Consensus for experimental design in electromyography (CEDE) project

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Consensus for experimental design in electromyography (CEDE) project: Single motor unit matrix

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ABSTRACT:

The analysis of single motor unit (SMU) activity provides the foundation from which information about the neural strategies underlying the control of muscle force can be identified, due to the one-to-one association between the action potentials generated by an alpha motor neuron and those received by the innervated muscle fibers. Such a powerful assessment has been conventionally performed with invasive electrodes (i.e., intramuscular electromyography (EMG)), however, recent advances in signal processing techniques have enabled the identification of single motor unit (SMU) activity in high-density surface electromyography (HDsEMG) recordings. This matrix, developed by the Consensus for Experimental Design in Electromyography (CEDE) project, provides recommendations for the recording and analysis of SMU activity with both invasive (needle and fine-wire EMG) and non-invasive (HDsEMG) SMU identification methods, summarizing their advantages and disadvantages when used during different testing conditions. Recommendations for the analysis and reporting of discharge rate and peripheral (i.e., muscle fiber conduction velocity) SMU properties are also provided. The results of the Delphi process to reach consensus are contained in an appendix. This matrix is intended to help researchers to collect, report, and interpret SMU data in the context of both research and clinical applications.

INTRODUCTION

A single motor unit (SMU) is comprised of an alpha motor neuron and the muscle fibers it innervates; SMUs are the final common pathway by which an activation signal from the central nervous system is transformed into contractile activity (Sherrington (1906)). Given the one-to-one association between an action potential generated by a motor neuron and those evoked in muscle fibers, electromyography (EMG) recordings of SMU activity provide a window into the nervous system (Merletti *et al.*, 2008).

The first methods introduced to record SMUs included concentric needle and fine wire electrodes (Adrian & Bronk, 1929; Joynt, 1994; Duchateau & Enoka, 2011). The recordings from intramuscular EMG electrodes can provide significant information about the discharge characteristics of SMUs in clinical populations and experimental studies, allowing a direct assessment of the variables responsible for the control of muscle force. However, such methods are invasive, and therefore not always feasible. Due to recent developments in signal processing methods, it is now possible to perform a non-invasive assessment of SMU activity with the aid of high-density surface electromyography (HDsEMG) electrode grids. Given their higher spatial resolution, HDsEMG recordings have enabled the concurrent analysis of both SMU discharge characteristics and the conduction velocity of muscle fiber action potentials on a greater number of SMUs than is possible with conventional intramuscular EMG techniques (Farina *et al.*, 2016). Given these advantages, the number of research groups that use HDsEMG recordings to characterize SMU activity has increased considerably during the last years. Nonetheless, HDsEMG still presents a number of limitations (i.e., lower SMU yield in women and difficulty in assessing deeper muscles) that must be acknowledged (Besomi *et al.*, 2019; Gallina *et al.*, 2022).

Despite some differences, when assessing SMU data, several features are common to both intramuscular and HDsEMG methods. Both require an algorithm that is able to identify and separate SMUs from an interference EMG signal. Although various semi-automatic SMU decomposition algorithms have been developed in recent years (Doherty & Stashuk, 2003; McGill *et al.*, 2005; De Luca *et al.*, 2006; Holobar & Zazula, 2007; Negro *et al.*, 2016b), in most cases the data still must be

edited manually to ensure accurate results. Once the data have been reviewed, the discharge times of SMU action potentials can be characterised in terms of such variables as the average number of action potentials discharged per second by a single motor unit (mean discharge rate), the variability in the number of action potentials discharged per second by a single motor unit, the force at which a motor unit begins to discharge action potentials repetitively (recruitment threshold), and the speed at which an action potential propagates along a muscle fiber (conduction velocity). However, there is no consensus yet on the specific ways in which these parameters should be calculated and reported. This, has compromised the quality of the knowledge in the field.

The aim of this matrix is to describe the main uses, advantages, and limitations of both intramuscular EMG and HDsEMG SMU recordings, and to provide indications on the recommended use of these techniques to characterise SMU action potentials. This matrix was developed by an international consensus of experts as part of the Consensus in Experimental Design in Electromyography (CEDE) Project using a Delphi process (Besomi *et al.*, 2019).

METHODS

The method used for expert group selection and the process employed for the development of the CEDE matrices can be found in previous CEDE articles (Besomi *et al.*, 2019; Besomi *et al.*, 2020; Hodges, 2020; McManus *et al.*, 2021; Gallina *et al.*, 2022). As with the previous CEDE matrices, the steering committee and the lead investigator prepared a draft of the matrix, which was then sent to the other CEDE members to reach consensus of the content following a Delphi process. All participants of the Delphi process are listed as co-authors. The Human Research Ethics Committee of The University of Queensland, Australia provided ethical approval for this project.

Development of the draft

The steering committee (RME, AH, DFar and KM), the coordinator of the project (MB) and the lead investigator (EM-V) prepared a first draft of the matrix. The matrix is arranged in nine sections: 1) Electrode type used to identify SMUs, 2) SMU decomposition techniques, 3) Contraction type used to assess SMU activity, 4) Longitudinal SMU tracking, 5) Analysis of SMU decomposition results, 6) SMU discharge characteristics, 7) Measures of assocation between discharge times, 8) Peripheral SMU

properties estimated with surface EMG grid electrodes, and 9) SMU action potential amplitude. Each section comprised various combinations of the following content: reporting, recommendations, advantages, limitations, considerations, cautions and definitions.

Delphi process

The Delphi process is a widely accepted method to achieve consensus (Waggoner, Carline and Durning, 2016). The approach used in our matrix was similar to the one employed in previous CEDE projects and is described in detail elsewhere (Besomi *et al.*, 2019, 2020; McManus *et al.*, 2021). In the first round, 17 members of the CEDE team were invited to review the first draft of the matrix and provide feedback. Two members withdrew from the process because they mentioned that this matrix was not within their expertise. The criteria to obtain consensus are described in previous CEDE project matrices (Besomi *et al.*, 2019; Besomi *et al.*, 2020; McManus *et al.*, 2021; Gallina *et al.*, 2022). The steering committee, coordinator and lead investigator oversaw the project and integrated comments but did not participate in the Delphi process. The Delphi questionnaires were sent online using a centrally supported survey tool (Checkbox Survey Software; www.checkbox.com) from the University of Queensland. The percentage of participants rating each item as either appropriate (score 7–9), uncertain (score 4–6), or inappropriate (score 1–3) were determined and the median and interquartile range (IQR) were calculated.

RESULTS

From the 15 experts who agreed to participate in the Delphi process, 14 (93.3%) replied to the first-round questionnaire. Version 1 comprised 39 items. After round one, four sections were ranked with insufficient consensus, and another three sections were substantially modified based on feedback and these were included in the second-round questionnaire. Round two, which was resubmitted to the 15 original experts comprised seven sections. Fourteen experts (93.3%) completed the second-round questionnaire. A summary of the results of the Delphi consensus process is presented in Appendix 1. The final SMU matrix endorsed by the CEDE project team is presented in Table 1 (SMU recordings), Table 2 (SMU decomposition techniques: processing, analysis, contraction type and longitudinal motor

unit tracking), Table 3 (SMU discharge characteristics), Table 4 (measures of association between SMU discharge times) and Table 5 (SMU peripheral properties and MUAP amplitude).

DISCUSSION

This matrix provides a number of recommendations related to the recording, reporting, and interpretation of SMU data. We focused on the details that are most commonly reported across SMU studies: 1) electrodes used to record SMU activity, 2) algorithms used to identify SMUs, 3) conditions in which SMUs can be recorded, 4) analysis of SMU results and reporting of SMU discharge characteristics, 5) measures of association between discharge times, and 6) muscle fiber properties and SMU action potential amplitude. It is important to note that the purpose of this matrix is not to replace formal training with SMU recordings and decomposition techniques. It, should however serve as a guide to promote standardized application of the procedures and reporting of SMU data.

