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A Scoping Review of Current and Emerging Techniques for Evaluation of Peripheral Nerve Health, Degeneration, and Regeneration: Part 1, Neurophysiology

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Abstract

Peripheral neuroregeneration research and therapeutic options are expanding exponentially. With this expansion comes an increasing need to reliably evaluate and quantify nerve health. Valid and responsive measures that can serve as biomarkers of the nerve status are essential for bot h clinical and research purposes for diagnosis, longitudinal follow-up, and monitoring the impact of any intervention. Furthermore, such biomarkers can elucidate regeneration mechanisms and open new avenues for research. Without these measures, clinical decision-making falls short, and research becomes more costly, time-consuming, and sometimes infeasible. As a companion to Part 2, which is focused on non-invasive imaging, Part 1 of this two-part scoping review systematically identifies and critically examines many current and emerging neurophysiological techniques that have the potential to evaluate peripheral nerve health, particularly from the perspective of regenerative therapies and research.

Keywords: Nerve regeneration, Peripheral nerve imaging, Muscle imaging, Quantitative, MR Neurography, Neuromuscular Ultrasound

Introduction

Pathology of peripheral nerves has a high lifetime prevalence and affects approximately 2.5% of the population, resulting in enormous societal morbidity and mortality[1,2]. The pipeline of therapies designed to promote peripheral reinnervation or slow degeneration in a multitude of conditions is expanding dramatically. This is typified by the explosion in Amyotrophic Lateral Sclerosis (ALS) trials to slow or reverse degeneration, immune therapies in inflammatory conditions, and the numerous therapeutic avenues in peripheral nerve injury including stimulation, stem cells, growth factors, and varied surgical techniques[3–7]. This context demands a re-evaluation of standard clinical and pre-clinical measurements of nerve health and the innovation of measures that can serve as novel biomarkers. However, choosing such measures is often not obvious.

The quality of peripheral neuronal measurement profoundly impacts the discovery and translation of neuroregenerative therapies. It is fundamental in study design and even small improvements in a biomarker can allow previously infeasible studies to commence. The extent to which research is shelved, delayed, or fails due to the lack of practical and high-quality metrics is unknown but may be substantial. Additionally, the increased mechanistic understanding that comes with the information provided by such measures can itself generate new therapeutic concepts and lines of research.

The objective of this scoping review is twofold: first, to create a compendium of neurophysiological techniques that currently provide, or have a reasonable potential to provide, valuable insights into nerve health, both in the clinical and research settings; and second, to comment on the underlying mechanisms, clinimetrics[8] (if available), and practical considerations for implementing these techniques in practice. The suitability and efficacy of a specific measure greatly depend on the context of its application, from basic science research through to clinical practice; as such, the intent of this paper is not to account for the wide array of potential goals or testing environments to conclude which test is best. While it's impractical to cover every aspect of appropriateness and performance in each situation, by synthesizing and evaluating available information on these techniques, this review aims to contribute to a better understanding of how they can be effectively used to inform diagnosis, treatment, and research design related to nerve health, as well as to highlight potentially fruitful and emerging avenues of basic and translational science. The breadth of scope necessarily limits the depth of analysis, but provides the necessary foundation from where more focused, valid, and directed investigations can be made. Nevertheless, when deciding on biomarkers of nerve health, the entire range of nerve evaluation approaches must be considered in conjunction with, or in place of, neurophysiology and/or imaging, including histological and lab-based assays, clinical examinations, patient reported outcomes, survival, and behavior[9].

Any metric of nerve health aims to approximate the true state of the nerve or a current gold standard measurement. The state of a nerve (its health) can be categorized in terms of histological structural abnormalities and how well it functions physiologically. Although beyond the scope of this review, an understanding of these areas is critical when deciding upon appropriate diagnostic measures or biomarkers and there are numerous excellent reviews that cover these areas in detail[10,11]. For the purposes of this review, peripheral nerves are considered to either project, or reside entirely, outside of the brain and spinal cord.

We focus heavily on the peripheral motor domain, including muscle measures, for a few reasons: over the decades, better tools have been developed to study the motor system more than sensory or autonomic systems; consequently, the motor system has been by far the most studied and so takes up a greater proportion of the review; and, at present, functional recovery is usually concerned more with motor function (notwithstanding the importance of sensory function and pain in overall recovery). Nevertheless, we reference several important peripheral sensory nervous system techniques, both for small and large fibers, given their certain unique features pertinent to nerve health.

Many of the techniques are currently in use and discussed first, but many reside in the emerging pipeline including some that are barely nascent but with truly exciting promise. After describing the methodology, we critically assess standard neurophysiological techniques before addressing an array of more quantitative and "emerging" approaches identified with the aid of a structured scoping review of the literature.

Methods

We carried out a scoping review of the literature in PubMed, Embase, Web of Science, and Google Scholar[12] to answer the question "what neurophysiological techniques currently assess peripheral nerve health in clinical and research practice, and what are the techniques that show promise?", generated using the PCC method, as feasible (Population, Concept, Context). Population is not relevant here, but the concept is that of electrophysiological assessment of nerve, and the context includes potential, performance, and practicality of applying the technology to nerve assessment. Because some techniques may not have yet been applied to peripheral nerve, and the objective is to capture techniques that have the potential to evaluate nerve health via a search strategy with high sensitivity, a formal systematic review of literature alone would fall short of the stated goal. In line with PRISMA-ScR[13,14] and with aid from librarians, the search strategy aimed to be as comprehensive as possible within the constraints of time and resources to identify both published and gray literature sources of evidence. An initial limited search was performed on PubMed with an analysis of the title, abstract, and associated index terms. This was augmented by a semi-structured iterative discussion among authors and colleagues resulting in a separate list for inclusion of current and emerging or potential techniques, which was merged with results of the initial limited PubMed search. A second search used these identified words and terms across all 4 databases. A Google Scholar search supplemented the 3 literature databases and involved screening of the first 400 records. The final process involved reviewing the references of selected articles for further relevant sources. There were no restrictions on language, dates, or article type. Search terms related to neurophysiology (such as electromyography, action potential, electromagnetic) were connected via "AND" Boolean statements to terms related to nerve health (such as injury, regeneration, denervation, neuropathy). This was refined using "NOT" statements to increase specificity and reduce the result numbers while maintaining search sensitivity. Terms included in the "NOT" statement were also collected during the initial limited PubMed search (generally referring to unrelated disorders and anatomy). Any technology that can detect electromagnetic signals from, or associated with, nerve was deemed eligible for further consideration. We included evaluation of muscle as a surrogate for nerve health due to its tight relationship with motor axons. Standard neurophysiology techniques were excluded but discussed in the review with reference to established textbooks; however, novel implementation of standard techniques were included in the search results. As mentioned, this review predominantly focuses on large fiber and motor nerve but incorporates and recognizes the importance of sensory and small fiber nerve. The results from the final search were

grouped into technology categories for inclusion and critical assessment in the discussion. Articles within each technology grouping were then discussed and selected based on the importance of their contribution to our understanding of the performance characteristics, practicality, and underlying mechanisms of each technique. Study authors represent a broad collaboration who draw upon decades of cumulative experience in nerve health within the varied fields of neurophysiology, neurosurgery, neurology, neuroscience, and engineering. The final search was completed in March 2023 with Covidence and Zotero citation software, as well as the individual online databases, used to store and process search results and articles. Risk of bias within the review was mitigated through the application of broad search criteria, a priori search protocol, and involvement of cross-disciplinary and multi-institutional authors; although, some subjectivity in assessment will be unavoidable.

Results

The results of the scoping search and categories of technology are detailed below in the flow diagram (Fig 1). Searching in the 4 databases returned 1,122 texts. Titles and abstracts of all texts were reviewed by authors, as well as the references of those selected as most informative about performance characteristics and mechanisms. This resulted in 702 texts deemed relevant for the relationship between electrophysiology and nerve health. For ease of analysis and focused repeat searches, these 702 texts were grouped into 9 main categories including, 1) novel uses of standard or classical quantitative neurophysiology (including techniques such as motor unit estimation and single fiber EMG), 2) surface electromyography and mechanomyography, 3) electrical Bioimpedance, 4) microneurography, 5) excitability testing, 6) ultrasound and ultrasound stimulation, 7) magnetic, or MRI, stimulation and magnetoneurography, 8) biointerfaces and electrodes, 9) intraoperative neurophysiology and evoked potentials. Authors discussed and critically evaluated the articles within each category and selected those most relevant to performance characteristics, mechanisms, and practicality to be included for discussion and referencing in the review.

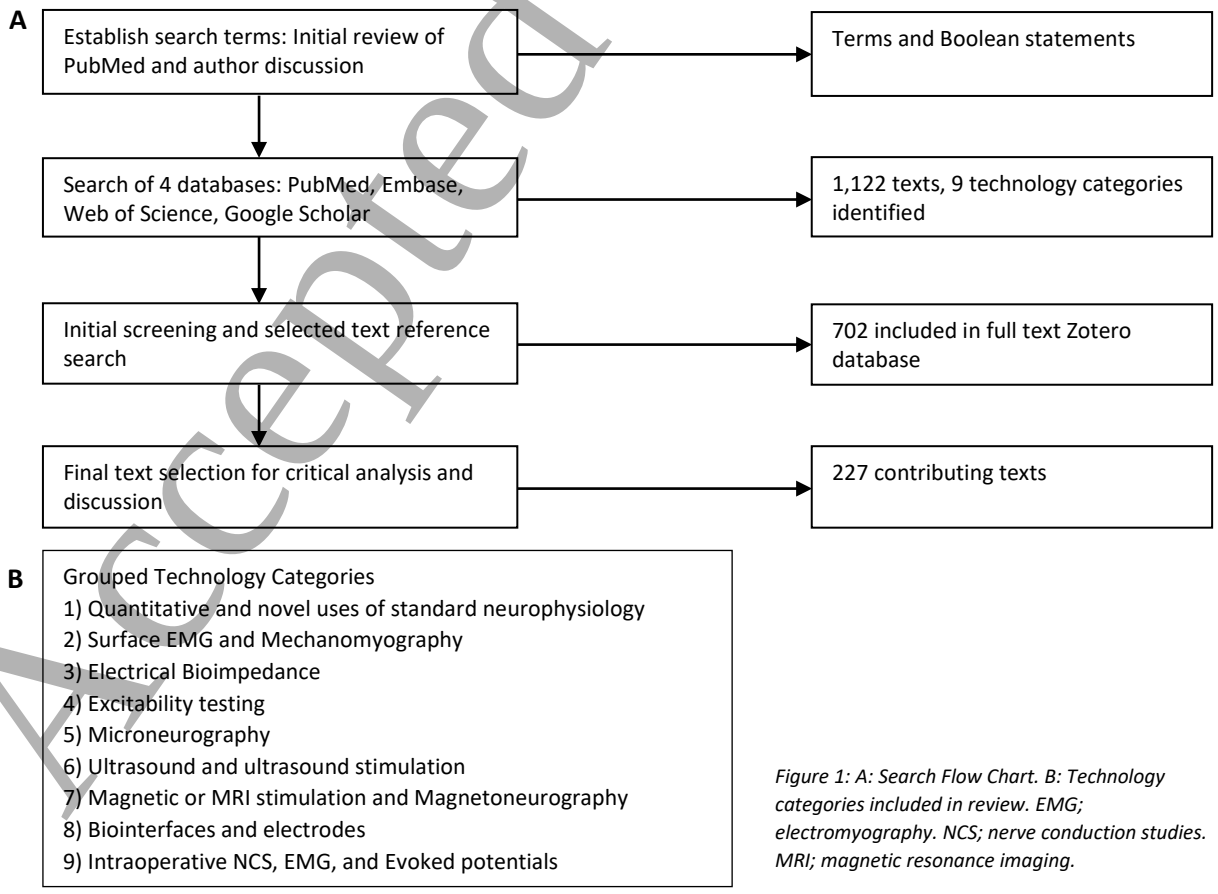

Figure 1: A: Search Flow Chart. B: Technology categories included in review. EMG; electromyography. NCS; nerve conduction studies. MRI; magnetic resonance imaging.

1 Neurophysiology

The measurement of axonal content and neuronal functioning over time is a task for which neurophysiology as a field should be ideally suited. However, standard nerve conduction studies and electromyography were predominantly developed to diagnose the category of pathology (muscle or nerve), and to a lesser extent to comment on severity, location, and chronology[15]. Augmentation of standard techniques is required through quantitation and novel approaches to recording and stimulating.

Many excellent reviews examine the benefit, limitations, and more common quantitative methods of standard nerve conduction and electromyography in the assessment of neuromuscular disease and less often nerve injury specifically[16–18]. We briefly summarize and critically examine current techniques before extending the analysis to include more advanced approaches identified within the results of the scoping review process that show promise as biomarkers of nerve health, degeneration, and regeneration.

1.1 Standard Nerve Conduction Studies (NCS)

NCS have been standardized and validated over many years[10,16,19] and involve stimulation of myelinated alpha-motor and large sensory axons and extracellularly recording from muscle or nerve . The information obtained allows inferences to be made regarding axonal loss or dysfunction. Stimulating all motor axons in a nerve and subsequently recording the grouped electrophysiological response from the muscle fibers of its respective motor units is termed a compound muscle action potential (CMAP). Similarly, stimulating all axons in a nerve and subsequently recording the grouped electrophysiological response of its individual axons is termed a compound nerve action potential (CNAP). Both measures are important quantitative metrics upon which many approaches are based. To record these extracellular potentials, typically, a variety of surface electrodes are used in human studies, while monopolar needles tend to be more practical in animal studies, given their smaller size; however, alternate electrodes are discussed below in a separate section.

Fortunately, muscle conveniently acts as a 'megaphone' for motor axons because each axon innervates hundreds of muscle fibers whose depolarizations make up the CMAP, allowing us to examine the motor system somewhat more easily, with CMAPs being approximately 100-1000-fold larger than CNAPs[10,19]. Characteristics of the response, such as latency, amplitude, and shape bare a relationship with the numbers and functioning of axons. In the context of regeneration, it is of particular interest that the amplitude of a CMAP and CNAP provide quantitative information relating to the number of axons within the nerve, with varying levels of accuracy depending on context, described in several renowned neurophysiology texts[20–22]. This quantitative aspect of even standard nerve conduction studies contrasts with standard EMG, which is broadly a more subjective and focal assessment.

1.1.1 Late Potentials

During standard motor NCS, action potentials spread proximally and distally. Distally, this activates the muscle but proximally, in the spinal cord, several motor neurons may respond by each generating a new axonal action potential that travels to the same muscle which in turn leads to the generation of muscle fiber action potentials along the fibers of each activated motor unit. These consequently contribute to the recorded, delayed extracellular potential, termed an F response (F is for foot, where it was originally noted). This characteristic can be exploited to examine the integrity of the entire motor nerve and

comment, to a degree, on proximal conduction block. F responses are used in one form of quantitative motor unit number estimate (MUNE) described later but are otherwise mainly used for detecting the presence of proximal pathology.

