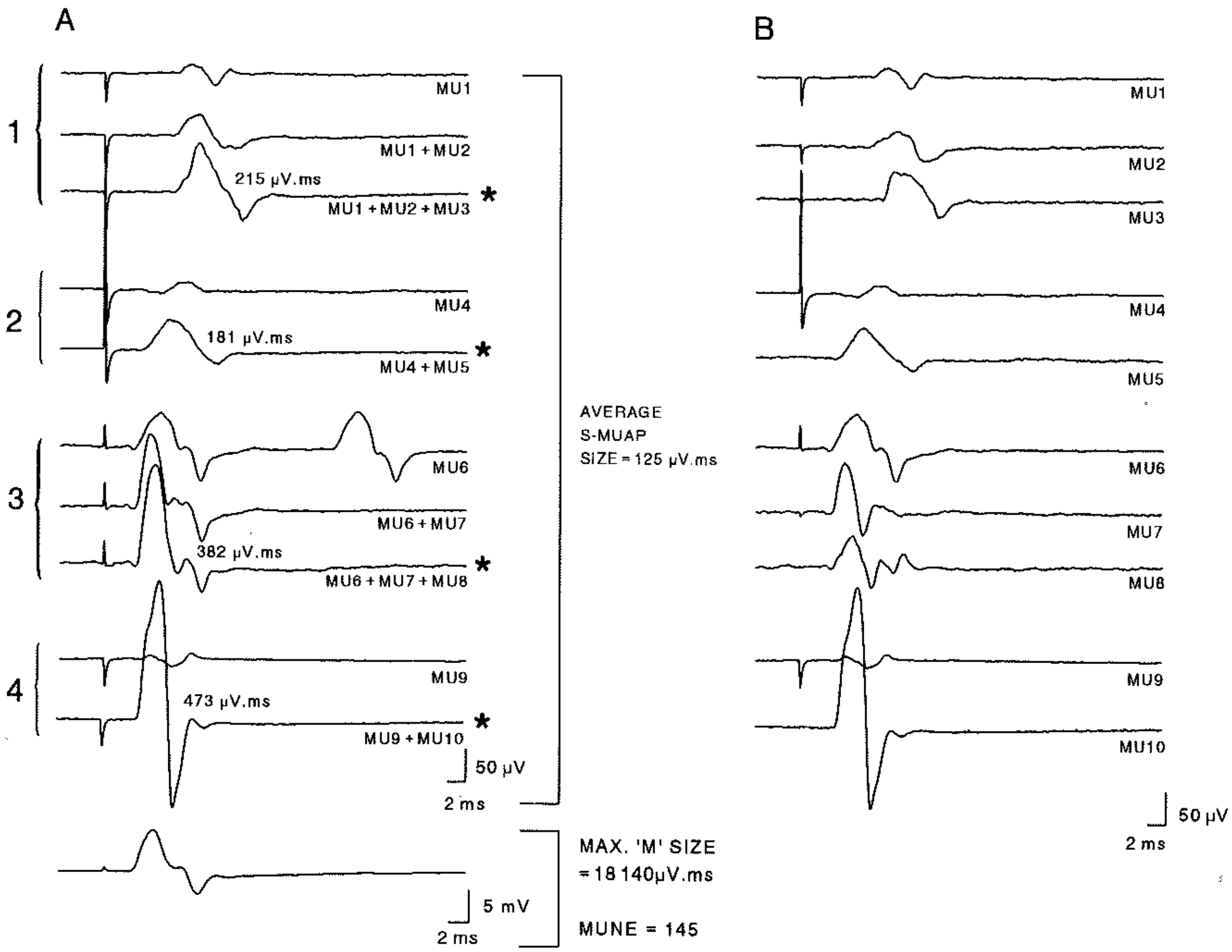

FIGURE 2. An illustrative example of the adapted multiple point stimulation (AMPS) method from the left median innervated thenar muscles in a 55-year-old subject. **(A)** Typical trial of AMPS. From the first stimulus site (1), at the elbow, three surface-recorded motor unit action potentials (S-MUAPs) were evoked by increments of stimulus intensity. From the second (2), third (3), and fourth (4) stimulus sites (6.5 cm, 5 cm, and 4 cm, respectively, from the distal crease of the wrist), seven other S-MUAPs were collected. From the third stimulus site, the sixth S-MUAP (MU6) was followed by its F-response which was identical in shape and size. The mean motor unit size was calculated from the four compound motor responses (*). In this case, the sum was 1251 µV · ms (215 µV · ms + 181 µV · ms + 382 µV · ms + 473 µV · ms) which, divided by the number of units, gives a mean of 125 µV · ms. The maximum M-potential, obtained by supramaximal stimulation of the median nerve, had a negative peak area of 18,140 µV · ms, so the thenar motor unit number estimate (MUNE) was 145 motor units (18,140 µV · ms: 125 µV · ms). **(B)** Morphology of every S-MUAP was reconstructed, in this example, by subtraction of each compound motor response from the next largest obtained from the same stimulus site. By so doing, it can be proven that one S-MUAP, elicited from two different stimulation points, is not included twice in the estimate.

means (right MUNE: 305 ± 124 motor units; left MUNE: 317 ± 127 motor units; right average S-MUAP size: 77.1 ± 27.7 µV · ms; left average S-MUAP size: 82.4 ± 24.1 µV · ms) and they were not significantly different (NS).

Sizes of Motor Units. The mean S-MUAP size in our series was 87 ± 27.6 µV · ms (Table 1). Figure 4B shows that average S-MUAP sizes increased in terms of age and that the best curve-fit was with an exponential function ($r = 0.28$, $P < 0.05$) but the correlation coefficient was poorer than that for MUNEs and age of the subjects.