SMU recordings have evolved over the years, from the use of intramuscular electrodes to that of surface EMG (Rau & Disselhorst-Klug, 1997; Duchateau & Enoka, 2011). Given the advantages and popularity of grid electrodes, it might be tempting to assume that this technique should be the current standard for the analysis of SMUs. However, this matrix demonstrates that intramuscular recordings still have an important role to play in the analysis of SMU activity. As clearly shown in this matrix, there are a number of conditions and analyses in which intramuscular methods are preferred over HDsEMG, such as the assessment of activity in deep muscles, recordings from individuals with thick subcutaneous tissue, and the analysis of near-fiber potentials. Therefore, the preferred recording method depends on the research question. Moreover, the two techniques can also be used concurrently; for example, grid electrodes combined with intramuscular EMG (Yavuz *et al.*, 2015; Thompson *et al.*, 2018) and thin-film high-density intramuscular EMG (Muceli *et al.*, 2015; Negro *et al.*, 2016a).

The development of signal processing algorithms to identify SMUs from the interference intramuscular and surface EMG signals has also evolved over time. As summarized in this matrix, the most important aspect to consider is the validity and accuracy (ability to distinguish between true SMU discharges and falsely detected SMU discharges) of these algorithms in identifying the discharge times of SMUs. Due

to their higher selectivity, decomposition methods applied to intramuscular EMG enable the accurate identification of SMU discharge times employing semi-automatic decomposition tools, such as EMGlab (McGill *et al.*, 2005). These algorithms first identify SMUs automatically and then allow the user to add or remove SMU discharges that were not detected by the software. With the emergence of decomposition algorithms for HDsEMG recordings, such as those that use blind source separation (Holobar & Zazula, 2007; Negro *et al.*, 2016a), this process has been automated, but the quality of the analysis requires careful evaluation. To address this need, we provide recommendations on how to check the accuracy of the data both when intramuscular EMG and HDsEMG are used, and we also offer advice on the way in which these accuracy measures should be reported. It is possible that future developments in artificial intelligence techniques may be able to decrease the computational load required for the SMU decomposition algorithms and make it possible to perform a fully automatic decomposition without the need to edit the output manually. This will ultimately decrease the time required to perform SMU analyses, which is crucial in clinical applications.

Another important issue that was considered for the development of this matrix was the conditions in which SMU recordings could be performed. In the past, SMU recordings were mostly limited to low force isometric contractions, which facilitate the identification of SMU action potentials. More recent studies have examined more challenging conditions, such as strong and fast isometric contractions (Del Vecchio *et al.*, 2019b) and dynamic contractions (Glaser & Holobar, 2019; Oliveira & Negro, 2021) in addition to tracking weakness in patients diagnosed with neurodegenerative disease (Howells *et al.*, 2018). Greater care needs to be taken under these conditions as it is more difficult to satisfy the requirements necessary for the identification of SMU discharge times. For example, the activity of multiple SMUs can merge into one SMU spike train and dynamic changes in action potential waveforms can reduce the ability of the decomposition algorithm to discriminate the activity of SMUs. Despite these challenges, it is likely that further development of decomposition algorithms, such as the implementation of real-time updating of SMU filters (Wen *et al.*, 2021), will improve the separation of SMUs from the interference signal.

In this matrix we also acknowledge the lack of standardization in the reporting of SMU data. Besides issues with terminology, which are addressed in the terminology matrix (McManus *et al.*, 2021), investigators tend to calculate and report the discharge characteristics of SMUs in different ways, which complicates the comparison of data between studies (Elgueta-Cancino *et al.*, 2022). We provide recommendations on how to calculate and report most time-domain discharge characteristics, such as recruitment and de-recruitment thresholds, mean, median, and peak discharge rates, and double discharges (doublets).

Measures of association (correlation and coherence) between SMU discharge times provide important information about the sources of common and independent synaptic input to SMUs within and across muscles (Laine *et al.*, 2015; Negro *et al.*, 2016b). As with the reporting of discharge characteristics, these measures have sometimes been treated as interchangeable, despite their means of calculation dictating that they reflect different physiological processes. Here we provide recommendations on how to report, calculate, and when to employ both time-domain (i.e., short-term synchrony) and frequency-domain (i.e., coherence) associations in SMU discharge times. We refer the reader to the terminology matrix (McManus *et al.*, 2021) for a more detailed definition of each of these measures.

We also discuss muscle fiber properties that can be obtained from SMU recordings. With the emergence of HDsEMG, it is now possible to estimate SMU territories and conduction velocities. Although this information was also covered in the HDsEMG matrix (Gallina *et al.*, 2022), it is important to emphasise the utility of these approaches and the caution that is required when using surface EMG data to infer properties at the level of the muscle fibers. This is particularly true for the estimation of SMU territories, for which further studies are required to validate this approach.

Finally, we also acknowledge the limitations of amplitude estimates to infer SMU properties. Knowledge of these limitations is important for those who aim to use intramuscular EMG recordings of SMU action potential amplitude and area as a diagnostic aid in, for example, neuromuscular disorders (Tankisi *et al.*, 2020). As discussed in the current matrix, the amplitude normalization matrix (Besomi *et al.*, 2020), and in multiple studies assessing the validity of EMG recordings to infer changes in SMU properties (Del Vecchio *et al.*, 2017; Martinez-Valdes *et al.*, 2018), EMG amplitude is influenced by a

number factors unrelated to SMU size and recruitment (Farina *et al.*, 2004). This applies to both intramuscular EMG and HDsEMG recordings. Therefore, the CEDE team decided to not recommend that amplitude estimates be used for the assessment of changes in SMU properties, but instead acknowledge that future studies are needed to assess the validity of these measurements.

CONCLUSION

SMU recordings provide the most direct information about the neural drive strategies used by the central nervous system to control muscle force. However, great care is needed when determining the discharge times of SMUs from interference EMG signals to ensure that the analysis yields physiologically meaningful data. Moreover, adequate reporting and unified criteria are required to allow comparison of findings across studies. The aim of the present matrix is to tackle these issues by providing recommendations on how to record, report, analyse, and interpret SMU data. The matrix is intended to serve as a guide for the standardized application of such measurements in both research and clinical applications. Due to the continual development of SMU recording and signal processing techniques, we expect that some of our recommendations will need to be updated in future versions of this matrix.

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Declaration of Competing Interest

Dario Farina is a scientific advisor for the company OT Bioelettronica, Torino, Italy, and for Meta/Facebook Reality Labs, NY, US. No other known competing financial interests or personal relationships are reported.