Reflex studies are delayed motor responses measured after stimulation of sensory axons. The soleus H-reflex (H is for Hoffman) is the most performed of these and represents the electrical equivalent of the ankle jerk. Action potentials received at the dorsal horn result in depolarization of anterior horn motor neurons and a subsequent contraction of the soleus muscle. As with F waves, the H-reflex can comment on proximal pathology to a degree and is generally used as a marker for detecting large fiber polyneuropathies, radiculopathies, and other proximal pathology[16], with less obvious application in the assessment of degeneration and regeneration.

1.1.2 Limitations of Standard NCS

Perhaps the main flaw of standard NCS is that only a relatively few, easily accessible, and isolated muscles and nerves can be examined due to a general reliance on surface stimulation and recording. The poor coverage of proximal and deep nerves has long been tolerated as clinical decision making was often not impacted significantly and the solution was complex, typically requiring invasive efforts. However, with the advent of promising neuroregenerative therapies, more targeted measurement has become increasingly pressing.

Conduction block can be difficult to demonstrate with standard NCS if the location is too proximal or distal. If distal to the site of stimulation, conduction block can appear as axon loss, in which case motor point or direct muscle stimulation that bypasses the nerve can be informative. On the other hand, if proximal to the site of stimulation, block can be hard to detect. In this case, we rely on a disproportionately large CMAP compared to strength, and subjective EMG recruitment patterns. F waves and reflex studies can evaluate for proximal block to a degree; however, quantification is not possible, it is insensitive, and few nerves are amenable to study in this way.

It is important to note that the validity of the CMAP as a motor axonal measure suffers because of physiological effects of collateral reinnervation, which can obscure the effect of axonal loss[16,23,24]. Furthermore, diffuse endplate regions and greater muscle fiber distance from the recording electrode in large muscles, as well as effects of pathology of the neuromuscular junction or muscle, contribute to obscuring the relationship between the CMAP and the underlying health of corresponding motor axons[25]. On the other hand, CNAP amplitude, if feasible, is directly dependent on the number of axons with a diameter greater than 9 µm[26].

1.2 Evoked Potentials

Somatosensory evoked potentials (SSEPs) are conceptually like nerve conduction studies but carried out over a long path that includes central and peripheral pathways[27]. Responses are recorded over the peripheral nerve, trunk, and scalp, and are usually elicited through electrical stimulation but natural stimulation of sensory receptors such as pain and temperature can also be performed. SSEPs usually assess central sensory pathway functional integrity but they do contain information about the involved portion of peripheral nerve. These signals are less robust than traditional NCS and require extensive averaging. Variation in amplitudes can be informative but reliance is placed more on latencies for detection of pathology[19,28]. Overall, the lack of precision and reproducibility make these studies ancillary at best when evaluating nerve health, although one of the few metrics specific to sensory axons.

Motor evoked potentials (MEPs) can again be conceptualized as a motor NCS that records from the muscle but involves both central and peripheral pathways[29,30]. Standard MEPs involve stimulation of the motor cortex or spinal roots[31] electrically (painful, performed intraoperatively) or magnetically (readily tolerated, performed in clinic). Electrical stimulation produces a series of signals that relate to central conduction, including the earliest wave, the D (direct) wave from direct activation of the pyramidal axons and the I (indirect) waves, reflecting indirect activation of pyramidal cells. Transcranial magnetic stimulation (TMS) generally elicits only I waves and tends to be less stable than electrically evoked responses[32], discussed in more detail below, making it also poorly suited to a precise quantitative evaluation of peripheral nerve health.

1.3 Standard Electromyography (EMG)

The anatomical motor unit (A-MU) is generally considered to comprise the neuronal cell body, motor axon, and innervated muscle fibers[33]. Following discharge of a neuronal cell body, axonal depolarization is transmitted and subsequently amplified by depolarization of the many MU muscle fibers to produce an electrophysiological motor unit (E-MU)[33], which can be used as a surrogate representation for the underlying A-MU. Any recorded E-MU potential (E-MUP) is just one view of the E-MU that can alter significantly depending on the characteristics and position of the recording electrodes. While the precise relationships between these three concepts (A-MU, E-MU, and E-MUP) remains to be clearly defined[33], the EMG signal from the muscle clearly relates to the health of the motor axons within the corresponding nerve and as such can provide an important insight into nerve health.

Standard "gestalt" EMG assessment involves the use of needle electrodes to sample areas of muscle while at rest, and during slight to full contraction[16]. Concentric or monopolar needle electrodes are usually used, which have recording surface areas between approximately 0.02-0.07mm2 and 0.2-0.4mm2, respectively. At various levels of contraction, several features of a selection of MUPs are subjectively placed on an ordinal scale, such as size, shape, stability, and MU firing rate. Inferences about the overall quality of the nerve supplying the muscle are subsequently made. At rest, potentials related to spontaneous muscle fiber discharges can be observed and categorically graded, and theoretically, this correlates to the overall level of muscle denervation[16]. Subsequently, slight activation is requested, which allows the examiner to subjectively or semi-objectively assess characteristics of transiently observed MUPs. Electrode recording area size affects MUP characteristics[34,35],[34,35], and therefore the type of electrode used is important. This is especially true of amplitude, which is determined by only a few fibers within about 0.5mm of the electrode[34]. As more MUs are recruited, it is usually no longer possible to observe individual MUPs due to superimposition[17,36]; however, information can still be gleaned from interference patterns (IPs) recorded at up to full contraction[37]. The degree of superimposition and the amplitude of the envelope (a line drawn around the interference pattern) contain information that can refine prior inferences[17,18]. Clinically, the electrodiagnostician will ordinally grade the degree to which the baseline of the IP is obscured[16,17]. If incompletely obscured, the muscle is considered mildly denervated, whereas if discrete MUPs are present, it is considered severely denervated. Another early but more objective measure simply creates an envelope around the signal recorded during full contraction, often achieved by computing the root mean square (RMS) value within a window that "slides across" the signal. This allows quantification of area and amplitude, which can be compared to reference limits to grossly categorize muscle as neurogenic (large amplitude) or myopathic (small amplitude)[18,38].

1.3.1 Weaknesses of Standard EMG

Compared to other metrics, there is an inherent reliance on the skills and experience of the clinician in planning, performing, and interpreting EMG studies using this "gestalt" approach, resulting in a large operator bias, high error rates,[17,36,38] and inconsistent diagnosis[39]. Assessment of E-MUPs suffers not only from its qualitative nature, but also only a few can be assessed in this manner and only at very low levels of activation. Subjective estimates are made for many measures including cut-offs for amplitude and slope, firing rates, size and shape, and stability. Additionally, the grading of this limited sample is not only subjective but also ordinal, leading to poor precision and reliability. Furthermore, when assessing maximal voluntary contraction (MVC), full effort is required, otherwise a subset of motor units will not be included in the assessment, which is also relevant when there is coexistent CNS pathology.

Another limitation of standard EMG is the use of electrodes with small recording area, resulting in the oversized contribution of muscle fibers within 1-2mm of the recording surface. This leads to variable signal characteristics with even small needle movement and subsequent compromised validity.

Attempts to quantify denervation through grading spontaneous activity not only suffer from the previously described subjectivity and ordinal grading but also due to muscle membrane excitability reducing over time, muscle fiber atrophy resulting in reducing signal amplitude, and a proportion of fibers never depolarizing spontaneously[40].

Standard neurophysiological techniques, including NCS and EMG, are tried and tested clinical and research techniques that offer some objective and semi-objective quantification of nerve health. However, significant limitations have been identified and augmentation with more quantitative techniques, as reviewed below, would likely enhance precision and reliability.

1.4 Non-Standard Neurophysiology

Beyond well-established standard approaches to NCS and EMG, many useful techniques have been developed over decades which have important roles in the evaluation of peripheral nerve health. We review established and innovative methods of quantitative EMG (QEMG) and for obtaining a Motor Unit Number Estimate (MUNE), as well as alternate neurophysiological approaches, electrodes, and novel stimulating and recording strategies identified within the formal scoping review process. As mentioned, it is important to distinguish between what can be done and in which environment. For any given measure, appropriateness of technique and performance characteristics vary substantially between studies performed in clinical practice, in large clinical trials, in small human pilot studies, and in large animal, small animal, and ex-vivo studies. It is beyond the scope of this review to fully address appropriateness in each context, but the following discussion of strengths and limitations aims to support decisions in this regard and offer insights into emerging approaches and areas of research.

1.4.1 Quantitative EMG (QEMG)

In the most widespread clinical application of gestalt EMG discussed above, features of individual MUPs, or IPs are subjectively assessed and scaled ordinally on the mild to severe spectrum based on clinically derived reference values[16], while MUP stability is rarely considered. Quantification methods can enhance inferences made by not only improving objectivity, reproducibility, and precision but also utilizing a greater number of metrics relevant to nerve health. In general, QEMG has focused on defining the *probability* that a muscle, individual MUPs, or the entire IP, is 'myopathic' or 'neurogenic'. The precise extent of axon loss, a surrogate for overall nerve health, within a muscle categorized as 'neurogenic' has been of secondary concern. Nevertheless, metrics used to establish probability-based

categorization of muscle are frequently continuous numeric values that strongly relate to the muscle's state of innervation. In the field of neuroregeneration, the benefit of more detailed quantification is becoming clear, both clinically and for research. Broadly speaking, quantification can be achieved through analysis of the individual MUP trains (MUPT) that make up the composite signal, or alternately, through analysis of the global EMG signal (composite signal or IP). We briefly review each of these approaches, highlighting the challenges and potential for their application to the evaluation of nerve health.

1.4.1.1 MUPT-based QEMG

Basic QEMG analysis of individual MUs requires the extraction of MUPTs and focuses on the assessment of a template or representative MUP. Manual extraction of MUPTs from a composite signal is time consuming and has its limits. It usually involves identifying and extracting 20 or more MUPTs, using level or window triggering and careful needle positioning during low activation levels, which are then each simply represented by a trigger-averaged MUP template[16], and is therefore usually limited to the study of only the first few recruited MUs. Statistics can then be calculated for features such as MUP size, shape, stability, or combinations of these such as area/amplitude (thickness), and MU firing pattern and number.

Software implementations of algorithms that attempt to extract MUPTs more automatically and comprehensively have been made available[36,41,42]. These automated algorithms can be categorized into those that attempt to extract all of the MUPTs comprising an EMG signal[42] and those that aim to extract a representative sample of the most identifiable MUPTs[36,41]. The former approach requires significantly more computational power and time given the complexity of a needle recorded signal that can include up to 25 overlapping MUPs[10,33], let alone surface recorded signals. The choice depends on context, especially time and knowledge; however, the number of MUs with fibers within range of the recording electrode is an important metric, and the ability to fully characterize all the recorded MUPs may add significant value in assessing nerve health. To date, no algorithms have experienced widespread adoption for various reasons, but a few are available commercially and can be relatively easily implemented[42,43]. Nevertheless, the benefits of rapid quantitative approaches are becoming clear, not only for research, but also clinically as advances in therapy demand greater accuracy and reliability than standard EMG to optimize clinical decision-making.

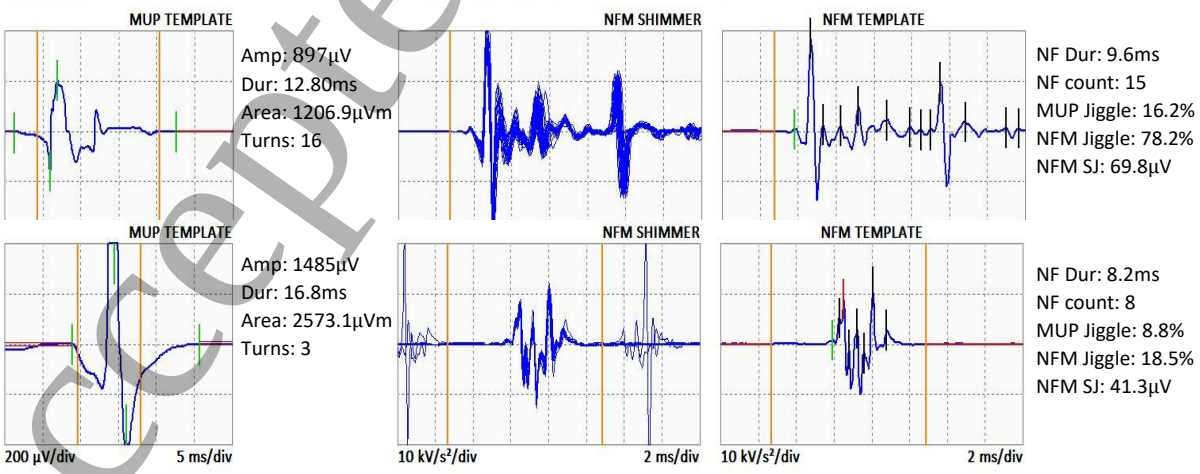

Figure 2. *Different stages of reinnervation. Top, reinnervation manifests with a complex shaped MUP with increased near fiber parameters. Bottom, the MUP has matured and stabilized. The near fiber changes may represent one of the earliest detectable changes in motor neuronal degeneration and reinnervation. From left to right: MUP template, NFM shimmer and NFM template are displayed. The top to bottom vertical lines demarcate the NFM duration. NF: near fiber. NFM: near fiber MUP. SJ: segment jitter.*

Use of automated algorithms has the additional benefit of further signal and feature transformations and abstractions[44]. For instance, near-fiber EMG (NFEMG)[32] is the novel approach of representing an E-MU as a near fiber MUP (NFMUP), which more clearly presents the relative contributions of individual muscle fibers near to the electrode recording surface. Suitably large and symmetric peaks within NFMUPs are associated with propagating MU fiber action potentials of single or small groups of near MU muscle fibers[45]. NFMUP duration and dispersion are related to the temporal dispersion across propagating MU fiber action potentials and if increased can reflect increased MU fiber diameter, axonal branch demyelination and/or increased axonal sprouting. All can be signs of denervation and/or reinnervation and offer the capability of rapidly providing information related to a muscle's quality of innervation[46]. NFMUP duration and dispersion are correlated with the number of MUP turns and the number of MUP phases but measure more directly temporal dispersion across propagating MU fiber action potentials. In addition, when using automated algorithms, it is possible to assess the stability of a single E-MU across multiple motor neuron discharges. Traditionally, this is done by calculating MUP jiggle[47] across a set of isolated MUPs selected from a MUPT. However, an emerging and potentially more precise technique involves measurement of NFMUP jiggle and NFMUP segment jitter[45,46] across a set of isolated NFMUPs selected from a MUPT. NFMUP jiggle is measured using the same formulation as MUP jiggle and like MUP jiggle focuses on NFMUP shape stability, while NFMUP segment jitter focuses on temporal stability by measuring temporal differences between matched segments of temporally-adjacent isolated NFMUPs, similar in concept to fiber pair SFEMG jitter measurement. Indeed, when assessing for degeneration or its reversal, regeneration, E-MU instability ('apparent jitter') may be one of the earliest and most sensitive measure. Figure 2 demonstrates NFMUP analysis and its potential use as a metric in nerve health.