Figure 4C shows the exponential decrease of maximum M-potential areas with aging. Maximum M-potential areas and age of the subjects were correlated with a coefficient of -0.52 ($P < 0.001$).

Comparison with the F-response Method. Table 1 compared mean thenar MUNE and mean S-MUAP size in 54 control subjects in whom both AMPS and F-response methods were applied.

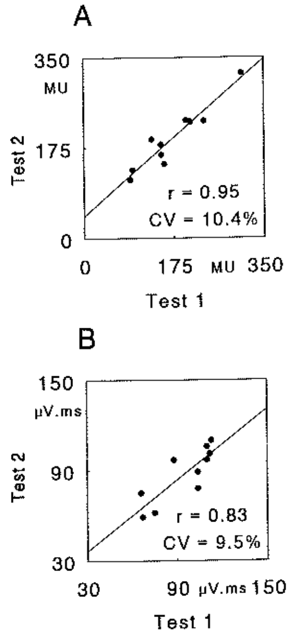

FIGURE 3. Reproducibility of the adapted multiple point stimulation method assessed by the coefficient of variation between data collected on 2 successive days (test 1 and test 2) from 10 control subjects. **(A)** Motor unit number estimates from the two trials were significantly correlated (r = 0.95, P < 0.001) and the coefficient of variation (CV) was 10.4%. **(B)** Average surface-recorded motor unit action potential sizes from the two trials were also significantly correlated (r = 0.83, P < 0.001) and the CV was 9.5%.

With the F-response method, the mean thenar MUNE in volunteers was 253 ± 107 motor units and the mean S-MUAP size was 96.8 ± 25.6 µV · ms. Figure 6 shows the correlation between the thenar MUNEs derived using both techniques (r = 0.83, P < 0.001). However, paired Student's t-tests indicated that mean thenar MUNE was significantly greater (P < 0.05) and mean S-MUAP size significantly smaller (P < 0.05) with the AMPS method than with the F-response method.

DISCUSSION

From a clinical point of view, a technique for estimating the number of motor units in a muscle or a

Table 1. Comparison of results from adapted multiple point stimulation (AMPS) and F-response methods (means ± SD).

Parameter	AMPS	F-response
Average surface-recorded motor unit action potential size (µV · ms)	87 ± 27.6 (n = 59)	96.8 ± 25.6 (n = 54)
Thenar motor unit number estimate	278 ± 113 (n = 59)	253 ± 107 (n = 54)