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Table 1. Considerations for single motor unit recordings

Electrode type	Surface grid of electrodes (High-density surface EMG; HDEMG)	Intramuscular fine-wire electrode	Intramuscular needle electrode
Electrode design reporting	- Number of electrodes - Shape of the grid (i.e., rectangular, square, linear), with the number of rows and columns. - Diameter of each electrode - inter-electrode distance (specify center-to-center or edge-to-edge) - Reference electrode - Pre-amplification - material (e.g., Ag/Cl, gold) - Use of a dry linear array to determine the propagation direction of motor unit action potentials (MUAPS) to align the grid electrode with the orientation of the muscle fibres - Location of grid electrodes relative to innervation zones, if measured - Report anatomical landmarks used to position the grid electrode	- Wire type - Materials used to construct the electrode - Length of exposed conductor (wire) - Approximate separation between electrodes - Insertion guidance method - Depth of insertion - Recording montage (bipolar, monopolar) - Muscle region where the wire was inserted - Report anatomical landmarks used to position the electrode - Mention if placement was verified, such as with ultrasound imaging	- Needle type (e.g., monopolar, concentric, quadrifilar). - Materials used to construct the electrode - Needle size/gauge - Perpendicular insertion - Depth of insertion - Electrode recording area - Muscle region where the needle was inserted - Mention if the needle was held in place or stabilized - Report anatomical landmarks used to position the electrode
Electrode design recommendations	- ≥ 32-channel grid is recommended to increase single motor unit (SMU) identification accuracy - Inter-electrode distance ≤ 10 mm to increase selectivity of recordings and allow interpolation - Grid positioning over the innervation zone is recommended in order to maximize the diversity of MUAP shapes and improve the discriminative power of SMU identification algorithms	- Multichannel signals can be recorded using separate electrodes or wires placed at different muscle locations. - Multichannel intramuscular signals (i.e., quadrifilar wire or thin-film electrodes) can generally be decomposed more reliably, as MUAPs that are difficult to distinguish in one channel can often be distinguished more easily in another channel	- Multichannel signals can be recorded with a quadrifilar needle (4 electrodes) or using separate electrodes at different muscle locations. - Multichannel intramuscular signals can generally be decomposed more reliably, as MUAPs that are difficult to distinguish in one channel can often be distinguished more easily in another channel.

General principles for reporting SMU recording procedures	- Sampling rate in space and time (Merletti & Muceli, 2019) - Gain - Time-domain filter: High-pass and low-pass cut-off frequencies, filter order, and type (e.g., Butterworth) - Was a notch filter (50 Hz or 60 Hz) used? - Type of spatial filter (e.g., monopolar, differential, Laplacian, principal component analysis (PCA), double differential, quadrupolar)	- Sampling rate - Gain - Time-domain filter: High-pass and low-pass cut-off frequencies, filter order and type (e.g., Butterworth). - Was a notch filter (50 Hz or 60 Hz) used?	- Sampling rate - Gain - Time-domain filter: High-pass and low-pass cut-off frequencies, filter order and type (i.e., Butterworth). - Was a notch filter (50 Hz or 6 0Hz) used?
General principles for recording single motor unit activity (recommendations)	- Sampling rate ≥2000 Hz - High signal-to-noise ratio. Remove any channels with low signal to noise ratio before running the decomposition algorithm - Adjust gain to avoid clipping and saturating signals, especially in amplifiers with analogue-digital converters with lower resolution (i.e., <16-bit) - Gain should allow clear MUAP visualization at low force magnitudes - Filter EMG signals with a 3 db band-pass of at least 10-500 Hz - Analog low-pass filter should be set at half of the sampling rate or less - Consider increasing high-pass cut-off frequency (e.g., 20 Hz) if movement artefacts are present. - Record monopolar signals to maximize flexibility during offline analysis - If signals are going to be processed (decomposed) in single differential mode, it is recommended to record these signals in single differential mode so that the recording amplifier can provide a higher common-mode-rejection-ratio (CMRR) compared with the differentiation made by signal processing software (due to imperfections in channel-to-channel gain matching)	- Sampling rate ≥10000 Hz - Oversampling (>10000 Hz) provides greater temporal resolution without the need for interpolation, but at the cost of increased storage requirements. - High signal-to-noise ratio - Adjust gain to avoid clipping and saturating signals, especially in amplifiers with analogue-digital converters with lower resolution (i.e., <16-bit) - Different filters can be considered depending on the application, please see (Tankisi *et al.*, 2020) for specific information about filtering in different conditions. - 3 db analog band-pass filter between 500 Hz and 5000 Hz is commonly applied. - Analog low-pass filter should be set at half of the sampling rate or less - Consider increasing high-pass cut-off frequency (e.g., 20 Hz) if movement artefacts are present.	- Sampling rate ≥10000 Hz - Oversampling (>10000 Hz) provides greater temporal resolution without the need for interpolation, but at the cost of increased storage requirements. - High signal-to-noise ratio - Adjust gain to avoid clipping and saturating signals, especially in amplifiers with analogue-digital converters with lower resolution (i.e., <16-bit) - Different filters can be considered depending on the application, please see (Tankisi *et al.*, 2020) for specific information about filtering in different conditions. Common filters applied for motor unit recordings: - 3 db analog band-pass filter between 2 Hz and 10000 Hz for monopolar and concentric needles (Tankisi *et al.*, 2020) - 3 db analog band-pass filter between 500 Hz and 10000 Hz for single-fibre EMG (Tankisi *et al.*, 2020) - Analog low-pass filter should be set at half of the sampling rate or less - Consider increasing high-pass cut-off frequency (e.g., 20 Hz) if movement artefacts are present

	- For SMU identification with blind source separation algorithms, non-linear pre-processing methods should be avoided as they alter the linear mixing model of EMG which is assumed by many blind source separation methods (Holobar & Zazula, 2007; Negro *et al.*, 2016a)		
General considerations for selection of electrodes (based on SMU properties to be studied)	**PROS** - Non-invasive - Depending on the number of electrodes, the concurrent activity of up to tens of MUs can be identified - Analysis of 2D MUAP distribution - Measurement of peripheral muscle fibre properties, such as conduction velocity - Recordings are possible during anisometric/slow dynamic muscle contractions, but caution is required as MU identification in these conditions can be challenging - Potential to identify MUs at high force magnitudes, including 100 % MVC and fast isometric contractions	**PROS** - Selective electrode that allows real-time identification of single MUs - Both superficial and deep muscles can be assessed - Signal quality does not depend on subcutaneous tissue thickness - Electrodes move with the muscle fascicles and, unlike solid needles, wires are flexible and stronger contractions can be performed without too much discomfort	**PROS** - Selective electrode that allows real-time identification of single MUs - Analysis of near-fibre action potentials (examination of contributions from fibres located close to the recording needle electrode) to assess jiggle and jitter, which provide information about neuromuscular transmission stability (Piasecki *et al.*, 2021) - Can be moved to record from different muscle regions - Standard EMG method for diagnosis in clinical neurophysiology/neurology [see (Tankisi *et al.*, 2020) for technical details of clinical use] - Both superficial and deep muscles can be assessed - Signal quality does not depend on subcutaneous tissue thickness
General considerations for selection of electrodes (based on SMU properties to be studied)	**CONS** - It is not possible to identify MU activity from deep muscles - Accuracy and number of identified MUs depends on subcutaneous tissue thickness and muscle architecture. This limitation significantly constrains the recruitment of study participants and the muscles that can be studied.	**CONS** - Invasive, and therefore special skills are required to insert electrodes - Can only identify a few MUs from a small region of the muscle - Electrode can be repositioned only slightly once inserted - Potential to discriminate MUs during strong contractions depends on the selectivity of the electrode and is difficult at force magnitudes close to the maximum	**CONS** - Invasive - Can only identify a few MUs from a small region of the muscle - Potential to discriminate MUs at high-intensity contractions depends on the selectivity of the electrode and is unlikely to be possible at force magnitudes close to the maximum - Discomfort/pain at high force magnitudes - Discomfort /pain may occur when inserted through fascial layers and into deeper muscles

		- Some discomfort/pain is possible at high force magnitudes - Discomfort /pain may occur when inserted through fascial layers and into deeper muscles - Movement artefacts can limit accuracy of MU discrimination during dynamic tasks, particularly in deep muscles - Risk of infection if sterilization and contamination protocols are not followed	- Generally, not suitable for anisometric/dynamic contractions due to needle movement - Risk of infection if sterilization and contamination protocols are not followed

Table 2. Single motor unit decomposition techniques: processing, analysis, contraction type and longitudinal motor unit tracking