The analysis of MUPTs can also be divided into approaches that use conventional morphological features from the time domain and approaches that extract spectral features from the frequency domain[36,48,49]. Using continuous scales, these features can be modeled conventionally by multivariate and Bayesian approaches[50], and slightly less transparent methods such as dimension reducing

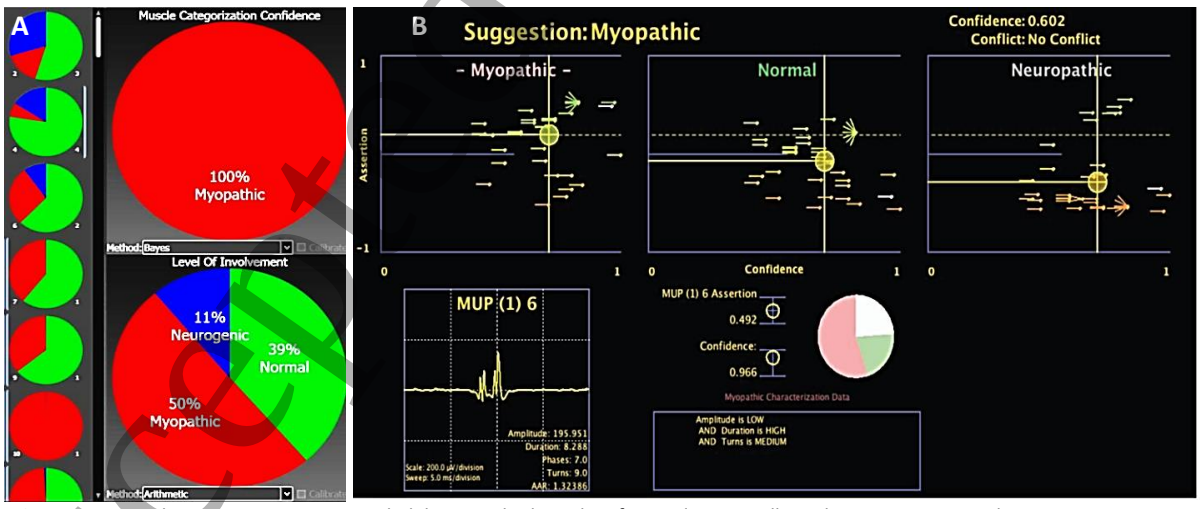

***Figure 3**. MUPT characterizations using probabilistic methods to classify muscle. **A**: Small pie charts represent single MUP characterizations, top large pie chart represents confidence of overall muscle characterization, bottom represents estimated level of involvement. **B**: Specific MUP characterization, supporting myopathic. (Farkas et al., 2010, courtesy of Begell House Inc.).*

algorithms, including principal component analysis[51], and learning algorithms, including linear discriminant analysis[52], support vector machines[53], and artificial neural networks[54,55]. Recent examples

of advanced methods that have had some success include Xie et al.[56], who applied fuzzy integral theory in a multi-domain approach incorporating MUP morphology, frequency domain, and time-frequency domain wavelet transform feature sets. Figure 3 exemplifies how sets of MUPT characterizations can be probabilistically aggregated and displayed to provide an overall classification of normal, myopathic, or neurogenic for the muscle under study[57].

1.4.1.2 Composite Interference Pattern Analysis (IPA)

IPA is a global quantitative approach to examining EMG signals[58]. This approach extracts information from EMG signals at greater force than MUPT techniques, quantifying several additional IP parameters related to muscle innervation. Unlike MUPT techniques, IPA is also capable of commenting on abnormalities secondary to disorders of fatigue, conduction block, and central drive[17].

The IP can be characterized by quantifying either signal frequency or time domain morphological components. Frequency domain analysis is based on the theory that can be modelled as being composed of combinations of sinusoids and any given signal can be resolved into its constituent sinusoids, including their frequency, amplitude , and phase[59]. Generally, short duration and fast rise time MUPs are composed of higher frequency components, while MUPs with long duration and slow rise times contain lower frequency components. Frequency spectrum analysis can be relatively easily carried out through Fourier analysis. The power (squared amplitude) of each frequency component can be plotted against its frequency (power spectral analysis, PSA), with the area under the curve representing total power within the signal[60], reflecting MU number, size, and firing rates. Early studies ignored the changes over time of EMG signals (non-stationarity); however, technological advances allow inclusion of this important aspect, resulting in potentially significant additional utility in quantification of nerve health (Fig 4). Early time-invariant PSA and turns-amplitude (TA) analysis[61] were not found to be sensitive at identifying partially denervated muscle compared to MUP duration[62]. However, when non-stationarity (change over time) is included, time–frequency analysis techniques correlate highly with MUPT-based morphology[63,64], while also possessing the capability to identify small components overlooked in parameters such as MUP duration[65]. We are not aware of composite scores combining time-frequency based analysis, with IP and MUP morphological metrics.

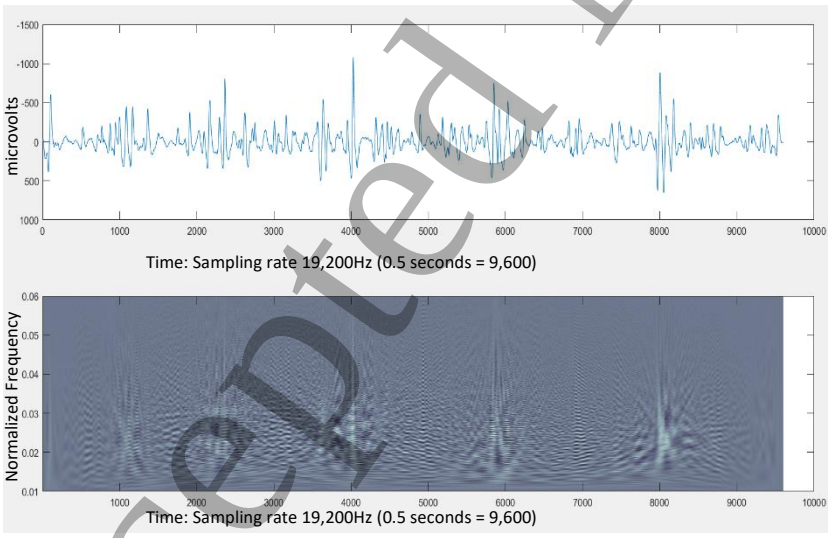

Figure 4. *Time Frequency Analysis. Top: 0.5 seconds of concentric needle recording of normal muscle (sampling frequency 19,200Hz). Bottom: the corresponding wavelet-based time-frequency analysis demonstrating added richness when including the time domain into frequency analysis (y-axis: normalized to the Nyquist frequency).*

Original time domain morphologically-based IPA is not founded on mathematical abstractions but rather the signal itself, including turns-per-second and mean amplitude-per-turn, both developed decades ago[61,66]. Generally, more turns in a signal and lower mean amplitude of these turns correlates with myopathy and the converse with neuropathy[18,38]. Although rapid, and potentially more accurate than MUP

analysis[67], reference clouds are required for each muscle but rarely developed due to the significant effort required. Consequently, its use has been limited to specific clinical scenarios, such as the thyroarytenoid for patients with unilateral vocal fold paralysis[68], where turn number alone correlates with severity of denervation in laryngeal surgery evaluation[69,70]; although a single study applied TA to the evaluation of nerve regeneration and found a significant improvement in accuracy over standard EMG[71]. Nandedkar et al[65] built on this concept and developed an integrated tool called EQUIP (expert quantitative IP analysis) that refines the TA cloud-based concept to better mimic subjective IPA by also capturing high frequency activity and envelope amplitude, standardized by activity level. Comparison studies of EQUIP to TA and subjective expert IPA have been favorable[61,72].

Overall, the increased precision offered by quantifying EMG signals is likely of great value both clinically and as a biomarker for research studies if time, resources, experience, and subject compliance allow. However, the degree of its benefit is unclear and may vary from one context to another but the application of advanced statistical methods to novel and classical multi-feature analysis represents an interesting area of further research.

1.4.2 Motor Unit Number Estimates (MUNE)

Few measures attempt to quantify a component of nerve health more accurately and objectively than a MUNE. An abrupt decline in the number of alpha-motor axons translates into a reduced CMAP amplitude/area in just a few days, but over weeks to months, compensatory collateral reinnervation will occur. A single motor axon can extend its intramuscular branches to expand the number of muscle fibers innervated by up to seven times[23,73,74], most clearly seen in the giant MUPs after childhood polio, restoring CMAP amplitude/area even when the majority of axons have been lost.

To address this, McComas[23,75] developed a method to obtain a MUNE. Originally, this method involved approximating the average amplitude of individual MUPs, termed single motor unit potentials (SMUPs) by measuring the increment in the CMAP amplitude with progressively stronger stimuli (aka, an incremental MUNE). The average amplitude of these SMUPs is then divided into the supramaximal CMAP to provide an estimate of the number of motor units within the muscle. Issues predominantly with sampling error and alternation have led to several variations to techniques to obtain a MUNE[25,76–82]. Alternation refers to the phenomenon whereby the threshold necessary to induce an action potential in an axon fluctuates within a range, resulting in probabilistic activation of MUs with any given fixed stimulus level.

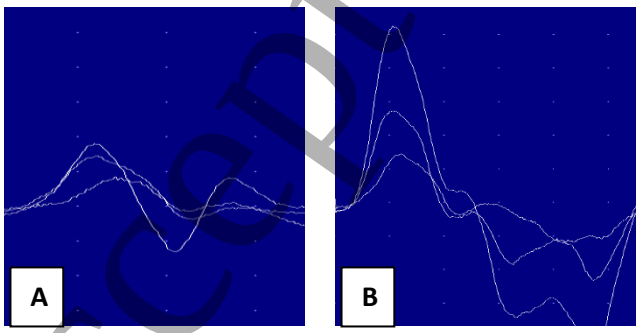

***Figure 5**. Examples of incremental stimulation in modified MPS MUNE. (A) 3 incremental stimulations in a healthy ADM. (B) 3 incremental stimulations in an ADM affected by radiculopathy. The larger steps in B will result in a larger mean SMUP and lower MUNE overall. MPS: multipoint stimulation. MUNE: motor unit number estimate. ADM: abductor digiti minimi. Scale: 20ms per horizontal division and 100uV per vertical division.*

The technique to obtain a multipoint stimulation MUNE[77,83] (MPS-MUNE) involves identifying a sample of SMUPs through evoking single SMUPs at several sites along the nerve, avoiding issues with alternation. The length of nerve required is not always feasible and so the original technique to obtain a MPS-MUNE was modified[84–86], merging the incremental and MPS techniques (Fig 5). This essentially involves performing several limited incremental studies at only 3-4 sites to obtain the requisite sample of SMUPs, relying on the fact that alternation can usually

be distinguished within the first few units recruited. The MPS-based MUNE technique suffers from not only counting the lowest threshold units (that tend to be the largest[75]), but also fails to consider phase cancellation, although partially accounted for in the modified incremental version. Unlike the original incremental approach, temporal relationships between latencies and phases that are present in the CMAP are no longer present between the SMUPs. The CMAP is not the simple arithmetic sum of the amplitudes or areas of the SMUPs due to phase cancellation. The original relationships between phases and peaks should be preserved when SMUPs are summated to calculate the average SMUP size from which the MUNE is derived. Averaging the sample of SMUP waveforms digitally, datapoint by datapoint, after selecting onset times, attempts to account for superimposition and results in improved estimates[87]. It has also been postulated, however, that axons with the lowest voluntary activation threshold are only representative of the smallest motor units, biasing toward a larger MUNE[79]. Empirically, there is little evidence of bias in one direction or the other[77].

The spike-triggered averaging MUNE technique[78] (STA-MUNE) relies on patient activation at low levels to allow measurement of individual MUPs that are identified using a needle, avoiding alternation. The averaged surface recording associated with these identified MUPs then represent the sample of SMUPs used to calculate the STA-MUNE, along with the CMAP recorded in the usual way. Nevertheless, not all concerns are addressed with this method[88]; phase cancellation is again not accounted for, the use of a needle is disadvantageous, and there is reliance on subject cooperation and ability, making it less suitable for children, animals, and certain adults. The issue of phase cancellation can again be mitigated to some degree by aligning SMUP onsets and digitally averaging the sample of waveforms[87,89]. Sampled SMUPs are likely of small size and SMUPs related to larger later recruited MUs are excluded, although a decomposition enhanced version may allow more valid sampling through measurements at greater force[89].

F-Wave methods[80] take advantage of the fact that a small subset of motor neurons produce late responses, avoiding alternation. However, moderate to severely denervated muscles may produce no or insufficient F waves, phase cancellation is ignored, there can be effects from upper motor neuron dysfunction (large and complex), and it is unclear whether the subpopulation of motor neurons that produce F waves make for a representative sample.

The above methods attempt to avoid alternation, however, the statistical (Poisson) method is founded on alternation, while additionally allaying some sampling and size concerns by obtaining MUs over a wider stimulation range[82]. Stimulation is given repeatedly at 3-4 levels of intensity. At each level, there will be a population of motor units near threshold resulting in a variability that theoretically estimates the size of those SMUPs at threshold, which can in turn be divided into the CMAP to produce a MUNE. This method may be more susceptible than other techniques to MUP instability causing the SMUP estimate to be artifactually low, a problem that increases with degree of denervation and reinnervation. Given that all SMUPs do not have identical size, as is assumed in the statistical method, the Bayesian methodology was developed[90], which allows for variability within and between SMUPs.

Overall, empirically, there is little difference in results between the methods[76]. Historical anatomic techniques that evaluate number of axons innervating hand muscles[33,91–95] and tibialis anterior show fair correlation with MUNE values[33]. Newer studies based on choline acetyltransferase do not address specific muscles but estimates of about 1700 efferent fibers innervating the hand which approximates the sum of available MUNE estimates[33,94]. Of note, within the anatomic studies, a key finding of an increasing proportion of sensory to motor axons when moving distally suggests complex control requires

more nuanced sensory feedback, an important consideration for any approach to neurorestoration. Other than being noninvasive (except for spike-triggered averaging), a MUNE has another key advantage over histology because it detects when a motor unit is not functioning; waiting for degeneration of the axon or cell body is not required for a drop out to be detected. Furthermore, mechanisms can be clarified by MUNE, such as whether motor unit size or number is affected by a therapeutics in ALS[77], for instance.

1.4.3 Variations of techniques to obtain MUNE-like information

MUNIX results from a mathematical model based on the relationship between the CMAP area and power, as well as the surface interference pattern[96]. The tolerability, rapidity, applicability to large muscles, and inclusion on commercially available machines make it particularly appealing. However, it is an index and as such may best be applied for tracking over time[97].