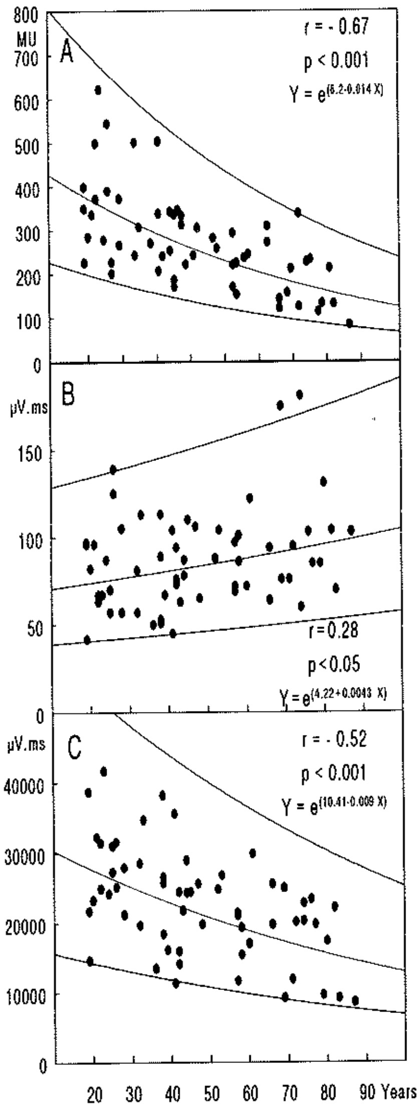

FIGURE 4. Influence of age, from 19 to 87 years on the abscissa, on various values. The best curve-fit coefficients among linear, exponential, and polynomial regressions were chosen. Prediction limits with a P-level of 0.05 give the lower and upper limits of normal. **(A)** Motor unit number estimate (MUNE) values declined progressively and the best curve-fit coefficient was with an exponential function (r = −0.67, P < 0.001). **(B)** Average surface-recorded motor unit action potential sizes increased in terms of age, according to an exponential function (r = 0.28, P < 0.05). This last coefficient was poorer than the correlation between MUNEs and the age of the subjects. **(C)** Correlation between the maximum M-potential size and the age of the subjects. The best curve-fit was also exponential (r = −0.52, P < 0.001).

muscle group would be welcome provided it is reliable, fast, noninvasive, and easily practicable in the clinical setting. To be reliable, a motor unit estimation method must be reproducible and must avoid alternation or any selection bias toward motor units of a particular size which might not be

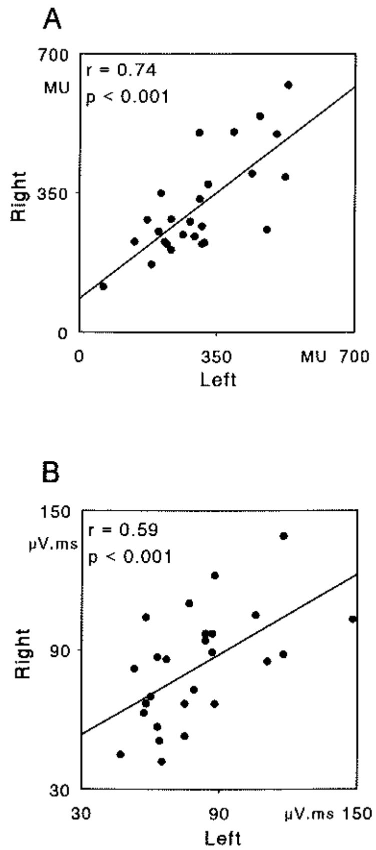

FIGURE 5. Relationships between the right and left thenar motor unit number estimates **(A)** and average surface-recorded motor unit action potential sizes **(B)** in 26 control subjects.

representative of the whole.[16] To meet these aims, various techniques have previously been developed.

The original McComas technique[17] did not avoid alternation. Aware of that limitation, Galea et al.[13] are currently proposing a fully automated computerized system in reply to that objection. Theoretically, both manual and automated incremental stimulation techniques are heavily biased toward low-threshold motor units. But McComas et al.,[17] in their original description of the manual incremental technique, found no systematic differences with respect to the lowest and highest threshold increments in the compound motor response. These results have been recently confirmed with the automated incremental technique,[13] by use of which no differences were found in the areas of the lowest threshold and highest threshold S-MUAPs. Automated incremental stimulation is equally applicable to distal and proximal muscle groups but with proximal muscles, potential biases arise from the location of detection electrodes in larger muscles or in muscle groups with complex innervation zones.[13] Compared with the manual technique, the automated technique has some advantages. It is not time consuming, it is free from subjective bias in the recognition of new S-MUAPs and in the manipulation of stimulation intensity, and it avoids the lengthy period of training required for the manual technique.[13] Finally, another drawback of the original McComas technique is that the gain often has to be reduced to 100 µV/division or less to keep the complete evoked response on the oscilloscope screen and, at that gain, recruitment of the last motor units may be difficult to detect.