SMU decomposition techniques	High-Density surface EMG SMU decomposition techniques	Intramuscular EMG SMU Decomposition techniques
General principles for processing of EMG signals for motor unit identification (Reporting)	- Report electrode grid position - Indicate the removal of any channel prior to decomposition - List any spatial filter used to process the signals (e.g., monopolar or differential) - Mention any time-domain filtering - Report decomposition technique (e.g., Blind-source separation, template matching, principal/independent component analysis) - List the decomposition software; for example, Precision decomposition (Nawab *et al.*, 2010), DEMUSE (Holobar & Zazula, 2007), DECOMPONI (OT Bioelettronica, Torino, Italy), dEMG Analysis Software (Delsys, Inc., Natick, MA), Convolutive Blind Source Separation (Negro *et al.*, 2016a), Custom. - Describe any constraints on acceptable data, such as maximal and minimal inter-spike intervals (ISIs), discharge rates or maximal discharge variability	- Report any time-domain filtering - Describe the spatial filter used (e.g., monopolar or differential) to process the signals recorded with multiple intramuscular electrodes (i.e., quadrifilar, thin-film) or in conjunction with surface EMG. - List the technique used to decompose SMU activity (i.e., Template matching, spike sorting) - Indicate whether the decomposition was automatic, semi-automatic, or manual - State the software employed to decompose signals, such as Spike [Cambridge Electronic Design (CED), Cambridge, UK], Precision Decomposition (Mambrito & De Luca, 1984), Decomposition-Based Quantitative Electromyography (Doherty & Stashuk, 2003), EMGLab (McGill *et al.*, 2005), Fuzzy Expert algorithm (Erim & Lin, 2008), EMG Long-term Decomposition (Zennaro *et al.*, 2003) - Acknowledge the use of an algorithm that includes the use of probability of SMU discharge (e.g., precision decomposition) - Mention the number of channels used for identification - Indicate if gradual changes in SMU identification template over time was allowed - Describe any constraints on acceptable data, such as maximal and minimal ISIs or discharge rates and maximal discharge variability - Report any manual inspection and editing performed on the results of automatic decomposition - List the method used to assess superpositions (Etawil & Stashuk, 1996; Marateb & McGill, 2009)

	- Mention the use of SMU spike train cross-correlation or similar methods to reduce the repeated identification of the same SMU - Indicate the use of accuracy indexes, such as Silhouette (SIL) threshold (Negro *et al.*, 2016a), pulse-to-noise ratio (PNR) (Holobar *et al.*, 2014), decompose-synthesize-decompose-compare (DSDC) (Nawab *et al.*, 2010) - Acknowledge any manual inspection and editing performed on the results of automatic decomposition - In case of long EMG recordings, report the length of the EMG epochs that were decomposed	
General principles for pre-processing of EMG signals for SMU identification (Recommendations)	- Remove channels that have excessive noise (i.e., signal noise should be no more than one half of the power of the signal (Del Vecchio *et al.*, 2020)) - A band-pass filter with corner frequencies at 10 and 500 Hz is recommended - The zero-phase filtering by second or higher order IIR notch filter with cut-off frequencies adjusted to the region (50 Hz: Europe, Asia, Pacific; or 60 Hz: USA) is recommended for monopolar recordings. When power line noise is substantial, higher harmonics can be also removed by decomposition software - Limit the duration of the decomposed signal to ≤100 s (for low fatiguing contractions) or shorter (for high fatiguing contractions). Due to changes in MUAP shapes over long time intervals, longer contractions should be decomposed as multiple overlapped segments followed by matching of SMU discharge times by cross	- If signals were recorded with a wide bandwidth to retain SMU architectural information, SMU detectability can often be enhanced by digitally high-pass filtering at 1 kHz prior to decomposition. - Limit the duration of the decomposed signal to ≤100 s (for low fatiguing contractions) or shorter (for high fatiguing contractions). Due to changes in MUAP shapes over long time intervals, longer contractions should be decomposed as multiple overlapped segments followed by matching of SMU discharge times by cross correlation across the epochs (Martinez-Valdes et al., 2020). - If updated MUAP templates were used to follow a SMU over time (long contractions), is important to confirm that this represents a gradual change in MUAP morphology rather than recruitment of a new unit.

	correlation across the epochs (Martinez-Valdes *et al.*, 2020). - If updated MUAP templates were used to follow a SMU over time (long contractions), this should be stated.	
General considerations regarding decomposition methods	**PROS** - Fast automatic decomposition - Spatial 2D MUAP representation allows the longitudinal tracking of individual SMUs when care is taken in placing the electrode across sessions (Martinez-Valdes *et al.*, 2017) - Spatial 2D maps show innervation areas and muscle fibre properties, such as conduction velocity in muscles with fascicles parallel to the skin - Up to tens of SMUs identified per contraction - Wide range of force magnitudes and conditions can be assessed	**PROS** - Most accurate EMG decomposition of MUAPs - Activity from deep and superficial SMUs can be detected - Real-time identification of MUAPs
General considerations regarding decomposition methods	**CONS** - Limited to superficial muscles and SMUs - Quality of the decomposition varies across participants and muscles - Fewer SMUs can be identified in muscles with fascicles parallel to the skin due to less spatially distinct waveforms (e.g., biceps brachii and vasti) - Difficult to assess accuracy of the decomposition - Automatic decomposition can add and miss ISIs - Decomposition algorithms can merge two different SMUs into one - Experienced operators are required to evaluate the ISIs - Signals recorded during strong contractions are difficult to decompose	**CONS** - Few SMUs can be identified (generally <10 per channel) - Generally limited to low-to-moderate force magnitudes - Signals recorded during strong contractions are difficult to decompose - Template-matching decomposition methods require extensive editing of ISIs - Visual inspection and editing of spike trains is time-consuming - Identification of multiple SMUs from these recordings is time consuming - MUAPs cannot be tracked across sessions

	- Visual inspection and editing of spike trains is time-consuming - Biased to subjects with low subcutaneous fat	
Contraction type used to identify motor units		
Submaximal isometric contractions	Yes. Explanation: Source separation techniques enable the reliable identification of SMU discharge times from low force magnitudes up to MVC in a wide range of isometric contractions (e.g., trapezoidal, triangular, or sinusoidal excitation profiles, fast and slow contractions).	Yes. Explanation: SMU identification with intramuscular electrodes is commonly performed during submaximal isometric contractions. Due to high selectivity, the number of identified SMUs is usually less than that obtained with surface grid electrodes, but the decomposed spike trains are usually more reliable than surface recordings. As these signals are decomposed with template-matching approaches from a single channel (or multiple selective channels), decomposition is commonly limited to low to moderate submaximal force magnitudes. Decomposition is possible at higher force magnitudes but requires extensive editing of SMU spike trains.
Submaximal isometric contraction until task failure	Caution. Explanation: Long contractions are difficult to decompose due to increases in SMU recruitment and changes in MUAP shapes. These contractions can be analysed either by decomposing different segments of the contractions and calculating the average population activity for each segment, or by decomposing overlapped segments and then matching discharge times belonging to the same SMU by cross correlation techniques (Martinez-Valdes *et al.*, 2020).	Caution. Explanation: As with surface electrodes, long contractions are difficult to decompose due to increases in SMU recruitment and changes in MUAP shape. More selective electrodes (needle) can help to follow the activity of a single SMU during this type of contraction. Nevertheless, it is difficult to control the position of needle. Wire electrodes can be taped with slack on the wire, allowing movement of the electrode with the muscle during the contraction and therefore, might be better suited to record submaximal fatiguing contractions. Nevertheless, as with HDEMG recordings, recruitment of new SMUs may impede the ability to follow a SMU continuously throughout the contraction.
Maximal isometric contractions	Caution. Explanation: It is difficult to discriminate among multiple SMU sources (e.g., different MUAP waveforms) during maximal contractions. However, it is possible in some muscles (e.g., tibialis anterior and gastrocnemius medialis) due to less spatially correlated recordings. Nevertheless, caution is required as it is difficult to test the accuracy of the	Caution. Explanation: The same limitations mentioned for surface electrodes apply for intramuscular electrodes during maximal contractions. The identification of SMU activity in this condition is extremely difficult with intramuscular electrodes. However, more selective recordings (e.g., needle, subcutaneous electrodes and quadrifilar electrodes) can isolate SMUs and follow their discharge times throughout the contraction. Discomfort and pain with solid-needle electrodes may limit the maximality of a contraction. Although wire electrodes are well tolerated during maximal isometric contractions, the integrity of wires inserted to deep muscles can be compromised at maximal force magnitudes.