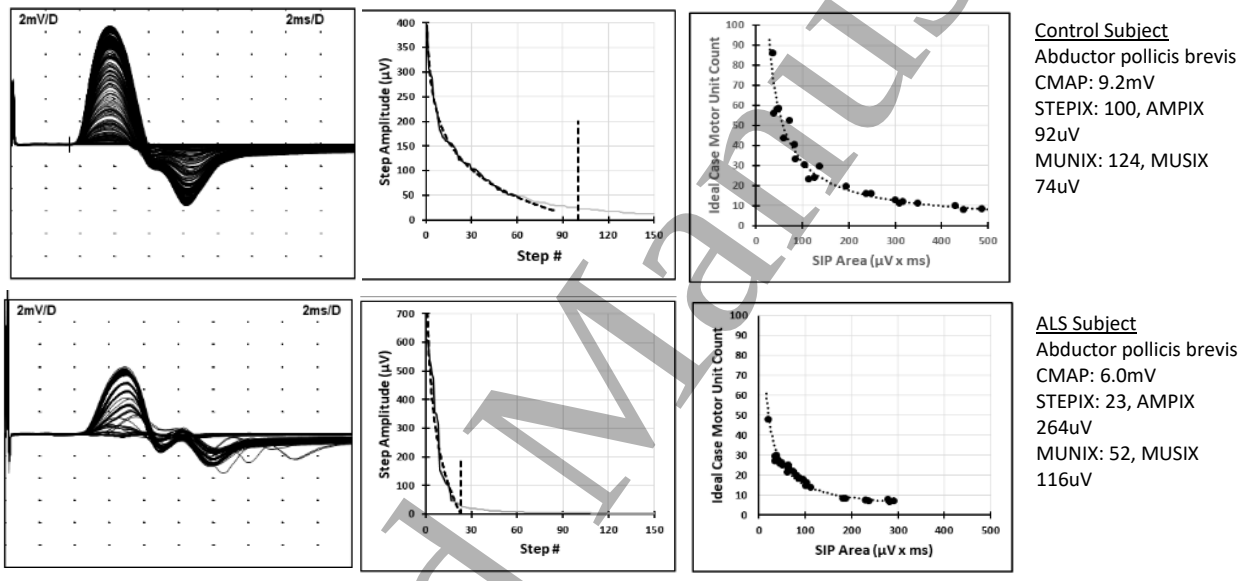

***Figure 6**. STEPIX. A new index describing an estimate of muscle innervation. TOP: healthy subject showing left, a full CMAP; middle, calculation of "step number" of about 100 motor units; and right, results of MUNIX from the same muscle (124). BOTTOM: Subject with ALS showing left, a smaller and less full CMAP; middle, calculation of "step number" of about 23 motor units; and right, results of MUNIX from the same muscle (52). ALS: Amyotrophic lateral sclerosis Courtesy of Sanjeev Nandedkar.*

The CMAP-Scan[98] is intuitively and visually appealing. The nerve is stimulated incrementally and the response amplitude against the stimulus intensity is plotted. What are subjectively obvious characteristic changes have been quantified in several ways[99,100], one of the most recent being MscanFit[101], which relies on a mathematical model to iteratively predict the number, size, and stimulation threshold of constituent MUs. Freeware and the ease of application are benefits of this approach and results appear to be consistent with other techniques for obtaining a MUNE[102]. Nandedkar et al[103] recently developed a deterministic approach to analyze CMAP scans, resulting in STEPIX (number of 'steps'), and AMPIX (MU size). Comparable results to MScanFit were found on initial investigations in ALS and normal subjects (Fig 6). One drawback is that CMAP scans subject patients to many stimulations (500). If validity were maintained, the application of algorithms to initial CMAP scan portions to reduce discomfort and time would likely greatly enhance applicability and uptake.

The spatial information provided by high density surface EMG (HDS-EMG) has been harnessed to estimate SMUPs, allowing a non-invasive calculation of a MUNE[104,105]. HDS-EMG utilizes spatial and

temporal information to identify valid SMUPs for inclusion in the sample of representative SMUPs, minimizing alternation and increasing the sampling of larger MUs. Chen[106] proposed a novel stimulation-free method to obtain a MUNE based on surface EMG decomposition, replacing the stimulated CMAP with a derivative of the surface EMG at maximum voluntary contraction (MVC). Research into this approach is ongoing but may add a very useful dimension for obtaining a MUNE should it prove accurate and reliable.

1.4.4 Non-MUNEs of MU loss

Theoretically, there should be a strong link between the proportion of MU loss and parameters of MUPs, however, the relationship between MUP characteristics and number of MUs within a muscle decouples due to several issues, including heterogenous geographic spread of motor unit loss, fascicular boundaries, health of residual MUs to collaterally sprout, use of population-based references[107], size principal effects[108] on recruitment in denervated muscle, and fiber hypertrophy[34]. Nevertheless, there remains a correlation that is useful when evaluating nerve health, and Macro-EMG[34,35] takes full advantage of this by quantifying aspects of MUs to which most methods of obtaining a MUNE are blind. Additionally, the ability to evaluate deep muscles is a distinct advantage and Macro EMG likely represents one of the best options for evaluating deep muscles that are not part of standard neurophysiology protocols. A related method is that of fiber density (FD), which refers to the number of time-locked spikes recorded by a SFEMG electrode, standalone, or incorporated in Macro-EMG[34]. FD relies on reinnervation; its validity as a proxy for MU number is reduced until this is complete, after which it correlates with MUNEs[109]. Proxies for fiber density might be reported concurrently with standard EMG using low-pass-double differentiation filtered MUPs (i.e. near fiber MUPs)[45].

In addition to MUP characteristics, the number of MUs represented in a standard IP has a relationship to the number of MUs in the muscle. As mentioned, there may be around 15-25 MUs contributing to most full needle recorded IPs. Should only 4-5 MUs be observed contributing at full force, this may represent a loss of as many as 80% of the MUs. Depending on the patchiness of loss, extrapolation to the muscle may require many samples.

A twitch force based MUNE[110–112] is less commonly used in studies but has shown some validity. It is grounded on the association of a motor unit firing with a measurable force when averaged over many discharges. The individual twitch force measured by special manometers allows an average twitch force to be calculated from a sample of MUs, similar in concept to calculating an average SMUP that can then divide into the maximum force.

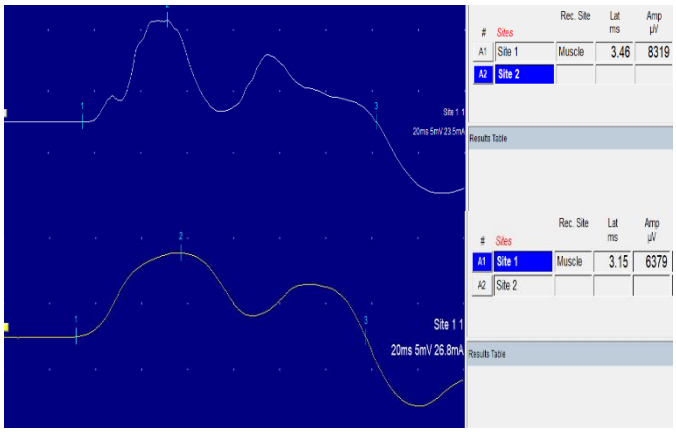

Figure 7*. CMAP recorded from the 15mm exposed cannula of a Macro EMG needle (top) deep to a standard surface electrode recording (bottom) of the Tibialis anterior. Note the slightly larger amplitude and sharper components.*

Nerve health is represented by more than an estimate of the number of its *motor* axons alone. Estimating the number of both sensory and motor axons in the nerve is more valid, such as a CNAP based axon number estimate[113–115]. A CNAP scan would be ideal but is difficult due to even large fiber increments being two or three orders of magnitude smaller than muscle recorded SMUPs. Nevertheless, distribution of conduction velocities (DCV) has been considered an

approximation[114,116–118], which shows the relative contribution to the CNAP from axons conducting in different velocity classes and makes inferences about axonal loss, although needing further development.

As discussed, standard techniques for obtaining a MUNE do not attempt to assess deep muscles. However, the use of the cannula of a needle electrode has potential application in this area (Fig 7), and this has been evaluated in one study using the cannula of the Macro-EMG electrode[119]. Given the reduced size of the Macro-EMG needle recording field compared to standard surface electrodes, the term motor unit density (MUD) has been used because the method realistically represents a tubular sample through the muscle of about 23mm by 4mm. Furthermore, the use of cannula electrodes could be amenable to incremental, MPS, and other methods for obtaining a MUNE, permitting almost any muscle to be quantified using this important metric.

1.4.5 Which method of obtaining a MUNE is most appropriate?

Superiority has not been established for either accuracy or reliability of one method over the others, mainly due to lack of appropriate histological correlates and comparison studies[76]. There is also a lack of a gold standard to which MUNEs have been correlated despite the attempts to compare histology to various methods of obtaining a MUNE discussed above[33] and the simulations[120] created to allow validation and comparison between techniques. Therefore, the context of application is important. Practical factors that impact choice of method include availability of equipment and expertise, ability for voluntary muscle activation needed for STA, pediatric or adult, cognitive or physical disability, availability of long nerve segments appropriate for MPS, deep target muscles not amenable to surface recording, time constraints, patient tolerance, clinical or research setting, and use in animal research (see below).

1.4.6 Limitations of techniques for obtaining MUNEs

Several limitations have been discussed above, such as alternation and phase cancellation, and many reviews discuss the wide array of concerns with the various techniques for obtaining MUNEs [25,75,76,79,82]. In the context of evaluating nerve health in general and attempts at the assessment of non-standard or deep muscles, many of these weaknesses are highlighted further. Beyond alternation described above, identifying an appropriate sample of valid SMUPs is challenged when assessing deep muscles not only by far field potentials (FFP) from non-target muscle SMUPs, but also by stationary FFPs from any of 6 conditions resulting in FFP generation[10] (such as those arising at musculo-tendinous junctions).

Assuming a SMUP was generated by the target muscle, most techniques sample a subpopulation of MUs, whether it is those that have the lowest stimulation threshold (MPS and Incremental), or those that are recruited first (STA). It has been shown that a MUNE can change by a factor of two depending on whether the MUs are sampled at a force of 10% versus 30% using the STA method[89], a result of the effect of the size principle[108].

Many techniques use a cut-off amplitude below which SMUPs are excluded (e.g., 25uV). There is a trade-off between improving specificity for SMUPs included in the MUNE calculation versus deflating the MUNE due to removing the many smaller distant SMUPs that make up the CMAP. This cut-off should likely change with differing electrode types and sizes.

Overall, even with standard methods for obtaining a MUNE, values exhibit a wide range in healthy controls, while also being age-dependent and having high variability between and within individuals[25,76].

This results in low cut-off values for determining what is a pathological loss of MUs, making these methods less sensitive diagnostically than other potential metrics such as QEMG parameters, and the poor reproducibility in highly innervated muscle reduces their value as a tracking tool. This is more so at mild levels of denervation because as denervation increases, methods for obtaining a MUNE usually become more and more reliable, which makes an argument for composite metrics dependent on severity and provides a topic for further research.

1.4.7 Practical approaches to MUNE of non-standard muscles.

Practically, there are a variety of techniques to maximize inclusion of as wide a variety of valid SMUPs while excluding as many non-target-muscle generated SMUPs as possible. The main approaches involve isolating both recording and stimulating sites as much as possible. Stimulating as distal as possible, preferably the nerve branches to the target muscle, reduces neighboring muscle activation, important for both the CMAP and SMUPs, and can be achieved through needle stimulation with ultrasound or stimulation guidance. The choice of electrode type and size can help to isolate the target muscle. For instance, use of a needle cannula embedded within the belly of the target muscle will maximize signal amplitude differences between target and non-target muscles, as will increasing high pass filter settings, albeit both will reduce the recording field. The more the recording field is reduced, depending on target muscle volume, the less valid the technique is for estimating the total number of MUs within a muscle, becoming more akin to an index or estimate of MU density. This is an issue with MUNEs in general as the surface recording electrode will likely only provide an estimate of the total number of MUs if the muscle is small, perhaps between 1-2 cm in diameter[10,76,121]. One method to overcome this might be the extrapolation from the estimated volume of muscle examined to the volume of the whole muscle, which can be obtained from ultrasound imaging[121]. Ultrasound has further potential utility in dynamically identifying each physical SMUP twitch as being within the target muscle, also allowing sampling of deep and superficial SMUPs to improve validity further.

2 Electrical Impedance

2.1 Electrical Impedance Myography (EIM)

The development of electrical impedance myography (EIM) provides a new window for evaluating nerve and has proven well-suited to the detection, staging, and monitoring of denervated muscle[122–126]. In EIM, a painless, alternating current is applied over a muscle using one pair of electrodes and voltages are simultaneously recorded using another electrode pair. The relationship between the current and voltage allows reactance and resistance to be determined, resulting in a spatial bioelectric profile of muscle that conceptually blurs the boundary between imaging and neurophysiological biomarkers. Over recent years, EIM has transformed into a growing field, encompassing multiple methods[127], and many potential applications. It has proven sensitive to changes in structure and composition of denervated muscle, with current research efforts focused on understanding histopathological features affecting EIM data over a range of conditions and electrode types[128–132], as well as correlation with complementary metrics such as quantitative utlrasound[133] and EMG[126]. Most recently, coupling of EIM with EMG (needle impedance EMG, iEMG) and EIM with ultrasound represent further examples of potential composite metrics of nerve health[134–138] that promise to increase both clinical and research efficiency and accuracy.

2.2 Electrical Impedance Neurography (EIN)

The electrical impedance of any tissue can be measured, including a region of nerve[139–142]. There is significant potential for impedance measurements of nerve directly to prove useful in assessing nerve

health. Being able to detect changes in the bioelectric properties of neuronal tissue in combination with a nerve's functional ability to conduct action potentials would provide a powerful synergy that could offer greatly improved metrics of nerve health. This capability offers an innovative solution to problematic intra-operative NAP measurement, with additional and important neuronal histological insights without the need for functioning axons.

2.3 Electrical Impedance Tomography (EIT)

Measurement of impedance has been extended to tomography in the form of neuronal electrical impedance tomography (EIT), essentially a real-time imaging technique using electrical impedance, which is included in this neurophysiological review due to its provenance as well as ability to record neuronal depolarization (Fig 8). Historically utilized to image cerebral electrical activity in healthy and pathological states, including stroke and epilepsy, as well as monitoring pulmonary ventilation, neuronal EIT has more recently been used to measure peripheral nerve depolarization at the fascicular level[143,144]. This extends the concept of EIN to another dimension and enhances the level of detail about structure and function within a nerve in a form that is capable of being miniaturized and integrated into implantable electronic devices for chronic monitoring.

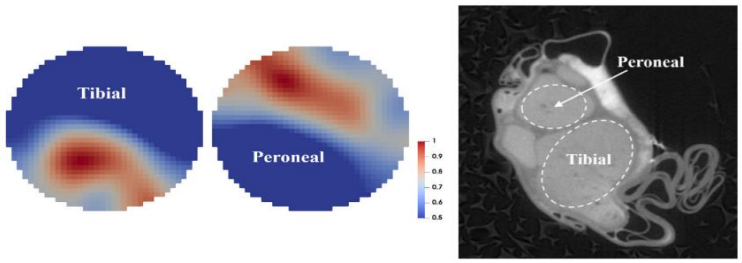

***Figure 8**. Left: EIT imaging of compound activity from tibial and peroneal fascicles. Right: MicroCT scan of same nerve. Enrico Ravagli et al 2019 Physiol. Meas. 40, 115007, available via license CC by 4.0.*

To summarize, the application of impedance measurement to the evaluation of nerve health is a new and exciting direction with much promise, offering a very different neurophysiological window into the health of nerve compared to standard measurement of action potentials and their associated electromagnetic signals.

3 Excitability

3.1 Nerve Excitability

Characterizing nodal and internodal neuronal membrane ion channel function using a variety of parameters, such as the strength-duration time constant, is playing an increasing role in the study of peripheral nerve health[15,145,146], detecting important changes invisible to other measures. Although the absolute or relative number of axons is not assessed, changes in excitability parameters could represent a first therapeutic response in neuroregeneration, especially measured longitudinally within a subject. For instance, the abnormal function of slow potassium channels in ALS has been noted as an early change[147], and excitability studies have proven sensitive to functional and reversible axonal loss in renal failure[148].