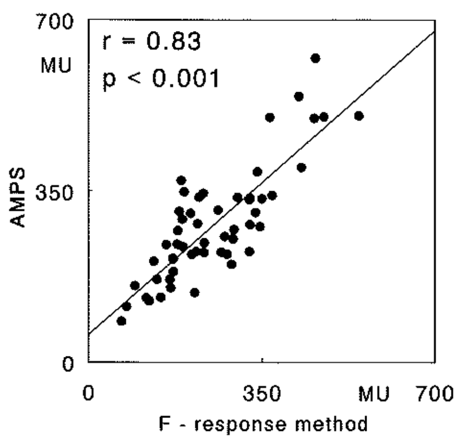

FIGURE 6. Correlations between the thenar motor unit number estimates using the adapted multiple point stimulation and the F-response methods in 54 control subjects.

The spike-triggered averaging (STA) technique[3,7,11] eliminates the problem of alternation since a needle electrode is used to select motor units recruited by voluntary muscle contraction. Nevertheless, this technique involves at least two possible selection biases. STA, according to Henneman's principle, deals chiefly with earlier recruited, lower threshold motor units which should generate the smallest S-MUAPs. However, STA might alternatively emphasize the larger motor units with a greater number of muscle fibers, because the likelihood of detecting activity with a single-fiber triggering electrode is then higher. Combining STA with EMG signal decomposition[5,11] might provide a more representative motor unit sample because the latter method comprises later recruited, higher threshold motor units. Selection bias toward larger motor units is also minimized by use of a concentric needle electrode. Other disadvantages of STA are that it is invasive, time consuming, and requires full cooperation of the patient. Finally, accurate measurements of S-MUAPs are also difficult because the precise onset of an averaged S-MUAP is not easy to detect.

Daube's statistical estimate[10] takes advantage of alternation since it is based on the probability with which an axon near threshold will fire. To be valid, this method requires that motor unit sizes follow a Poisson distribution. But in pathological cases, this prerequisite is not necessarily met, especially when motor unit sizes are enlarged following denervation. Thus, possible bias exists because of the unproved Poisson distribution of motor unit sizes.

Multiple point stimulation (MPS), described by Doherty and Brown,[11] is also a good alternative for avoiding alternation. Moreover, with MPS, the average S-MUAP size is based on surface potentials generated by true single motor units and not on a statistical estimate of S-MUAP size or an estimate derived by algorithms designed to correct for alternation.[11] However, MPS, like the original McComas technique, could be biased toward low-threshold motor units but the wide range in relative latencies, conduction velocities, and sizes of S-MUAPs observed with this technique[11] strongly suggests that there is no systematic bias toward any particular type of motor units by the selection of motor axons with threshold electrical stimulation. On the other hand, MPS depends on the skill of the operator to select appropriate S-MUAPs. If stimulation between the elbow and axilla is needed, simultaneous activation of median and ulnar nerves may occur. Moreover, it is sometimes difficult to evoke 10 distinct motor units at 10 different stimulation points along the accessible course of the median nerve. We anticipate this difficulty may be more pronounced when the number of axons declines as a result of axonal degeneration; MPS might not be applicable, particularly in patients presenting with severe axonal loss.

The F-response technique[5] is not completely free of alternation and there is a possible bias toward summated F-waves. This manual technique also is time consuming and requires great attention to identify and select S-MUAPs. Brown et al.[5] have developed a computer-based algorithm which automatically extracts S-MUAPs from the F-response. This automated approach is fast, easily applicable, and well tolerated.

MUNEs derived by computer-based systems[5,10,13] or by twitch tension[8,22] require special equipment or proprietary software for data analysis.

For all the above-mentioned reasons, we have sought an alternative method which would minimize objections and be easily practicable, namely the AMPS method, which has its own advantages as well as disadvantages.

AMPS is considered to be a technique which avoids or minimizes the risk of alternation. Every motor axon is excited with a distinct threshold (Fig. 1) in an orderly and reproducible manner. Compound motor responses remain unchanged, without any fractionation, after successive stimuli. Because the number of stimulation increments is limited, every S-MUAP is clearly identifiable, being elicited in an all-or-nothing manner. If there is still uncertainty, subtracting from each compound motor response the preceding one obtained at the same stimulus site should identify every S-MUAP without a problem (Fig. 2B). Moreover, when an F-response follows the first direct S-MUAP (about once or twice in 10 trials), a control situation exists since both should be identical in shape and size (MU6, Fig. 2A).