	decomposition at these contraction intensities.	
Submaximal dynamic contractions	Caution. Explanation: The relative movement of the electrodes over the skin and changes in muscle length during dynamic contractions change MUAP shapes and compromise decomposition algorithms. New approaches based on blind-source-separation techniques (i.e., cyclostationary convolution-kernel-compensation (CKC) (Glaser & Holobar, 2019)) have been developed to compensate for changes in MUAP shape during shortening and lengthening contractions, and have been able to identify SMUs under these conditions. However, this technology requires more extensive testing.	Caution. Explanation: Even when intramuscular wire electrodes can move with the muscle during changes in length, MUAP shapes change, and this challenges template-matching methods. Although previous studies have only assessed SMUs during slow shortening and lengthening contractions over a limited range of motion (Pasquet *et al.*, 2006), discrimination of MUAPs during dynamic contractions is possible by adjusting templates for some tasks and muscles.
Maximal dynamic contractions	No. Explanation: Contractions at maximal intensities in both small and large ranges of motion are not currently possible due to the extensive recruitment of SMUs and high discharge rates along with large changes in MUAP shapes.	No. Explanation: Contractions at maximal intensities in both small and large ranges of motion are not currently possible due to the extensive recruitment of SMUs and high discharge rate along with the large changes in MUAP shapes.
Longitudinal motor unit tracking		
Real-time SMU tracking within a session	Caution. Explanation: Although blind-source separation methods (Convolution-Kernel-Compensation, CKC) have been used for real-time decomposition, these techniques require an offline calibration phase (contraction) to learn SMU filters.	Yes. Explanation: The selectivity of intramuscular and subcutaneous electrodes makes it possible to isolate the discharge times of a single SMU without the aid of any decomposition method. These discharge times can be visualized or heard in real time and the feedback can be used to control a contraction and detect the activity of a specific SMU in various conditions (e.g., fatiguing contractions, pain, or electrical stimulation). However, this approach requires participants to exert

	Afterwards, SMU filters can be applied to new EMG recordings to yield SMU discharge times (providing that the muscle geometry and position of electrodes have not changed). (Glaser *et al.*, 2013). Other methods are also being currently explored (Chen *et al.*, 2020; Wen *et al.*, 2021)	low force magnitudes (to record a single unit) or that the MUAP shapes clearly differ between units. Nevertheless, manual checking is required for a reliable result. Real-time SMU tracking is commonly used in clinical practice.
Tracking within a session (across different repetitions)	Yes. <u>Explanation:</u> When the recording conditions are kept constant in a session (e.g., similar target force magnitude and muscle length), decomposition of HDEMG signals can identify similar populations of SMUs across trials. When the same SMU needs to be identified at different target force magnitudes, then cross-correlation of the spatial 2D representation of MUAPs (or similar quantifications of SMU match between contractions) is recommended. (Martinez-Valdes *et al.*, 2017)	Yes. <u>Explanation:</u> It is possible to track the same SMU within a session with intramuscular and subcutaneous fine wire electrodes and with needle electrodes. However, it is not possible to track the same SMU across trials when intramuscular electrodes are repositioned
Across sessions	Yes. <u>Explanation:</u> HDEMG provides a 2D spatial sampling of the electrical activity of MUAPs. The large number of channels makes it possible to discriminate between different SMUs. The spatial distribution of each MUAP enables the longitudinal tracking of single SMUs in the absence of significant changes in muscle morphology or architecture (Del Vecchio *et al.*, 2019a). However, tracking accuracy of training interventions that last >4 wks or for neuromuscular diseases needs to be verified. Tracking accuracy increases with the number of channels. (Martinez-Valdes *et al.*, 2017)	No. <u>Explanation:</u> Due to high selectivity and the small recording area, it is almost impossible to detect the same SMU across sessions with intramuscular, subcutaneous, and needle electrodes. This limitation explains the high variability of intramuscular SMU recordings during longitudinal studies.
Analysis of decomposition results		

Details that should be reported following decomposition	- Number of SMUs identified per contraction and participant - Number of discarded SMUs and why they were discarded. Mention criteria used (see below). - SMU decomposition accuracy threshold (Pulse-to-noise ratio, Silhouette, two-source method, Decompose-Synthesize-Decompose-Compare). - If the discharge times were edited, mention how this was done and by whom - Report the number of SMUs and discharges that were edited - Report any limits on ISIs, such as removal of values below or above fixed thresholds. - In muscles with few synergists (e.g., tibialis anterior, first dorsal interosseous) show examples of common fluctuations in force and low-pass filtered discharge rates (when possible). - In longitudinal studies, report the consistency of the placement of the electrode grid (e.g., marking skin across sessions, transparent paper, consistency in participant's position).	- Number of SMUs identified per contraction and participant. - Number of discarded SMUs and why they were discarded. Mention criteria used (see below). - SMU decomposition accuracy (Inter-operator agreement, self-consistency, rotated signals, a posteriori accuracy assessment). - If the discharge times were edited, indicate how and by whom. - Report the number of SMUs and discharges that were edited - Indicate any limits on ISIs, such as removal of values below or above fixed thresholds. - In muscles with few synergists (e.g., tibialis anterior, first dorsal interosseous) show examples of common fluctuations in force and low-pass filtered discharge rates (when possible)
Recommendations following decomposition	- Quantifying accuracy * for convolution kernel compensation (CKC) a Pulse-to-noise ratio > 30 dB is recommended (Holobar *et al.*, 2014) * for convolutive blind-source separation a Silhouette > 0.9 is recommended (Negro *et al.*, 2016a) * for precision decomposition a Decompose-Synthesize-Decompose-Compare >95% is recommended (Nawab *et al.*, 2010) - Editing of erroneous ISIs is strongly recommended; however, it is important to	- Several methods for quantifying accuracy have been proposed, although none has so far gained universal acceptance. Among the intramuscular methods for decomposition accuracy we can find: *Inter-operator agreement: When semi-automatic or manual decomposition is used, two expert operators compare results and assess agreement between identified discharge times (Pilegaard *et al.*, 2000) *Rotated signals: The intramuscular signal and a time-rotated version of this signal are decomposed independently and the rate of agreement between the results is calculated (Zennaro *et al.*, 2002) *Self-consistency: MUAP train accuracy based on discharge time and shape consistency (Parsaei & Stashuk, 2013)