3.2 Muscle Excitability

Inferences as to nerve health are also possible through surrogate muscle excitability parameters[149], which classically rely on the differential susceptibility to stimulation amperage and duration, between nerve and muscle. These metrics have been shown to correlate to varying degrees with acute and chronic denervation changes on EMG[150,151], offering an alternate viewpoint from which not only nerve health can be evaluated, but also the receptivity of muscle itself to reinnervation. Over time, the

excitability of muscle tissue reduces, and this measurable process may correlate with the level of fiber degeneration, fibrosis, and fatty change that progresses over time in chronically denervated muscle tissue. Viability of muscle is an inherently important aspect of neuroregeneration.

Excitability studies are in the early stages of application to nerve health, degeneration and regeneration and much work is required in this area; however, it may be one of the first markers of change and, notably, is likely capable of providing important information on muscle receptivity to regenerating nerve..

4. Microneurography

CNAPs fail to discriminate the contribution of subcategories of axons including small caliber fibers that refer spontaneous pain and autonomic function. Microneurography allows single-fiber recordings[152] using 0.2mm tungsten needles with a tip diameter of between 1-5µm. While traditionally regarded as time-consuming and difficult, its place in research is established and the recent focus on the measurement of neuropathic pain and its treatment has increased interest in this technique. Microneurographic research can be divided into three groups[152]: (1) fusimotor system; (2) sensory innervation of the human skin (3) sympathetic innervation and efforts to further progress each area are required, as is the development of this important skill more broadly. Alternate present-day measures of small fiber nerve function usually address diffuse small fiber neuropathies,[153] and a non-invasive quantitative measure that precisely measures focal small fiber activity is otherwise lacking. Further, with the advent of ultrasound guided microneurography[154], the roles for microneurography have been greatly expanded, potentially including clinic-based evaluation for painful neuroma after nerve injury as well as research-based longitudinal tracking of small fiber activity in response to therapy.

5. Magnetoneurography

The ability to non-invasively investigate biomagnetic signals anywhere in the body would clearly be of great benefit, not least for peripheral nerve evaluation. This technology has been established for years

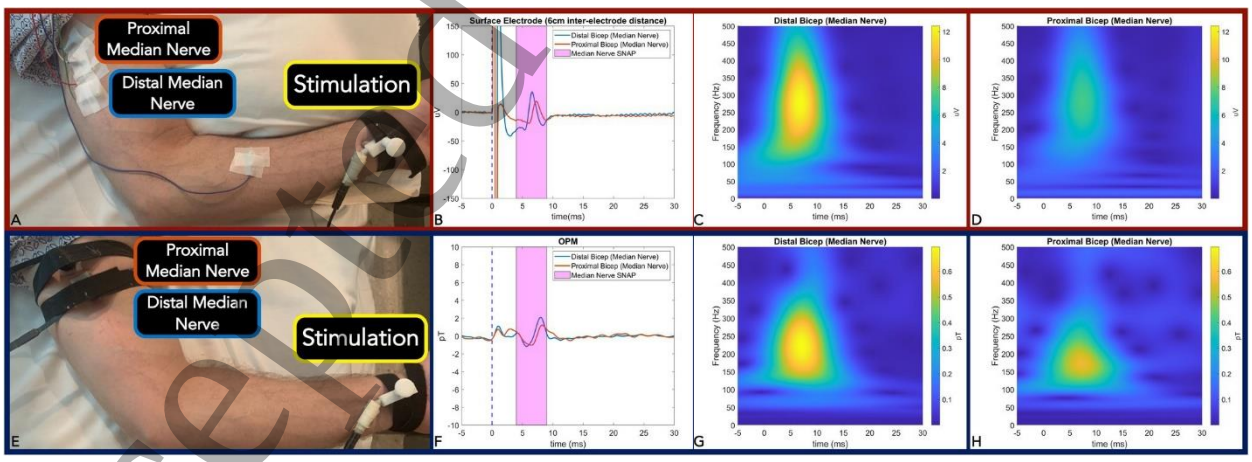

Figure 9. *Panel (A, E): Measurement setup of Median nerve SNAP with surface electrode (A) and equivalent action current by OPM (E). Panel (B, F): Time-locked average comparison (from two sites: distal bicep = blue line; and proximal bicep = red line) between surface electrode (6cm inter-electrode distance) (B) and OPM (F) demonstrate identical 0.8 ms temporal dispersion for both modalities. SNAP action potential/currents are marked in the magenta shaded area. Panel (C, D): Surface electrode time-frequency analysis for SNAP measured at the distal bicep (C) and proximal bicep (D). Panel (G, H): OPM time-frequency analysis for SNAP measured at distal bicep (G) and proximal bicep (H). OPM, optically pumped magnetometers; SNAP, Sensory nerve action potential; µV, microvolt; pT, picoTesla; ms, milliseconds. Bu Y et al., 2022, available via CC BY 4.0.*

and successfully applied to the brain and heart (magnetoencephalograms and magnetocardiograms) but

application to peripheral nerve is not a new concept. Wikswo[155] produced some of the first magnetic field measurements of nerve impulses followed by numerous incremental improvements in application to the human nerve that continue to be ongoing[156–158]. Ionic current flow (as measured by conventional surface electrodes) also generates a tiny magnetic field, which can be detected by magnetometers, the most applied of which being superconducting quantum interference device (SQUID) magnetoencephalography (MEG) systems[158] and more recently optically pumped magnetometers[159,160] (OPMs, Fig 9). The ability to non-invasively produce high resolution pictures of intra-axonal as well as inward currents in deep nerves (depth-independent) throughout the entire neural pathway, including plexus, roots, spine, and brain would clearly be of significant value[161]. Testing that combines real time image-guided magnetoneurographic recording with non-invasive stimulation (magnetic or ultrasonographic) at any location in the body represents an exciting albeit seemingly distant future goal. Nevertheless, limitations are clear and predominantly include expense, time, availability, expertise, and size of equipment[156,158,159].

6. Alternate Stimulation Techniques

Pushing electrical current into neural tissue has been the favored approach to neuronal depolarization for over a century but this approach is hindered by well-known limitations including, artifact and current spread that results in poor spatially confined excitation. However, depolarization can be accomplished by several other means, each technique having benefits and drawbacks and at different stages of translation toward clinical and research contexts. We briefly review and assess interesting alternate emerging approaches to generating a nerve action potential beyond standard electrical stimulation.

6.1 Ultrasound stimulation

Ultrasound promises to be one of the most exciting and cost-effective modalities for evaluating nerve health, having the potential to not only locate and assess a target nerve but also to both induce, modulate, and inhibit action potentials through highly focused ultrasound (FUS: focusing stimulation as accurately as 100μm)[162–165,165–168]; although, there is some ongoing debate as to how and even if FUS stimulates large fiber axons[168,169]. The mechanism of stimulation is unclear but mechanical stretch with ion channel opening, sonoporation from cavitation, and thermal effects are potentially involved in inducing neuronal depolarization[163,165,169,170]. Recent research in cell culture has shown that ultrasound excites primary murine cortical neurons through a mechanical mechanism mediated by specific calcium-selective mechanosensitive ion channels[171]. Activated channels appear to cause calcium to build up, resulting in membrane depolarization and a burst firing response, which is in turn augmented further by calcium- and voltage-gated channels. Other mechanical effects are not required for this excitation. Inhibition or over expressing of these channels leads to reduced response or stronger stimulation, respectively[171]. Regardless of the mechanism, although predominantly viewed from a neuromodulation and therapeutic angle in literature, potential for ultrasound stimulation in evaluating nerve health is high due to its ability to target neural tissue types selectively, with high spatial specificity, and at depth, avoiding the limitations of stimulation artifact and current spread. Research is building strong momentum in this area.

6.2 Magnetic stimulation

Magnetic stimulation relies on Faraday's principle that states a changing current will induce a time-varying magnetic field, which itself is able to generate a current in a second circuit[172]. It was first used to stimulate peripheral nerve[173] through the rapid discharge of a capacitor; however, the main application has been transcranial[174]. The technique is appealing given its promise to be able to non-invasively

stimulate nerves anywhere in the body due to the lack of attenuation of the field, as well as avoiding the need to inject current and associated stimulation artifact. Nevertheless, compared to electrical stimulation, the technique is limited by its ability to administer a controlled focal stimulus and remains inconsistent, imprecise, and time-consuming[175–178]. Micro-coil magnetic probes have been developed for intracortical neural stimulation but have the potential to be successfully repurposed for peripheral nerve stimulation and recording, especially intraoperatively, as discussed further in the section 9 below[179–181].

In an age of high gradient amplitudes and switching rates, Magnetic resonance imaging (MRI) itself can stimulate peripheral nerve[182,183]. Although problematic for improvements in imaging performance, this side effect could potentially offer another non-invasive opportunity for both stimulation and recording of peripheral nerve simultaneously, but, in contrast to FUS, much work is required before MRI has the capability to provide dual stimulating and imaging capabilities.

6.3 Optical stimulation

Neuronal depolarization through optical stimulation has been studied since 1971[184]. This area of study has evolved over the decades with in-vivo action potentials being recorded in nerve and muscle that as a result of low-level, pulsed infrared laser light[185]. Advantages of this technique over electrical stimulation have been widely expounded, emphasizing in particular the lack of stimulation artifact, as well as its selective precision of stimulation, without requirement for physical contact, and its safety profile[185–189]. The physiological process that explains this phenomenon has been clarified through analysis of potential photobiological effects resulting from the absorption of light by tissue, including thermal, pressure, electrical, and photochemical[186]. A thermal transient (photothermal effect) was found to be the most likely mechanism causing neuronal activation, necessitating an increase in temperature of between 3.6-6.4℃. It is unclear precisely how the thermal transient initiates a depolarization but may be secondary to alterations in capacitance[190,191], nanoporation[192], or heat sensitive channels[193].

Some concerns include the risk of thermal damage and expense. Thermal damage was of initial concern given that the radiant exposure (energy needed per area) for tissue injury was only about twice that required for neuronal depolarization[189,194]. One attempt to mitigate this was by applying a subthreshold electrical stimulus concurrent with the optical stimulation, reducing the required pulse energy significantly[189]. The widespread use of laser technology in telecoms within the range of 1,500 nm led one group to assess the effects of varying wavelength on neuronal activation[187]. They found that a cost-effective and practical approach might entail using these cheaper lasers and that different wavelengths may be suited to different depths of analysis.

One issue with optical stimulation that is pertinent to the evaluation of nerve health is the difficulty it has in activating all axons within a nerve[186], limiting its sensitivity and ability to quantify axonal content. In contrast to this, the lack of artifact and subsequent ability to stimulate close to the recording site allows a small intraoperative window to be used that would likely lead to greater utilization of nerve action potential recordings with intraoperative monitoring of peripheral nerve surgery as well as in research settings.

7. Electrodes

It is not possible to adequately evaluate most of the peripheral nervous system through standard surface electrodes. The ability to both record and stimulate using alternate electrode types, including invasive needle or cuff electrodes, wireless technology, as well as high-density surface electrodes provides an opportunity to effectively augment nerve health evaluation in both human and animal

research. Nevertheless, the geometry of recording electrodes has received little attention by way of previous systematic studies[195].

7.1 Specialized EMG electrodes

Commercially available Macro and Single-fiber (SF) electrodes have been discussed above[35,74]; the macro cannula electrode provides a more comprehensive view of the MUP, while the SF electrode characterizes the relationship between muscle fibers and the MUP. Bespoke four-electrode embedded needles[196] have been used to record three simultaneous views (MUPs) of each E-MU. Unique signatures are created using template matching and firing statistics, allowing identification far more accurately and decomposition at high contraction levels. Fine wire electrodes have long been used in gait analysis[197] and have the ability to remain in place for years in the control of prostheics[198]. Although providing very useful longitudinal and rich data on muscle, they suffer from small recording fields and risk of infection.

Other types of electrodes are currently under development, such as combined EMG/EIM needles[199] (Fig 10), which takes advantage of the needle's ability to accurately place electrodes close to an area of interest, enhancing accuracy and precision. The combination of multiple modalities into a single electrode promises to provide a more complete electromyographic profile of muscle. The four electrical impedance electrodes not only provide important new insights in passive elements of muscle that are invisible to EMG but are capable of greatly enhancing signal decomposition, in addition to providing macro and micro level MUP information. Inclusion of a single fiber electrode would make this a truly complete EMG electrode.

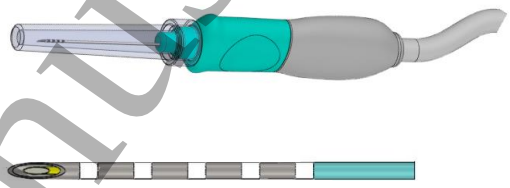

Figure 10. *Impedance-electromyography (I-EMG) needle electrode with details of electrodes. The EMG electrode is located at the tip of the needle. Haystack Diagnostics, Inc (in development).*

7.2 Needle stimulation and recording

Although not standard, near-nerve needle recording and stimulation have been performed for decades[115] and given the increased need for sensitive measures from non-standard nerves in the context of neuroregeneration studies, their improved validity in this context is likely relevant to many studies involving neuroregeneration. The CNAP is a fundamental biomarker of nerve health that can be recorded using needle electrodes with guidance from stimulation, direct visualization, or more recently ultrasound[19,115,200–202]. Reference values for amplitudes are not currently available because of increased variability as the amount and type of tissue, and therefore resistance, between electrode and nerve reduces[115,203]. However, it is possible to record a CNAP with as few as 10 axons with diameters greater than 7um[26], allowing one of the earliest means of detecting nerve regeneration. Regenerating axons change over time from having small diameter and slow conduction velocity to having near normal diameter and conduction velocity, but only once mature and connected with an appropriate end organ[204]. The recording area should be chosen based on context and can range from microneurography electrodes[205] (see above) through to the entire bare cannula. The smaller the recording surface, the greater the amplitude but the smaller the recording field, as with EMG recordings.

Another importance of needle electrodes lies in their ability to accurately target stimulation, if placed properly, essential when stimulating proximally or pre/post sites of injury, allowing for assessment of the proportions of neuropraxia and axonotmesis, as well as the assessment of deep or proximal muscles/nerves. Direct muscle stimulation can also reveal the extent and chronicity of denervation (see

strength-duration measurements below), differentiating between myopathic and neurogenic conditions[206] and potentially providing insight into muscle receptivity to reinnervation. Risks of stimulating nerve with needles has been assessed and generally found to be safe[10].

7.3 Surface Electrodes

In general, standard surface electrodes provide comfort but lack sensitivity due to signal attenuation by intervening tissue. Subtle morphological changes of E-MUPs cannot be discerned, rendering quantification and analysis difficult[207], and CNAPs rapidly become unrecordable with depth[10,19]. High-density surface EMG techniques overcome some of the issues when recording E-MUPs. Systems have improved to the degree that they are now capable of resolving spatial and temporal characteristics of a subset of E-MUPs[196,207,208]. Instrumentation complexities have hindered clinical uptake but use in research is gathering pace[209] and not only due to its painless nature. It provides many opportunities for gathering significant amounts of useful data, including longitudinal monitoring, wireless data capture, and muscle activation patterns[210,211].