Theoretically, the AMPS method as well as MPS and the original McComas techniques may all be biased toward low-threshold motor units. However, as evidence against this possible bias, are the arguments developed by McComas et al.,[17] Galea et al.,[13] and Doherty and Brown[11] summarized above.

In this article, we systematically compare two methods, AMPS and F-response. Correlation coefficients for both series of MUNEs reach 0.83 ($P < 0.001$) (Table 1, Fig. 6). Since the AMPS method takes cancellation into account, average S-MUAP sizes are significantly smaller ($P < 0.05$) and thenar MUNEs greater ($P < 0.05$) than those obtained using the F-response method. Nonetheless, results of MUNEs obtained with AMPS are close to those reported with the previously described techniques[4,10,11,13,18] (Table 2), with the exception of lower estimates published by Stein and Yang.[22] In Table 2, standard deviations of the two methods used in the present study are higher than with other techniques, but in our study, the age range is larger than, for example, in studies by Galea et al.[13] and Doherty and Brown.[11] Since there is a loss of motor units with age, the larger the age range, the higher the standard deviation. Moreover, we reviewed the results in terms of age using three groups with 24 subjects from 19 to 39 years of age, 18 subjects from 41 to 58, and 17 subjects from 60 to 87 and found the mean thenar MUNEs to be 353 ± 120 motor units, 258 ± 64 motor units, and 194 ± 74 motor units, respectively, in which standard deviations are reduced compared to data in Tables 1 and 2. We also showed (Fig. 3) that when MUNEs are performed on 2 successive days in the

Table 2. Motor unit number estimates (MUNEs) in median innervated thenar muscles (means ± SD).

Investigator	MUNE	Method
Brown[4] (1972)	253 ± 34	Manual incremental stimulation
Sica et al.[18] (1974)	340 ± 87	Manual incremental stimulation
Stein and Yang[22] (1990)	170 ± 62	Manual incremental stimulation
	135 ± 27	Spike-triggered averaging
	122 ± 38	Microstimulation
Galea et al.[13] (1991)	228 ± 93	Automated motor unit estimate
Daube[10] (1988)	315 ± 48	Statistical estimate
Doherty and Brown[11] (1993)	288 ± 95	Multiple point stimulation
Wang and Delwaide (1995)	278 ± 113	Adapted multiple point stimulation
Wang and Delwaide (1995)	253 ± 107	F-response

same subjects, the results are reproducible. Thus, the standard deviations seem to be more influenced by variability of individual values than by a variability inherent to the technique. Therefore, it is advisable to fix the lower and upper limits of normal by using prediction limits, as indicated in Figure 4, rather than by using the standard deviation of the whole group.

Another advantage of AMPS is that it is nearly always possible to obtain a MUNE. In our group of 59 volunteers, we did not fail in anyone for technical reasons. Moreover, in our experience with pathological cases presenting with severe axonal loss, even though it may be more difficult to find as many stimulation points from which to evoke distinct S-MUAPs than it is in normal subjects, it is usually easy to derive MUNEs. Indeed, as we know from Brown and Milner-Brown's data,[6] there is less overlap of motor unit thresholds when denervation is severe and, as a consequence, the alternation problem is less noticeable. It is often possible to recruit more than three motor units, free of alternation, from the same point so that three different stimulation points along the course of the median nerve are usually enough for derivation of a MUNE.

As complementary advantages, it should be mentioned that AMPS can be performed in about 15 min, does not require special equipment, and is noninvasive and painless as only surface electrodes are used.

Certain disadvantages should, however, be recognized. The AMPS method is not a hands-off technique, so the skill of the practitioner must be taken into account. A subjective bias in recognition of new S-MUAPs is possible. Moreover, the same motor unit may be included in the estimate twice if the same axon is recruited at two different stimulation sites. To take this objection into consideration, it is advisable, as proposed by Ballantyne and Hansen,[2] to reconstruct every individual S-MUAP by subtracting traces from each other to make sure that S-MUAPs are different (Fig. 2B). However, such a procedure would extend the examination time and cannot be done on all EMG machines.