	consider the task performed (e.g., isometric or anisometric contraction), condition assessed (e.g., pain, fatigue) and the population under study (e.g., neuromuscular disorders, older adults). If possible, check ISI editing results with fluctuations in force to avoid deleting or adding discharges incorrectly as changes in discharge rate usually follow fluctuations in force. - Report how ISI editing was done and by whom (e.g., manually, semi-automatic, by one operator, or two blinded operators) - Report number/percentage of SMU discharges that were added/removed - Report the discharge characteristics of discarded SMUs - Show examples of the concurrent fluctuations in SMU discharge rates (single SMUs or cumulative spike train) and force (more evident at high force magnitudes). If possible, report the level of correlation between the associated fluctuations. - Observe and report if doublets are present (particularly during dynamic contractions) - Longitudinal tracking of SMUs requires high cross-correlation coefficient of 2D MUAP signatures (typically >0.80 for 64 EMG channels). When double matches are found, the SMU pair with the highest correlation coefficient should be selected. Nonetheless, an experienced operator should always visually inspect MUAPs to verify the match.	*A posteriori accuracy assessment: Bayesian framework analysis based on the estimated statistical properties of the MUAP trains and background noise that considers all the shape- and time-related information in the signal (McGill & Marateb, 2011) - It is recommended that at least one of these methods be employed to check decomposition accuracy. - Editing of erroneous ISIs is strongly recommended; however, it is important to consider the task performed (e.g., isometric or anisometric contraction), condition assessed (e.g., pain, fatigue) and the population under study (e.g., neuromuscular disorders, older adults). Check ISI editing with fluctuations in force to avoid deleting or adding discharges incorrectly. - Report how ISI editing was done (e.g., manually, semi-automatic, by one operator, or two blinded operators) and by whom. - Report number/percentage of SMU discharges that were added/removed - Report the discharge characteristics of discarded SMUs - Show examples of the concurrent fluctuations in SMU discharge rates and force (more evident at high force magnitudes). If possible, report the level of correlation between the associated fluctuations. - Observe and report if doublets are present (particularly during dynamic contractions)

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SMU discharge characteristics	Recruitment and derecruitment thresholds	Mean/average firing rate/discharge rate/rate coding	Discharge rate at recruitment and derecruitment	Peak discharge rate	Variability (SD interspike interval (ISI), coefficient of variation (CoV) for ISI, SD discharge rate, CoV for discharge rate)	Double discharges or doublets
Reporting SMU discharge characteristics	Report: - Force [%MVC, Newtons (N)] or torque [Nm] at which the SMU began and ended discharging action potentials repetitively [(discharge times separated by <200 ms (Farina *et al.*, 2009)]. - The rate of change in force/torque during the task in which the thresholds were measured - The contraction velocity and type (e.g., shortening/concentric or lengthening/eccentric) for dynamic contractions	Report: - The period over which the mean was calculated (e.g., ascending ramp, plateau) - The duration of the period over which the mean was estimated - If discharge rate was quantified directly from discharge times, ISIs, mean of inverse ISI (1/ISI) or from a smoothed signal. If the latter, report the filter or windowing used on the time-series of ISIs. - Median discharge rate with interquartile ranges (IQRs) when the data have a skewed distribution	Report: - The number of discharges or ISIs used in the calculation - If discharge rate was quantified directly from discharge times, ISIs, mean of inverse ISI (1/ISI) or from a smoothed signal. If the latter, report the filter or windowing used on the time-series of ISIs - Median discharge rate at recruitment/derecruitment with interquartile ranges (IQRs) when the data have a skewed distribution	Report: - The number of discharges or ISIs used in the calculation - The period over which peak discharge rate was calculated (e.g., peak force signal) - If peak discharge rate was quantified directly from discharge times, ISIs, mean of inverse ISI (1/ISI) or from a smoothed signal. If the latter, report the filter or windowing used on the time-series of ISIs.	Report: - The period over which variability was calculated (e.g., ascending ramp, plateau) - The duration of the period over which mean variability was estimated - If variability was quantified directly from discharge times, ISIs, mean of inverse ISI (1/ISI) or from a smoothed signal. If the latter, report the filter or windowing used on the time-series of ISIs. - Provide information on how coefficient of variation for discharge rate/ISI was calculated (i.e. CoV for ISI = (SD for ISI / mean ISI) x 100), SD of DR =	ISI for doublets has been usually defined as 2.5–20 ms. However, it has been recently suggested that doublets need to be defined as ISIs that are significantly shorter than the mean ISI for a given motoneuron (McManus *et al.*, 2021) - Report when they occur, the number of doublets observed, and consistency across repetitions

					SQRT [(SD of ISI)2 / (mean ISI)3] - Interquartile ranges (IQRs) of ISI when the data have a skewed distribution	
SMU discharge characteristics, recommendations	- Exclude ISIs > 200 ms when estimating recruitment and derecruitment thresholds (Farina *et al.*, 2009)	- Calculate discharge rate during a sustained steady contraction (i.e., where force magnitude or muscle activity (EMG) are relatively constant) - Before smoothing, resample ISI time series to a constant sampling period (ISIs are calculated at SMU discharge times, therefore their sampling frequency varies in time)(Berger *et al.*, 1986) - Report discharge rate as median and IQR in conditions where the data have a skewed distribution	- Use the first or the last few discharges [e.g., 6 (Farina *et al.*, 2009)] or ISIs to determine discharge rate at recruitment and derecruitment - Exclude ISIs >200 ms (Farina *et al.*, 2009) - Calculate discharge rate at recruitment/derecruitment as median and IQR in conditions where the data have a skewed distribution	- Use gradual ramp-contractions or brief fast contractions to measure peak discharge rate - It can be quantified as the average rate over ≤ 6 discharges or as the average of the 5 shortest ISIs or estimated from a function fitted to the ISIs (Farina *et al.*, 2009) - Calculate peak discharge rate as median and IQR in conditions where the data have a skewed distribution	- Requires high decomposition accuracy (>90% sensitivity), with edited ISI trains. - Calculate discharge rate variability during a sustained steady contraction when force magnitude or muscle activity (EMG) are relatively constant - Calculate discharge rate variability as IQR in conditions where the data have a skewed distribution	- It is recommended to examine for the presence of doublets when there are large variations in force magnitude or EMG activity (i.e., fast contractions with steep increases in force magnitude). However, it is important to note that doublets might still occur during sustained contractions (Sogaard *et al.*, 2001). Therefore, caution is required when editing spike trains to avoid eliminating physiological doublets.

Table 4. Measures of association between single motor unit discharge times

Measures of association between SMU discharge times	Short-term synchronization	Common drive	Coherence
General principles (definitions)	A tendency for two or more SMUs to discharge together or within a few milliseconds of one another, with a rate of occurrence above that expected due to chance. Assessed by cross-correlation peak widths of ≤10 ms between spike trains of two simultaneously recorded SMUs (Sears and Stagg, 1976; Kirkwood et al., 1982). Measured in the time domain.	Concurrent fluctuations in discharge rate between pairs of SMUs over time. Measured in the time domain.	linear association between the discharge times of pairs or populations of SMUs. Measured in the frequency domain and calculated with the magnitude squared coherence estimate, which is the square of the absolute value of the cross-spectrum of two signals (i.e., discharge times of a pair of SMUs or cumulative spike train of two groups of SMUs) divided by the power in each spectrum.
Reporting of measures of association	- Show exemplary cross-correlograms and the associated cumulative sum (CUSUM) - Show where the CUSUM derivative trace exceeds 10 and 90% of the difference between its maximal and minimal values. Histogram bins within this region represent synchronous discharge times. *Synchronization indexes: - Common-input strength (CIS) index (Nordstrom *et al.*, 1992); the number of extra counts in the synchronous peak above that expected due to chance, normalized to the duration of the trial. - K' index (Sears & Stagg, 1976); ratio of the number of synchronous spikes relative to the number expected by chance divided by the average count in the peak region relative to the off-peak region. - E index (Datta *et al.*, 1991); number of extra counts within the peak above that expected	- Report the filter used to smooth the ISI trains and procedure used for ISI resampling to a constant sampling frequency before smoothing. - Report cross-correlation value [(Common drive index (De Luca & Erim, 1994)] of each motor unit pair with the largest correlation coefficient within ± 100 ms of zero lag.	- Report the number of SMUs used to calculate coherence (e.g., pairs, cumulative spike train) and their average discharge rates - Indicate the method used to calculate coherence [e.g., integral of specific coherence in each frequency band (McManus *et al.*, 2016)] - State the windows used (duration, type and overlap) to estimate coherence - Show examples of coherence spectra with the 95% confidence interval - Report statistical method used to indicate significance of coherence (Negro & Farina, 2012).