Stimulation using surface electrodes is commonly done within standard neurophysiology as well as in neuromuscular electrical stimulation[212] (NES). The combination of multichannel high-density surface arrays and alternate stimulation parameters including strength, duration, and shape, allow for improved monitoring of muscle excitability[213–215].

Although not a neurophysiological measure, mechanomyography (MMG) can be used in conjunction with stimulation and, like twitch-force MUNE, has many similar properties; therefore, it has been included briefly in this review. MMG non-invasively measures mechanical vibrations produced by skeletal muscle. As muscle contracts, vibration is detected on the skin in several ways[216]: accelerometer, piezoelectric sensor[217], laser distance sensor, or microphone (acoustic myogram[218]). MMG has been used to assess muscle function, fatigue, and control of prosthetic devices and interest has increased recently due to advances in sensor and signal analysis, along with MMG's indifference to electrical artefact that challenges many electrode recordings. It has recently been applied intraoperatively to monitor decompression at the root level[219], as well as at entrapment sites[220], with favorable performance characteristics, although appearing to show complete and immediate resolution of conduction block intraoperatively in 100% of ulnar and peroneal entrapment cases. However, validation against standard nerve conduction studies intraoperatively is lacking and necessary. The potential of this technique to monitor motor nerve recovery and muscle reinnervation by way of non-invasively and sensitively detecting nascent units over time holds promise but is as yet unstudied.

7.4 Biointerfaces

Frequently employed for Vagus nerve stimulation, neural cuffs and clips have evolved extensively recently, moving from stiff rigid structures to flexible electronics that allow wrap around contact and reduce inflammatory responses[221–224]. Various configurations of microneedles can be implanted to penetrate different depths[143,225,226] (Fig 11, Utah Array); however, concerns about injury and chronic implantation limit application in many scenarios. Such advances offer an array of potential neuronal monitoring and modulating

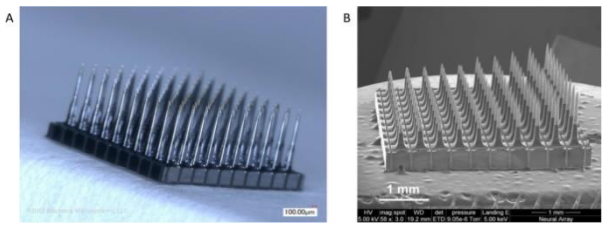

***Figure 11**. Utah penetrating (A) and slanted (B) electrode arrays. Available via license: CC BY 4.0, Blackrock Neurotech, Inc. Chandrasekaran et al, 2021.*

possibilities, allowing the collection of rich, accurate, and longitudinal real time passive and stimulated data. Nevertheless, implantation and likely removal is required at some point, necessitating invasive procedures. Advances such as regenerative electrodes[227], whereby the nerve regrows through pores in the electrode, offer potentially higher precision in stimulation and integration.

Reliance on wires has always been problematic. Improved spatial resolution and the ability to interrogate small-diameter nerves is evermore necessary but miniaturization has required significant advances in technology and a move away from electromagnetic-based systems. One such approach involves 'neural dust', which is a wireless ultrasonic backscatter system that is capable of both powering and communicating with nerve or muscle implanted bioelectronics below the millimeter-scale[228]. External ultrasonic energy causes a piezocrystal to vibrate, which is converted to current that powers a transistor. The transistor's gate is modulated by any extracellular voltage change across recording electrodes, which in turn alters the crystal's vibration. Thereby, electrophysiological information is encoded mechanically in the crystal's vibration, which can be detected and reconstructed externally.

Ever more integrated biointerfaces are being developed that promise impressive advances in metrics. A final noteworthy interface involves a stretchable ultrathin hybrid myoblast-graphene interface that can record EMG signals as well as stimulate electrically or optically in vivo[224]. In summary, the importance of electrode design cannot be overstated as a vital component in the evaluation of nerve health and is likely to be at the frontier of new scientific endeavors going forward, which will subsequently require efforts at translation to the clinical arena.

8. Animal and Lab Neurophysiology

Distinguishing methods of animal neurophysiological examination from human is important because the contexts are fundamentally different, including subject cooperation, size, environment, skills and experience, equipment, questions being answered, and types of procedures allowable. Such differences require tailored approaches but also offer opportunities to provide enhanced neurophysiological metrics and improve on standard techniques; for instance, incremental twitch subtraction MUNE method (ITS-MUNE)[120]. The use of sedation allows significantly different parameters to be applied for many stimulating techniques including for methods of transcranial and transcutaneous electrical[229,230] and magnetic[231,232] stimulation. This enhances the diagnostic ability of many techniques in lab research as well as the mechanistic understanding that is vital for translation to humans.

Significant technical variation exists in lab-based neurophysiology, including techniques for obtaining even standard measures, such as a CMAP, and especially for more advanced methods, including NAP and MUNE. In the evaluation of nerve health, this lack of standardization mainly relates to type and position of recording and stimulating electrodes, the quantitative methodology implemented and, notably, experience and skill levels. One of the often overlooked and hardest skills to acquire is the recognition of artefacts and unwanted signals, and how to remediate or account for it. In general, the smaller the animal, the more artefact and movement play a role in study quality.

Subcutaneous needle electrodes have very different recording parameters as compared to surface electrodes, and there is significantly greater variability of CMAP with small movements due to the minimal amount of subcutaneous tissue within which the electrode sits[233]. The repeated use over time of needle electrodes in smaller rodents can also lead to muscle injury with subsequent alteration in recordings that may be misattributed to disease progression. On the other hand, compared to needle recording, surface recording suffers from more unwanted non-target muscle activity, and selectivity has

increased importance when it comes to studying reinnervation and nerve health. Further variation includes the use of ring versus surface electrodes, as well as electrode area and method of adhesion.

If collateral reinnervation has not had sufficient time to occur, as may be the case in acute denervation or mouse models involving rapid degeneration, a simple CMAP will quantify innervation well. However, application of MUNEs will often provide richer information. When performing MUNEs in research animals, determining what constitutes individual SMUP responses is the most difficult skill to acquire[77]. Without experience, the measure's accuracy and repeatability suffers, although it is unclear to what degree researchers are usually trained to obtain MUNEs prior to commencing a study[233].

It is generally not possible to obtain voluntary EMG or active forms of MUNEs (such as STA-MUNEs) in research animals as there is a requirement for cooperation. Approaches to overcome this involve EMG recording during light anesthesia[234], allowing recording, decomposition, and analysis of interference patterns, and potentially even acquisition of SMUPs for STA-MUNE methods. This approach allows greater understanding of the innervation than a simple CMAP, including MUP size, shape, and stability, and MU number and firing patterns. Invoking a walking reflex[235] in a sedated animal may represent another approach to overcoming this issue. EMG can also be recorded using long term intramuscular electrodes in freely moving animals, allowing important sequential and granular recordings of change over time[15,236,237]; the rectified amplitude of the EMG can serve as an indirect indicator of muscle activity, although it may underestimate muscle activity at high levels of activation[15,238].

Nerve excitation is also possible using pulsed infrared light, and it has been lauded due to the lack of stimulation artefact and enhanced spatial precision[186,239]. However, the proximity of the stimulation threshold to the threshold for thermal ablation remains close[194]. Although efforts have been made to overcome this using a conditioning electrical stimulation prior to photo stimulation[189], concern over the safety profile and penetration limit its use. Optogenetic stimulation also uses light instead of electricity to depolarize genetically modified neurons, expressing light-sensitive proteins (opsins) such as channelrhodopsin[240,241]. Opsins sensitive to different light wavelengths can be inserted via viral transduction, allowing selective targeting of nerve fibers innervating a specific muscle. The benefit of stimulating using light over the course of longitudinal studies is clear, including its lack of artefact. However, it requires the insertion of opsins, usually through viral vectors, and the transdermal penetrance of light has its limits. Much research is aimed at its role in functional stimulation and nerve regenerative capabilities[242]. Although this review has focused on the motor system, advances in sensory measurement have progressed beyond classical nerve conduction studies and microneurography discussed above, and particularly noteworthy are animal studies on the dorsal root ganglion (DRG), a key structure in peripheral nerve health. The DRG represents an opportunity to interface far more easily with the peripheral sensory system. An important example of this involves new non-invasive epineural DRG electrodes, which have recently been found to perform comparably to invasive electrodes in the laboratory, providing a clearer path to clinical translation[243]; although, as the study points out, further work on electrode density, configuration, and fixation is required and ongoing.

9 Intraoperative Neurophysiology

Human intraoperative recordings are intuitively appealing due to the direct access to nerves and theoretically should be an indispensable tool for nerve surgeons and surgeries where there is a risk of nerve injury. They have the potential to provide vital functional, diagnostic, and prognostic information to the surgical team that cannot be obtained in other ways[244]. However, current intraoperative

neurophysiological approaches generally focus on central pathways using evoked potentials and peripheral nerve specific measures are relatively rarely applied in peripheral nerve surgery. However, the effectiveness of techniques such as intraoperative NAP recording has been documented for decades[245,246] and is increasing[244,247–249]. The lack of uptake has stemmed from difficulty in obtaining recordings such as CNAPs, mainly due to stimulus artefact; although, several approaches have been taken to improve recordings, including the above mentioned alternate forms of stimulation that avoid stimulation artefact[185,246,250,251].

With direct access to nerve, intraoperative neurophysiology affords the ability to obtain somatosensory evoked potentials from along the peripheral nerve. This may be capable of revealing the presence and location of neuronal regeneration along the nerve[252–254], which would be vital information in surgical decision making by preventing unnecessary repair procedures. This information could potentially be obtained in clinic through needle electrodes, but the spread of stimulation from a percutaneously placed needle electrode is unknown and may result in false positives from nearby non-neuronal tissue stimulation. Additionally, the intraoperative set up is already in place for recording SSEPs and spatially selective neuronal stimulation is more easily ensured with direct visualization and low current[246].

Other measures such as CMAP, are usually done using intramuscular monopolar needles with bare cannulas, for which an all or none response is generally observed. Unlike clinic-based neurophysiology, there are currently no reference values for intraoperative CNAPs or CMAPs and intraoperative MUNEs are rarely, if ever, calculated. Bioelectrical impedance measurement, as discussed above, has not been attempted on nerve or muscle yet, which would offer a valuable and rapid new intraoperative perspective on nerve health, if technically feasible in the intraoperative environment. Microneurography has the promise of identifying regenerating axons before they are detectable by conventional NAP, as well as ectopic discharges from painful neuromas; however, significant challenges regarding practicality would need to be overcome to implement this intraoperatively given time taken and equipment required. Finally, alternate categories of electrode may help reduce stimulation artifact and improve NAP recording intraoperatively, including using magnetic stimulation and recording micro-coils, currently applied to intracortical stimulation[179,180]. Given the unrivalled access to peripheral nerve offered intraoperatively, there is a significant opportunity to obtain valuable information about nerve health via numerous potential intraoperative measurements if practical, reliable, and rapid approaches can be developed in the challenging surgical environment.

Conclusion

Advances in neurophysiological biomarker and outcome measures represent a powerful method of expediting progress in neurotherapeutic research and clinical practice.

The present scoping review serves as a complement to Part 2, which specifically concentrates on non-invasive imaging. and aims to accomplish two objectives. Through Part 1 of this scoping review, we were able to bring under one roof the array of neurophysiological techniques that currently provide, or have a potential to provide, valuable insights into nerve health both in clinical and research settings. We have also provided commentary, where feasible, on the fundamental mechanisms, performance characteristics, and pragmatic considerations for implementing these techniques.

We provide a comprehensive and updated resource on the wide spectrum of current and emerging neurophysiological approaches related to nerve health, degeneration, and regeneration research and

clinical care. Lastly, in addition to aiding in the selection of appropriate metrics of nerve health, we highlighted numerous areas of promise and nascent technologies that represent potentially fertile avenues of further research.

References

1. England, J. D. & Asbury, A. K. Peripheral neuropathy. *Lancet Lond. Engl.* **363**, 2151–2161 (2004).

2. Martyn, C. N. & Hughes, R. A. Epidemiology of peripheral neuropathy. *J. Neurol. Neurosurg. Psychiatry* **62**, 310–318 (1997).

3. Gordon, T. Peripheral Nerve Regeneration and Muscle Reinnervation. *Int. J. Mol. Sci.* **21**, 8652 (2020).

4. Bazarek, S. *et al.* Spinal motor neuron transplantation to enhance nerve reconstruction strategies: Towards a cell therapy. *Exp. Neurol.* 114054 (2022) doi:10.1016/j.expneurol.2022.114054.

5. Ghergherehchi, C. L. *et al.* Polyethylene glycol (PEG) and other bioactive solutions with neurorrhaphy for rapid and dramatic repair of peripheral nerve lesions by PEG-fusion. *J. Neurosci. Methods* **314**, 1–12 (2019).

6. Lanier, S. T., Hill, J. R., Dy, C. J. & Brogan, D. M. Evolving Techniques in Peripheral Nerve Regeneration. *J. Hand Surg.* **46**, 695–701 (2021).

7. Carvalho, C. R., Oliveira, J. M. & Reis, R. L. Modern Trends for Peripheral Nerve Repair and Regeneration: Beyond the Hollow Nerve Guidance Conduit. *Front. Bioeng. Biotechnol.* **7**, (2019).

8. Arezzo, J. C., Seto, S. & Schaumburg, H. H. Sensory-motor assessment in clinical research trials. in *Handbook of Clinical Neurology* vol. 115 265–278 (Elsevier, 2013).

9. Wood, M. D., Kemp, S. W. P., Weber, C., Borschel, G. H. & Gordon, T. Outcome measures of peripheral nerve regeneration. *Ann. Anat. - Anat. Anz.* **193**, 321–333 (2011).

10. Dumitru, D. *Electrodiagnostic medicine*. (Hanley & Belfus ; Mosby, 1995).

11. *Peripheral Neuropathy Volume 2 Edited by Peter J. Dyck and P.K. Thomas*. (London, 2005).

12. Bramer, W. M., Rethlefsen, M. L., Kleijnen, J. & Franco, O. H. Optimal database combinations for literature searches in systematic reviews: a prospective exploratory study. *Syst. Rev.* **6**, 245 (2017).

13. Tricco, A. C. *et al.* PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann. Intern. Med.* **169**, 467–473 (2018).

14. JBI Manual for Evidence Synthesis - JBI Global Wiki. https://jbi-global-wiki.refined.site/space/MANUAL.

15. Navarro, X. & Udina, E. Chapter 6 Methods and Protocols in Peripheral Nerve Regeneration Experimental Research. in *International Review of Neurobiology* vol. 87 105–126 (Elsevier, 2009).

16. Stålberg, E. *et al.* Standards for quantification of EMG and neurography. *Clin. Neurophysiol.* **130**, 1688–1729 (2019).

17. Sanders, D. B., Stålberg, E. V. & Nandedkar, S. D. Analysis of the electromyographic interference pattern. *J. Clin. Neurophysiol. Off. Publ. Am. Electroencephalogr. Soc.* **13**, 385–400 (1996).