The decline in motor unit estimates with age has been extensively studied anatomically and electrophysiologically using various techniques. Nevertheless, this important subject continues to be much debated. Two main hypotheses are discussed: either a decline in motor units starting beyond the age of 60 years or a continuous depletion of motor units throughout life.

From an anatomical point of view, Tomlinson and Irving[23] did not find evidence of loss of motoneurons in the human lumbosacral cord up to the age of 60, but Kawamura et al.[15] showed a progressive loss of large cells in the spinal cord from the age of 20. The decline in number of motor units may also be due to a loss of axons by wallerian degeneration. In one study of control human peripheral nerves, Arnold and Harriman.[1] found that wallerian degeneration first appeared at the age of 10 and increased steadily with age.

The decline in MUNEs with age has also been studied electrophysiologically. Campbell et al.[9] and Sica et al.,[18] using the manual incremental stimulation method, reported a loss of motor units starting after the age of 60. On the other hand, Brown,[4] with the same technique, interpreted his thenar muscle data as indicating a progressive decline of motor unit number throughout adult life. Later, applying the spike-triggered averaging technique to the biceps brachii muscle, Brown et al.[7] noted that increasing age was accompanied by progressive decline in the numbers of motor units, especially in the later decades of life.

Although preliminary, our results of thenar MUNEs obtained with AMPS are more consistent with a progressive reduction in the number of motor units with age from 19 to 87 than with a decline starting beyond the age of 60. This decline fits better with an exponential ($r = -0.67$) rather than a linear ($r = -0.61$) function (Fig. 4).

Other electrophysiological studies may help us understand neuromuscular aging. For example, Stålberg et al.[19,21] reported that single-fiber EMG fiber density (FD) values increase with age. In the

EDB muscle,[21] increases may be noted as early as the third decade. In quadriceps femoris,[19] FD increased linearly with age in both sexes. We know that FD is directly dependent on the anatomical organization of the motor unit and an increase in FD, in the context of aging, indicates reorganization of the motor unit, most likely due to reinnervation by collateral sprouting. Thus, an increase in FD may be interpreted as proof of progressive peripheral denervation throughout adult life. This denervation may be the consequence of loss of motoneurons with age but also of subclinical proximal nerve compression and/or peripheral entrapments, likely to develop with age. Probably both mechanisms, motoneuron degeneration and subclinical nerve compression, intervene.

Motor unit size related to age is also much debated. In our study, the correlation coefficient between the average S-MUAP sizes and subjects' ages (Fig. 4B) is only 0.28 and the slope is less steep than for MUNEs. Increase in average S-MUAP size likely results from collateral reinnervation or muscle fiber hypertrophy. However, the increase in average S-MUAP size does not seem marked enough in normal subjects to compensate for the reduction in number of motor units. As a result, the maximum M-potential area declines with aging (Fig. 4C). The increase in average S-MUAP size with age reported using AMPS is less significant than with other motor unit estimation techniques.[7,9,11] However, this problem has also been studied by Macro-EMG, concentric needle, and histochemistry techniques. For example, the Macro-EMG amplitude values recorded by Stålberg et al.[19,20] in biceps brachii and quadriceps femoris are quite close to our S-MUAP size data. On the other hand, Brown et al.,[7] in their analysis of concentric needle recordings from biceps brachii, mentioned that the shapes and sizes of the intramuscularly recorded motor unit potential are almost indistinguishable in successive decades. Finally, as indicated by histochemistry data from Stålberg et al.,[19] it seems that the mean muscle fiber diameter decreases with age. This decrease may certainly be responsible in part for the apparent lack of compensatory morphologic changes in motor unit action potentials with age as the motor unit number declines.

As shown in Figure 5, thenar MUNEs and average S-MUAP sizes in left and right hands are very similar with a linear correlation coefficient of 0.74 ($P < 0.001$) and 0.59 ($P < 0.001$), respectively. The paired Student's t-test indicates that the two means are not significantly different. Such an observation indicates that comparisons between both hands may be considered when assessing patients for a clearly lateralized pathology, despite the fact that failure to demonstrate a difference between the hands does not mean that there is no difference.

In summary, in the present study, we have updated a method for estimating the number and relative size of motor units which has three major advantages in comparison with other techniques: it is a fast, noninvasive method and no specific collection system or software is required. We propose AMPS as a reliable method because it is reproducible, it minimizes alternation, and it avoids any significant motor unit selection bias.

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