	due to chance relative to the total number of reference unit discharges. - Synchronization index (De Luca *et al.*, 1993); which uses first order recurrence times (assesses the nearest forward and backward discharge times) to avoid secondary peaks.		
Recommendations for measures of association	- Binary conversion of discharge times (assigning to each sample of recording either a 1 when a spike occurred or 0 when a spike did not occur) with 1 sample resolution - Generate cross-correlation histogram with bin size = 1 ms, lags ± 100 ms. - Identify peak region using the CUSUM derivative - Mean and SD of the off-peak bin counts (region outside ±40 ms range) as these discharge times are usually attributed to chance.	- Binary conversion of discharge times (assigning to each sample of recording either a 1 when a spike occurred or 0 when a spike did not occur) with 1 sample resolution. - SMU spike trains are typically convolved with a 400 ms Hann window and then high-pass filtered at 0.75 Hz.	- Binary conversion of discharge times (assigning to each sample of recording either a 1 when a spike occurred or 0 when a spike did not occur) with 1 sample resolution. - Use a large number of SMUs and calculate pooled coherence (compare all possible pairs) (Amjad *et al.*, 1997) or combine discharge times from multiple MUs before estimating coherence. - Significance thresholds should be defined and applied. - Use the same number of SMUs when comparing across conditions. -Coherence values should be normalized prior to making comparisons (since coherence has a skewed sampling distribution), therefore: 1) Convert coherence values into Fisher's Z-values (Fz), formula: $Fz = \operatorname{atanh}\sqrt{(c)}$, where c is coherence. 2) Transform Z-values into Z-scores $Z = Fz/\sqrt{(1/2L)}$, where L is the number of time segments used in the coherence analysis. 3) Remove inherent bias of each coherence profile by subtracting the maximal coherence value for frequencies >100 Hz.

General considerations for measures of association	**PROS** - Only one pair of SMUs per muscle is required to calculate short-term synchronization, however, estimates may vary across different SMU pairs (caution).	**PROS** - Only one pair of SMUs per muscle is required to calculate the common drive index, however, estimates may vary across different SMU pairs (caution).	**PROS** - Provides information about linear dependency between a pair or a group of SMUs in the delta (0.1-4 Hz), alpha (8-13 Hz), beta (14-30 Hz), and gamma (>30-80 Hz) bands, which are believed to be related to specific sources of modulation (Babiloni *et al.*, 2020).
General considerations for measures of association	**CONS** - The magnitude of correlation that can be estimated from the discharge times of two motor neurons depends on the frequency content of the synaptic input and the sampling/discharge rate. Therefore, the indexes are biased by average discharge rate (even when normalized). - Correlation estimates are confounded by discharge rate variability. - Correlation of SMU pairs provide low levels of correlation due to non-linearity of single SMU activity (undersampling of population activity). - Different indexes estimate short-term synchronization in different ways. - There is high variability among indexes of short-term synchronization calculated from different SMU pairs.	**CONS** - As with short-term synchronization, CDI compares common fluctuation for pairs of SMUs, therefore, correlation values tend to be small and not representative of the population. - The length of the filter (e.g., Hann window of 150 or 400 ms) influences the level of correlation between SMUs. - This index shows high variability across different SMU pairs.	**CONS** - Estimates of coherence are influenced by the number of SMUs used for the calculation (up to a saturation point). - Coherence measures derived from one pair of SMUs are not representative of the population. - Average coherence in different bandwidths can be influenced by discharge rate, but less than for the indexes of short-term synchronization.

Table 5. Single motor unit peripheral properties and single motor unit action potential amplitude

Peripheral SMU properties estimated with grid surface EMG electrodes	
Considerations for the measurement of SMU territories	The discharge times from individual SMUs can be used to trigger surface EMG signals (spike-triggered averaging technique) to estimate the 2D spatial representation of MUAPs and thereby assess the location of innervation zones, the orientation of muscle fascicles, and indirectly assess SMU territory. Moving plots (videos) showing spatial distribution of SMU activity over time, can help to visualize propagation of MUAPs along the fascicles. **Report** - Anatomical landmarks to denote the location of the grid electrode. - The use of dry linear arrays prior to placing the grid electrode. - Spatial filter used to visualize innervation maps. - The use of intramuscular EMG in combination with surface EMG. If both methods were combined, report the technique that was employed to identify MUAPs (e.g., spike-triggered averaging).
	Recommendations - Visualize MUAP propagation with dry linear arrays (single differential configuration) prior to placement of grid electrode - Align grid electrode in the direction of the muscle fascicles (i.e., with rows or columns)
	Caution - This method cannot assess actual 3D SMU size. - This method could be potentially used to estimate SMU cross-sectional diameter or length, but caution is required.
Considerations for the measurement of SMU conduction velocity	Following SMU decomposition, discharge times from individual SMUs can be used to trigger surface EMG signals via spike triggered averaging. The 2D spatial representation of MUAPs from HDEMG grid electrode can be used to quantify MUAP propagation speed along the muscle fibres. **Report** - Interelectrode distance, size and electrode location. - Technique used to calculate conduction velocity (e.g., time domain, frequency domain, see (Farina & Merletti, 2004) for review). - Spatial filter used to calculate conduction velocity (i.e., single or double differential). - Cross-correlation value between channels. - Number of channels used to calculate conduction velocity.
	Recommendations - SMU conduction velocity can be only reliably estimated from muscles with fascicles that run parallel to the skin (e.g., vastus medialis, biceps brachii). - Use ≥3 double-differential channels to estimate conduction velocity to reduce the variability of the estimation (Farina *et al.*, 2002) - Cross correlation coefficient of MUAPs across all channels should be reported. - The same columns/rows should be selected for repeated measurements across different testing sessions as conduction velocity estimates can vary across the electrode grid.

	Caution - The estimation of muscle fibre/motor unit size/recruitment with this method requires caution as several experimental conditions can alter conduction velocity without any changes in muscle fibre size. - The accuracy of motor unit conduction velocity estimates decreases with SMU depth. - Non-aligned fascicles can bias this estimate. - Discard motor units with conduction velocity estimates <2 m/s or >8 m/s as they are not physiological (Beretta-Piccoli *et al.*, 2019).
Estimation of MUAP amplitude	
General considerations	MUAP amplitude has been used to infer SMU size (i.e., lower-threshold SMUs may have lower MUAP amplitude compared to higher-threshold SMUs), but the variability is substantial. MUAP amplitude can be quantified with both grid surface electrodes and intramuscular recordings. Common measures include peak-to-peak amplitude, root-mean-square, and area. **Report** - Recording mode (e.g., monopolar, single-, or double-differential) used to measure MUAP amplitude. - The number of channels in the measurement (i.e., full electrode grid, single column/row). - Mention if SMU discharge times obtained from intramuscular or HDEMG recordings were used to trigger surface EMG signals (spike-triggered averaging (Kakuda *et al.*, 1991).
	Caution - Estimates of MUAP amplitude are influenced by the distance from the SMU to the recording electrode (intramuscular or HDEMG). - MUAP amplitude estimates are also modulated by inter-electrode distance, muscle architecture, subcutaneous tissue thickness, among other factors [see (Farina *et al.*, 2004) for a review]. Therefore, comparison across subjects and muscles requires caution (Martinez-Valdes *et al.*, 2018). - The estimation of SMU size from measures of MUAP amplitude is not generally recommended.

Abbreviations and definitions

CDI: Common drive index

CIS: Common input strength

CKC: convolution kernel compensation

CUSUM: Cumulative sum

DSDC: Decompose-synthesise-decompose-compare

ISI: inter-spike interval.

SMU: single motor unit

MUAP: motor unit action potential

MVC: maximum voluntary contraction

SIL: Silhouette threshold.