18. Nandedkar, S. D. & Stålberg, E. V. Quantitative measurements and analysis in electrodiagnostic studies: present and future. *Future Neurol.* **3**, 745–764 (2008).

19. Kimura, J. *Electrodiagnosis in Diseases of Nerve and Muscle: Principles and Practice*. (Oxford University Press, 2013).

20. Sorenson, E. J. Sensory Nerve Conduction Studies: Sensory Nerve Action Potentials. in *Clinical Neurophysiology* (eds. Rubin, D. I. & Daube, J. R.) 292–311 (Oxford University Press, 2016). doi:10.1093/med/9780190259631.003.0018.

21. KIMURA, J. Clinical electrophysiology of peripheral nervous system axons. in *The Axon: Structure, Function and Pathophysiology* (eds. Waxman, S. G., Kocsis, J. D. & Stys, P. K.) 0 (Oxford University Press, 1995). doi:10.1093/acprof:oso/9780195082937.003.0030.

22. Dumitru, D. & Delisa, J. A. AAEM minimonograph #10: Volume conduction. *Muscle Nerve* **14**, 605–624 (1991).

23. McComas, A. J., Fawcett, P. R. W., Campbell, M. J. & Sica, R. E. P. Electrophysiological estimation of the number of motor units within a human muscle. *J. Neurol. Neurosurg. Psychiatry* **34**, 121–131 (1971).

24. Wohlfart, G. Collateral regeneration in partially denervated muscles. *Neurology* **8**, 175–180 (1958).

25. Bromberg, M. B. Updating motor unit number estimation (MUNE). *Clin. Neurophysiol.* **118**, 1–8 (2007).

26. Krarup, C. & Buchthal, F. Conduction studies in peripheral nerve. *Neurobehav. Toxicol. Teratol.* **7**, 319–323 (1985).

27. Li, C., Houlden, D. A. & Rowed, D. W. Somatosensory evoked potentials and neurological grades as predictors of outcome in acute spinal cord injury. *J. Neurosurg.* **72**, 600–609 (1990).

28. Lorenzini, N. A. & Poterack, K. A. Somatosensory evoked potentials are not a sensitive indicator of potential positioning injury in the prone patient. *J. Clin. Monit.* **12**, 171–176 (1996).

29. Curt, A., Keck, M. E. & Dietz, V. Functional outcome following spinal cord injury: Significance of motor-evoked potentials and ASIA scores. *Arch. Phys. Med. Rehabil.* **79**, 81–86 (1998).

30. Wiertel-Krawczuk, A. & Huber, J. Standard neurophysiological studies and motor evoked potentials in evaluation of traumatic brachial plexus injuries – A brief review of the literature. *Neurol. Neurochir. Pol.* **52**, 549–554 (2018).

31. Mandeville, R. M., Brown, J. M., Gertsch, J. H. & Allison, D. W. Use of Posterior Root-Muscle Reflexes in Peripheral Nerve Surgery: A Case Report. *Neurodiagnostic J.* **56**, 178–185 (2016).

32. Ellaway, P. H. *et al.* Review of physiological motor outcome measures in spinal cord injury using transcranial magnetic stimulation and spinal reflexes. *J. Rehabil. Res. Dev.* **44**, 69 (2007).

33. Bromberg, M. B. The motor unit and quantitative electromyography. *Muscle Nerve* **61**, 131–142 (2020).

34. Stålberg, E. Macro EMG, a new recording technique. *J. Neurol. Neurosurg. Psychiatry* **43**, 475–482 (1980).

35. Jabre, J. F. Concentric macro electromyography. *Muscle Nerve* **14**, 820–825 (1991).

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

37. Mandeville, R. M., Brown, J. M. & Sheean, G. L. Semi-quantitative electromyography as a predictor of nerve transfer outcome. *Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol.* **130**, 701–706 (2019).

38. Stålberg, E. *et al.* Automatic analysis of the EMG interference pattern. *Electroencephalogr. Clin. Neurophysiol.* **56**, 672–681 (1983).

39. Narayanaswami, P. *et al.* Critically re-evaluating a common technique. *Neurology* **86**, 218–223 (2016).

40. Jiang, G.-L., Zhang, L.-Y., Shen, L.-Y., Xu, J.-G. & Gu, Y.-D. Fibrillation potential amplitude to quantitatively assess denervation muscle atrophy. *Neuromuscul. Disord.* **10**, 85–91 (2000).

41. Stålberg, E., Falck, B., Sonoo, M., Stålberg, S. & Åström, M. Multi-MUP EMG analysis — a two year experience in daily clinical work. *Electroencephalogr. Clin. Neurophysiol. Mot. Control* **97**, 145–154 (1995).

42. McGill, K. C., Lateva, Z. C. & Marateb, H. R. EMGLAB: An interactive EMG decomposition program. *J. Neurosci. Methods* **149**, 121–133 (2005).

43. Doherty, T. J. & Stashuk, D. W. Decomposition-based quantitative electromyography: Methods and initial normative data in five muscles. *Muscle Nerve* **28**, 204–211 (2003).

44. Abdelmaseeh, M., Smith, B. & Stashuk, D. Feature selection for motor unit potential train characterization: Feature Selection for MUPT Characterization. *Muscle Nerve* **49**, 680–690 (2014).

45. Piasecki, M., Estruch, O. G. C. & Stashuk, D. W. *Near-Fibre Electromyography*. http://medrxiv.org/lookup/doi/10.1101/2020.10.07.20208348 (2020) doi:10.1101/2020.10.07.20208348.

46. Garnés-Camarena, O., Díaz-Cano, G. & Stashuk, D. Motor unit electrophysiological changes in Guillain-Barré syndrome in the context of a COVID-19 infection. *Muscle Nerve* **64**, E23–E25 (2021).

47. Stålberg, E. V. & Sonoo, M. Assessment of variability in the shape of the motor unit action potential, the "jiggle," at consecutive discharges. *Muscle Nerve* **17**, 1135–1144 (1994).

48. Dobrowolski, A., Tomczykiewicz, K. & Komur, P. Spectral Analysis of Motor Unit Action Potentials. *IEEE Trans. Biomed. Eng.* **54**, 2300–2302 (2007).

49. French, A. S. & Holden, A. V. Frequency domain analysis of neurophysiological data. *Comput. Programs Biomed.* **1**, 219–234 (1971).

50. Pfeiffer, G. The diagnostic power of motor unit potential analysis: An objective Bayesian approach. *Muscle Nerve* **22**, 584–591 (1999).

51. Yamada, R., Ushiba, J., Tomita, Y. & Masakado, Y. Decomposition of electromyographic signal by principal component analysis of wavelet coefficients. in *IEEE EMBS Asian-Pacific Conference on Biomedical Engineering, 2003.* 118–119 (2003). doi:10.1109/APBME.2003.1302612.

52. Pino, L. J., Stashuk, D. W., Boe, S. G. & Doherty, T. J. Motor unit potential characterization using "pattern discovery". *Med. Eng. Phys.* **30**, 563–573 (2008).

53. Katsis, C. D., Goletsis, Y., Likas, A., Fotiadis, D. I. & Sarmas, I. A novel method for automated EMG decomposition and MUAP classification. *Artif. Intell. Med.* **37**, 55–64 (2006).

54. Christodoulou, C. I. & Pattichis, C. S. Unsupervised pattern recognition for the classification of EMG signals. *IEEE Trans. Biomed. Eng.* **46**, 169–178 (1999).

55. Katsis, C. D. *et al.* A two-stage method for MUAP classification based on EMG decomposition. *Comput. Biol. Med.* **37**, 1232–1240 (2007).

56. Xie, H., Huang, H. & Wang, Z. Multiple Feature Domains Information Fusion for Computer-Aided Clinical Electromyography. in 304–312 (Springer Berlin Heidelberg, 2005). doi:10.1007/11556121_38.

57. Farkas, C., Hamilton-Wright, A., Parsaei, H. & Stashuk, D. W. A Review of Clinical Quantitative Electromyography. *Crit. Rev. Biomed. Eng.* **38**, (2010).

58. Nandedkar, S. D. Emerging Techniques in the Electrodiagnostic Laboratory. *PM&R* **5**, S115–S122 (2013).

59. Fuglsang-Frederiksen, A. & Rønager, J. The motor unit firing rate and the power spectrum of EMG in humans. *Electroencephalogr. Clin. Neurophysiol.* **70**, 68–72 (1988).

60. Rønager, J., Christensen, H. & Fuglsang-Frederiksen, A. Power spectrum analysis of the EMG pattern in normal and diseased muscles. *J. Neurol. Sci.* **94**, 283–294 (1989).

61. Gilchrist, J. M. *et al.* Automatic analysis of the electromyographic interference pattern using the turns: amplitude ratio. *Electroencephalogr. Clin. Neurophysiol.* **70**, 534–540 (1988).

62. Fuglsang-Frederiksen, A. & RØnager, J. EMG power spectrum, turns-amplitude analysis and motor unit potential duration in neuromuscular disorders. *J. Neurol. Sci.* **97**, 81–91 (1990).

63. Sheean, G. L. Quantification of motor unit action potential energy. *Clin. Neurophysiol.* **123**, 621–625 (2012).

64. Sheean, G. L. Application of time-varying analysis to diagnostic needle electromyography. *Med. Eng. Phys.* **34**, 249–255 (2012).

65. Nandedkar, S. D., Sanders, D. B. & Stålberg, E. V. Automatic analysis of the electromyographic interference pattern. Part I: Development of quantitative features. *Muscle Nerve* **9**, 431–439 (1986).

66. Fuglsang-Frederiksen, A., Monaco, M. L. & Dahl, K. Turns analysis (peak ratio) in EMG using the mean amplitude as a substitute of force measurement. *Electroencephalogr. Clin. Neurophysiol.* **60**, 225–227 (1985).

67. Nirkko, A. C., Rösler, K. M. & Hess, C. W. Sensitivity and specificity of needle electromyography: a prospective study comparing automated interference pattern analysis with single motor unit potential analysis. *Electroencephalogr. Clin. Neurophysiol. Mot. Control* **97**, 1–10 (1995).

68. Statham, M. M., Rosen, C. A., Nandedkar, S. D. & Munin, M. C. Quantitative laryngeal electromyography: Turns and amplitude analysis. *The Laryngoscope* **120**, 2036–2041 (2010).

69. Smith, L. J., Rosen, C. A., Niyonkuru, C. & Munin, M. C. Quantitative electromyography improves prediction in vocal fold paralysis. *The Laryngoscope* **122**, 854–859 (2012).

70. Kneisz, L. *et al.* Objectivation of laryngeal electromyography (LEMG) data: turn number vs. qualitative analysis. *Eur. Arch. Otorhinolaryngol.* **277**, 1409–1415 (2020).

71. Hou, Z., Zhu, J. & Su, Z. The clinical evaluation of peripheral nerve regeneration using turns/amplitude analysis system in EMG. *Electromyogr. Clin. Neurophysiol.* **39**, 489–492 (1999).

72. Cao, J. & Sanders, D. B. Multivariate discriminant analysis of the electromyographic interference pattern: statistical approach to discrimination among controls, myopathies and neuropathies. *Med. Biol. Eng. Comput.* **34**, 369–374 (1996).

73. Brown, M. C. & Ironton, R. Sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles. *J. Physiol.* **278**, 325–348 (1978).

74. Stalberg, E. & Thiele, B. Motor unit fibre density in the extensor digitorum communis muscle. Single fibre electromyographic study in normal subjects at different ages. *J. Neurol. Neurosurg. Psychiatry* **38**, 874–880 (1975).

75. McComas, A. J. Reflections on motor unit number estimation. *Muscle Nerve* **51**, 623–623 (2015).

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

77. Shefner, J. M., Cudkowicz, M. E. & Brown, R. H. Comparison of incremental with multipoint MUNE methods in transgenic ALS mice. *Muscle Nerve* **25**, 39–42 (2002).

78. Bromberg, M. B. Motor unit estimation: Reproducibility of the spike-triggered averaging technique in normal and ALS subjects. *Muscle Nerve* **16**, 466–471 (1993).

79. Gooch, C. L. *et al.* Motor unit number estimation: a technology and literature review. *Muscle Nerve* **50**, 884–893 (2014).

80. Stashuk, D. W., Doherty, T. J., Kassam, A. & Brown, W. F. Motor unit number estimates based on the automated analysis of F-responses. *Muscle Nerve* **17**, 881–890 (1994).

81. Lomen-Hoerth, C. & Slawnych, M. P. Statistical motor unit number estimation: From theory to practice. *Muscle Nerve* **28**, 263–272 (2003).

82. Daube, J. R. Estimating the Number of Motor Units in a Muscle. *J. Clin. Neurophysiol.* **12**, 585–594 (1995).

83. Kadrie, H. A., Yates, S. K., Milner-Brown, H. S. & Brown, W. F. Multiple point electrical stimulation of ulnar and median nerves. *J. Neurol. Neurosurg. Psychiatry* **39**, 973–985 (1976).

84. Shefner, J. M. *et al.* Multipoint incremental motor unit number estimation as an outcome measure in ALS. *Neurology* **77**, 235–241 (2011).

85. Kasselman, L. J., Shefner, J. M. & Rutkove, S. B. Motor unit number estimation in the rat tail using a modified multipoint stimulation technique: Rat Tail MUNE. *Muscle Nerve* **40**, 115–121 (2009).

86. Wang, F.-C. & Delwaide, P. J. Number and relative size of thenar motor units estimated by an adapted multiple point stimulation method. *Muscle Nerve* **18**, 969–979 (1995).

87. Doherty, T. J., Stashuk, D. W. & Brown, W. F. Determinants of mean motor unit size: Impact on estimates of motor unit number. *Muscle Nerve* **16**, 1326–1331 (1993).

88. Calancie, B. & Bawa, P. Limitations of the spike-triggered averaging technique. *Muscle Nerve* **9**, 78–83 (1986).

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

90. Ridall, P. G., Pettitt, A. N., Henderson, R. D. & McCombe, P. A. Motor Unit Number Estimation-A Bayesian Approach. *Biometrics* **62**, 1235–1250 (2006).

91. Carvalho, V. C., Cintra, A. I. & Santo Neto, H. Motor units and functional anatomy of the human musculus opponens digiti minimi. *Anat. Anz.* **165**, 65–68 (1988).

92. Neto, H. S., Filho, J. M., Passini, R. & Marques, M. J. Number and size of motor units in thenar muscles. *Clin. Anat.* **17**, 308–311 (2004).

93. Neto, H. S., de Carvalho, V. C. & Marques, M. J. Estimation of the number and size of human flexor digiti minimi muscle motor units using histological methods. *Muscle Nerve* **21**, 112–114 (1998).

94. Gesslbauer, B. *et al.* Axonal components of nerves innervating the human arm: Arm Nerve Axonal Components. *Ann. Neurol.* **82**, 396–408 (2017).

95. Feinstein, B., Lindegård, B., Nyman, E. & Wohlfart, G. MORPHOLOGIC STUDIES OF MOTOR UNITS IN NORMAL HUMAN MUSCLES. *Cells Tissues Organs* **23**, 127–142 (1955).

96. Nandedkar, S. D., Nandedkar, D. S., Barkhaus, P. E. & Stalberg, E. V. Motor Unit Number Index (MUNIX). *IEEE Trans. Biomed. Eng.* **51**, 2209–2211 (2004).