PNR: Pulse to noise ratio.

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APPENDIX:

Appendix 1. Delphi rating scores (for both rounds 1 and 2). Each cell provides the median score and (in parenthesis) IQR in first row, then % and absolute frequency of appropriate (scores 7–9) followed by inappropriate (scores 1–3) in second row.

SMU recordings matrix items	**R**	**Rating scores – Median (IQR); % appropriate (n), % inappropriate (n)**		
Electrode type		***Surface grid of electrodes***	***Intramuscular fine-wire electrode***	***Intramuscular needle electrode***
Electrode design reporting	1	8 (1.8) 78.6 (11), 0 (0)	8 (0.8) 92.9 (13), 0 (0)	8 (0.8) 92.9 (13), 0 (0)
Electrode design recommendations	1	8.5 (1) 85.7 (12), 0 (0)	8 (1) 78.6 (11), 0 (0)	8 (2) 100 (14), 0 (0)
General principles for reporting on SMU recording procedures	1	8 (1.8) 71.4 (10), 0 (0)	9 (1) 100 (14), 0 (0)	8.5 (1.8) 85.7 (12), 0 (0)
General principles for recording single motor unit activity (Recommendations)	1	9 (2) 78.6 (11), 0 (0)	8.5 (1.8) 85.7 (12), 7.1 (1)	8 (1) 78.6 (11), 7.1 (1)
	2	8 (1) 92.9 (13), 7.1 (1)	8 (1) 85.7 (12), 7.1 (1)	8 (2) 85.7 (12), 7.1 (1)
PROS	1	8 (1.8) 92.9 (13), 0 (0)	8.5 (1) 92.9 (13), 0 (0)	8 (1) 100 (14), 0 (0)
CONS	1	8.5 (1.8) 100 (14), 0 (0)	**8 (2.8)** **64.3 (9), 21.4 (3)**	8 (2) 78.6 (11), 14.3 (2)
	2	9 (0.5) 100 (14), 0 (0)	8 (1) 100 (14), 0 (0)	8.5 (1) 100 (14), 0 (0)
MU decomposition techniques		***HDsEMG MU decomposition techniques***	***Intramuscular EMG MU decomposition techniques***	
General principles for processing of EMG signals for MU identification (Reporting)	1	8 (1) 92.9 (13), 0 (0)	8 (1) 92.9 (13), 0 (0)	
General principles for pre-processing of EMG signals for MU identification (Recommendations)	1	8 (2) 78.6 (11), 0 (0)	8.5 (1.8) 85.7 (12), 0 (0)	
PROS	1	9 (1) 92.9 (13), 0 (0)	9 (1) 100 (14), 0 (0)	
CONS	1	7 (1) 78.6 (11), 0 (0)	7.5 (1.8) 78.6 (11), 0 (0)	
Contraction type used to identify MUs		***HDsEMG MU decomposition techniques***	***Intramuscular EMG MU decomposition techniques***	
Submaximal isometric contractions	1	9 (1) 92.9 (13), 0 (0)	9 (1) 78.6 (11), 0 (0)	
Submaximal isometric contraction until task failure	1	9 (1) 92.9 (13), 0 (0)	8.5 (1.8) 85.7 (12), 1 (7.1)	
Maximal isometric contractions	1	9 (1) 100 (14), 0 (0)	9 (2) 85.7 (12), 0 (0)	
Submaximal dynamic contractions	1	9 (1.8) 92.9 (13), 7.1 (1)	8.5 (2) 92.9 (13), 0 (0)	
Maximal dynamic contractions	1	9 (0) 100 (14), 0 (0)	9 (0) 100 (14), 0 (0)	
Longitudinal MU tracking		***HDsEMG MU decomposition techniques***	***Intramuscular EMG MU decomposition techniques***	
Real-time SMU tracking within a session	1	8 (1) 100 (14), 0 (0)	8.5 (2) 85.7 (12), 0 (0)	
Tracking within a session (across different repetitions)	1	9 (1) 100 (14), 0 (0)	9 (0.8) 100 (14), 0 (0)	

Across sessions	1	9 (1.8) 92.9 (13), 7.1 (1)		9 (0.8) 100 (14), 0 (0)			
Analysis of decomposition results		***HDsEMG MU decomposition techniques***		***Intramuscular EMG MU decomposition techniques***			
Details that should be reported following decomposition	1	8 (1.8) 100 (14), 0 (0)		8 (2) 92.9 (13), 0 (0)			
Recommendations following decomposition	1	8 (2) 85.7 (12), 0 (0)		8 (2) 92.9 (13), 0 (0)			
	2	8 (1.8) 100 (14), 0 (0)		8 (1.8) 100 (14), 0 (0)			
MU discharge characteristics		***Recruit. and de-recruit. Thresh.***	***Mean firing rate /discharge rate***	***Discharge rates at recruit. and de-recruit.***	***Peak DR***	***Variability***	***Double discharges or doublets***
Reporting MU discharge characteristics	1	8 (1.5) 78.6, 0	8 (1) 78.6, 0	8.5 (1.8) 92.9, 0	8 (1) 92.9, 0	9 (1.8) 78.6, 0	8 (2.8) 91.4, 0
	2	9 (1) 92.9, 0	8 (1) 100, 0	8.5 (1) 92.9, 0	8 (1) 92.9, 0	8 (1.8) 92.9, 0	8 (2) 92.9, 7.1
MU discharge characteristics (Recommendations)	1	8 (1) 92.9, 0	8.5 (1.8) 85.7, 0	8.5 (1.8) 92.9, 0	8.5 (1.8) 78.6, 7.1	9 (1) 78.6, 7.1	8 (1.8) 78.6, 7.1
	2	9 (1) 100, 0	9 (1) 100, 0	9 (0.8) 100, 0	9 (1) 100, 0	9 (1) 100, 0	9.5 (1.8) 92.9, 0
Measures of correlation between MU discharge times		***Short-term synchronization***		***Common drive***		***Coherence***	
General principles (definitions)	1	8.5 (1.8) 85.7 (12), 0 (0)		8.5 (1) 85.7 (12), 0 (0)		9 (1) 92.9 (13), 0 (0)	
Reporting of correlation measures	1	8.5 (1) 85.7 (12), 0 (0)		8 (1) 92.9 (13), 0 (0)		8.5 (1.8) 85.7 (12), 0 (0)	
Recommendations for measures of correlation	1	8 (1) 78.6 (11), 0 (0)		8 (1.8) 85.7 (12), 0 (0)		8 (2) 92.9 (13), 0 (0)	
PROS	1	8.5 (1) 85.7 (12), 0 (0)		8.5 (1) 85.7 (12), 0 (0)		8.5 (1) 92.9 (13), 0 (0)	
CONS	1	8 (2) 100 (14), 0 (0)		8.5 (1.8) 92.9 (13), 0 (0)		8 (1) 92.9 (13), 0 (0)	
Peripheral MU properties estimated with grid surface EMG electrodes							
Considerations for the measurement of MU territories – Report	1	9 (1) 100 (14), 0 (0)					
Recommendations	1	9 (1) 100 (14), 0 (0)					
Caution	1	8 (1.8) 100 (14), 0 (0)					
Considerations for the measurement of MU conduction velocity – Report	1	9 (1.8) 92.9 (13), 0 (0)					
Recommendations	1	8 (2) 78.6 (11), 0 (0)					
Caution	1	8 (2) 78.6 (11), 7.1 (1)					
Estimation of MUAP amplitude							
General considerations – Report	1	8 (1.8) 92.9 (13), 0 (0)					
	2	8 (1.8) 78.6 (11), 0 (0)					
Caution	1	7.5 (2.8) 71.4 (10), 0 (0)					
	2	8.5 (1) 100 (14), 0 (0)					

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