97. Neuwirth, C. *et al.* Motor Unit Number Index (MUNIX): A novel neurophysiological marker for neuromuscular disorders; test–retest reliability in healthy volunteers. *Clin. Neurophysiol.* **122**, 1867–1872 (2011).

98. Blok, J. H., Ruitenberg, A., Maathuis, E. M. & Visser, G. H. The electrophysiological muscle scan. *Muscle Nerve* **36**, 436–446 (2007).

99. Sleutjes, B. T. H. M. *et al.* CMAP scan discontinuities: Automated detection and relation to motor unit loss. *Clin. Neurophysiol.* **125**, 388–395 (2014).

100. Kesim-Sahin, O. *et al.* Compound muscle action potential scan and MScanFit motor unit number estimation during Wallerian degeneration after nerve transections. *Muscle Nerve* **62**, 239–246 (2020).

101. Bostock, H. Estimating motor unit numbers from a CMAP scan: CMAP Scan MUNE. *Muscle Nerve* **53**, 889–896 (2016).

102. Jacobsen, A. B., Bostock, H. & Tankisi, H. Following disease progression in motor neuron disorders with 3 motor unit number estimation methods: MUNE in ALS. *Muscle Nerve* **59**, 82–87 (2019).

103. Nandedkar, S. D., Barkhaus, P. E. & V Stålberg, E. Analysis of the compound muscle action potential scan: Step Index (STEPIX) and Amplitude Index (AMPIX). *Clin. Neurophysiol.* S1388245722002450 (2022) doi:10.1016/j.clinph.2022.04.011.

104. van Dijk, J. P. *et al.* Motor unit number estimation using high-density surface electromyography. *Clin. Neurophysiol.* **119**, 33–42 (2008).

105. Peng, Y. *et al.* Motor unit number estimation based on high-density surface electromyography decomposition. *Clin. Neurophysiol.* **127**, 3059–3065 (2016).

106. Chen, M., Bashford, J. & Zhou, P. Motor Unit Number Estimation (MUNE) Free of Electrical Stimulation or M Wave Recording: Feasibility and Challenges. *Front. Aging Neurosci.* **14**, (2022).

107. Jabre, J. F., Pitt, M. C., Deeb, J. & Chui, K. K. H. E-Norms: A Method to Extrapolate Reference Values From a Laboratory Population. *J. Clin. Neurophysiol.* **32**, 265–270 (2015).

108. Henneman, E. THE SIZE-PRINCIPLE: A DETERMINISTIC OUTPUT EMERGES FROM A SET OF PROBABILISTIC CONNECTIONS. 8.

109. E.V. Stalberg. Single Fiber and Macro EMG: A Workshop. in (2003).

110. Dengler, R. *et al.* Amyotrophic lateral sclerosis: Macro-EMG and twitch forces of single motor units: Motor Units in ALS. *Muscle Nerve* **13**, 545–550 (1990).

111. Dengler, R., Konstanzer, A., Hesse, S., Schubert, M. & Wolf, W. Collateral nerve sprouting and twitch forces of single motor units in conditions with partial denervation in man. *Neurosci. Lett.* **97**, 118–122 (1989).

112. Milner-Brown, H. S., Stein, R. B. & Lee, R. G. Contractile and electrical properties of human motor units in neuropathies and motor neurone disease. *J. Neurol. Neurosurg. Psychiatry* **37**, 670–676 (1974).

113. Dorfman, L. J. Quantitative clinical electrophysiology in the evaluation of nerve injury and regeneration. *Muscle Nerve* **13**, 822–828 (1990).

114. Xiao, S. J., McGill, K. C. & Hentz, V. R. Electrophysiological assessment of focal nerve lesions. in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society* 1694–1695 vol.4 (1988). doi:10.1109/IEMBS.1988.94920.

115. Rosenfalck, A. Early recognition of nerve disorders by near-nerve recording of sensory action potentials. *Muscle Nerve* **1**, 360–367 (1978).

116. Hirose, G., Tsuchitani, Y. & Huang, J. A NEW METHOD FOR ESTIMATION OF NERVE CONDUCTION VELOCITY DISTRIBUTION IN THE FREQUENCY DOMAIN t.

117. Smith, K. J. A sensitive method for the detection and quantification of conduction deficits in nerve. *J. Neurol. Sci.* **48**, 191–199 (1980).

118. Walsh, M. E. *et al.* Use of Nerve Conduction Velocity to Assess Peripheral Nerve Health in Aging Mice. *J. Gerontol. Ser. A* **70**, 1312–1319 (2015).

119. Koning, P. de *et al.* Estimation of the number of motor units based on macro-EMG. *J. Neurol. Neurosurg. Psychiatry* **51**, 403–411 (1988).

120. Major, L. A., Hegedus, J., Weber, D. J., Gordon, T. & Jones, K. E. Method for Counting Motor Units in Mice and Validation Using a Mathematical Model. *J. Neurophysiol.* **97**, 1846–1856 (2007).

121. Piasecki, M. *et al.* The reliability of methods to estimate the number and size of human motor units and their use with large limb muscles. *Eur. J. Appl. Physiol.* **118**, 767–775 (2018).

122. Rutkove, S. B. Electrical Impedance Myography: Background, Current State, and Future Directions. *Muscle Nerve* **40**, 936–946 (2009).

123. Sanchez, B. & Rutkove, S. B. Electrical Impedance Myography and Its Applications in Neuromuscular Disorders. *Neurotherapeutics* **14**, 107–118 (2017).

124. Sanchez, B. & Rutkove, S. B. Present Uses, Future Applications, and Technical Underpinnings of Electrical Impedance Myography. *Curr. Neurol. Neurosci. Rep.* **17**, 86 (2017).

125. Sanchez, B., Martinsen, O. G., Freeborn, T. J. & Furse, C. M. Electrical impedance myography: A critical review and outlook. *Clin. Neurophysiol.* **132**, 338–344 (2021).

126. Ahad, M. & Rutkove, S. B. Correlation between muscle electrical impedance data and standard neurophysiologic parameters after experimental neurogenic injury. *Physiol. Meas.* **31**, 1437 (2010).

127. Kwon, H., Malik, W. Q., Rutkove, S. B. & Sanchez, B. Separation of Subcutaneous Fat From Muscle in Surface Electrical Impedance Myography Measurements Using Model Component Analysis. *IEEE Trans. Biomed. Eng.* **66**, 354–364 (2019).

128. Nagy, J. A., DiDonato, C. J., Rutkove, S. B. & Sanchez, B. Permittivity of ex vivo healthy and diseased murine skeletal muscle from 10 kHz to 1 MHz. *Sci. Data* **6**, 37 (2019).

129. Nagy, J. A., Semple, C., Riveros, D., Sanchez, B. & Rutkove, S. B. Altered electrical properties in skeletal muscle of mice with glycogen storage disease type II. *Sci. Rep.* **12**, 5327 (2022).

130. Kapur, K., Nagy, J. A., Taylor, R. S., Sanchez, B. & Rutkove, S. B. Estimating myofiber size with electrical impedance myography: a study in amyotrophic lateral sclerosis mice. *Muscle Nerve* **58**, 713–717 (2018).

131. Rutkove, S. B., Kwon, H., Guasch, M., Wu, J. S. & Sanchez, B. Electrical impedance imaging of human muscle at the microscopic scale using a multi-electrode needle device: A simulation study. *Clin. Neurophysiol.* **129**, 1704–1708 (2018).

132. Ahad, M., Fogerson, P. M., Rosen, G., Narayanaswami, P. & Rutkove, S. B. Electrical characteristics of rat skeletal muscle in immaturity, adulthood, and after sciatic nerve injury and their relation to muscle fiber size. *Physiol. Meas.* **30**, 1415 (2009).

133. Roy, B. *et al.* Exploring the relationship between electrical impedance myography and quantitative ultrasound parameters in Duchenne muscular dystrophy. *Clin. Neurophysiol.* **130**, 515–520 (2019).

134. Kwon, H., Rutkove, S. B. & Sanchez, B. Recording characteristics of electrical impedance myography needle electrodes. *Physiol. Meas.* **38**, 1748–1765 (2017).

135. Rutkove, S. B. *et al.* Design and pilot testing of a 26-gauge impedance-electromyography needle in wild-type and ALS mice. *Muscle Nerve* **65**, 702–708 (2022).

136. Kwon, H., Park, H. C., Barrera, A. C., Rutkove, S. B. & Sanchez, B. On the measurement of skeletal muscle anisotropic permittivity property with a single cross-shaped needle insertion. *Sci. Rep.* **12**, 8494 (2022).

137. Kwon, H., Guasch, M., Nagy, J. A., Rutkove, S. B. & Sanchez, B. New electrical impedance methods for the in situ measurement of the complex permittivity of anisotropic skeletal muscle using multipolar needles. *Sci. Rep.* **9**, 3145 (2019).

138. Murphy, E. K., Skinner, J., Martucci, M., Rutkove, S. B. & Halter, R. J. Toward Electrical Impedance Tomography Coupled Ultrasound Imaging for Assessing Muscle Health. *IEEE Trans. Med. Imaging* **38**, 1409–1419 (2019).

139. Morales-Sanchez, E., Prokhorov, F., Llamas, F. & Gonzalez-Hernandez, J. Nerves location using impedance measurements. in *(ICEEE). 1st International Conference on Electrical and Electronics Engineering, 2004.* 536–538 (2004). doi:10.1109/ICEEE.2004.1433943.

140. Cole, K. S. & Curtis, H. J. Electric Impedance of Nerve and Muscle. *Cold Spring Harb. Symp. Quant. Biol.* **4**, 73–89 (1936).

141. Cole, K. S. & Curtis, H. J. Electrical Impedance of Nerve During Activity. *Nature* **142**, 209–210 (1938).

142. Fouchard, A. *et al.* Functional monitoring of peripheral nerves from electrical impedance measurements. *J. Physiol.-Paris* **110**, 361–371 (2016).

143. Ravagli, E. *et al.* Fascicle localisation within peripheral nerves through evoked activity recordings: A comparison between electrical impedance tomography and multi-electrode arrays. *J. Neurosci. Methods* 109140 (2021) doi:10.1016/j.jneumeth.2021.109140.

144. Ravagli, E., Mastitskaya, S., Thompson, N., Aristovich, K. & Holder, D. Optimization of the electrode drive pattern for imaging fascicular compound action potentials in peripheral nerve with fast neural electrical impedance tomography. *Physiol. Meas.* **40**, 115007 (2019).

145. Bostock, H. The strength-duration relationship for excitation of myelinated nerve: computed dependence on membrane parameters. *J. Physiol.* **341**, 59–74 (1983).

146. Burke, D., Kiernan, M. C. & Bostock, H. Excitability of human axons. *Clin. Neurophysiol.* **112**, 1575–1585 (2001).

147. Bostock, H., Sharief, M. K., Reid, G. & Murray, N. M. F. Axonal ion channel dysfunction in amyotrophic lateral sclerosis. *Brain* **118**, 217–225 (1995).

148. Mansouri, B., Adybeig, B., Rayegani, M., Yasami, S. & Behshad, V. Uremic neuropathy and the analysis of electrophysiological changes. *Electromyogr. Clin. Neurophysiol.* **41**, 107–115 (2001).

149. Parry, C. B. W., Salter, M. & Millar, D. *Rehabilitation of the Hand*. (Butterworths, 1981).

150. Paternostro-Sluga, T., Schuhfried, O., Vacariu, G., Lang, T. & Fialka-Moser, V. Chronaxie and Accommodation Index in the Diagnosis of Muscle Denervation. *Am. J. Phys. Med. Rehabil.* **81**, 253–260 (2002).

151. MacKenzie, I. G. Strength-duration curves in poliomyelitis. (1948).

152. Hagbarth, K.-E. Microelectrode recordings from human peripheral nerves (microneurography). *Muscle Nerve. Suppl.* **11**, S28-35 (2002).

153. Terkelsen, A. J. *et al.* The diagnostic challenge of small fibre neuropathy: clinical presentations, evaluations, and causes. *Lancet Neurol.* **16**, 934–944 (2017).

154. Dunham, J. P., Sales, A. C. & Pickering, A. E. Ultrasound-guided, open-source microneurography: Approaches to improve recordings from peripheral nerves in man. *Clin. Neurophysiol.* **129**, 2475–2481 (2018).

155. Swinney, K. R. & Wikswo, J. P. A calculation of the magnetic field of a nerve action potential. *Biophys. J.* **32**, 719–731 (1980).

156. Mackert, B.-M. Magnetoneurography: theory and application to peripheral nerve disorders. *Clin. Neurophysiol.* **115**, 2667–2676 (2004).

157. Nakanishi, K. *et al.* Wide-range visualization of compound nerve action magnetic fields in the human median and ulnar nerves from the forearm to Erb's point. *Neurosci. Lett.* **356**, 151–153 (2004).

158. Adachi, Y. *et al.* Multichannel SQUID Magnetoneurograph System for Functional Imaging of Spinal Cords and Peripheral Nerves. *IEEE Trans. Appl. Supercond.* **31**, 1–5 (2021).

159. Boto, E. *et al.* A new generation of magnetoencephalography: Room temperature measurements using optically-pumped magnetometers. *NeuroImage* **149**, 404–414 (2017).

160. Bu, Y. *et al.* Peripheral Nerve Magnetoneurography With Optically Pumped Magnetometers. *Front. Physiol.* **13**, (2022).

161. Sasaki, T. *et al.* Visualization of electrophysiological activity at the carpal tunnel area using magnetoneurography. *Clin. Neurophysiol.* **131**, 951–957 (2020).

162. Downs, M. E. *et al.* Non-invasive peripheral nerve stimulation via focused ultrasound in vivo. *Phys. Med. Biol.* **63**, 035011 (2018).

163. Hoffman, B. U. *et al.* Focused ultrasound excites action potentials in mammalian peripheral neurons in part through the mechanically gated ion channel PIEZO2. *Proc. Natl. Acad. Sci.* **119**, e2115821119 (2022).

164. Kubanek, J. Neuromodulation with transcranial focused ultrasound. *Neurosurg. Focus* **44**, E14 (2018).

165. Wright, C. J., Haqshenas, S. R., Rothwell, J. & Saffari, N. Unmyelinated Peripheral Nerves Can Be Stimulated in Vitro Using Pulsed Ultrasound. *Ultrasound Med. Biol.* **43**, 2269–2283 (2017).

166. Riis, T. & Kubanek, J. Effective Ultrasonic Stimulation in Human Peripheral Nervous System. *IEEE Trans. Biomed. Eng.* **69**, 15–22 (2022).

167. Lee, S. A., Kamimura, H. A. S., Burgess, M. T., Pouliopoulos, A. & Konofagou, E. E. Real-Time Displacement and Cavitation Imaging of Non-Invasive Neuromodulation of the Peripheral Nervous System via Focused Ultrasound. in *2018 IEEE International Ultrasonics Symposium (IUS)* 1–4 (IEEE, 2018). doi:10.1109/ULTSYM.2018.8580011.

168. Guo, H. *et al.* Ultrasound does not activate but can inhibit in vivo mammalian nerves across a wide range of parameters. *Sci. Rep.* **12**, 2182 (2022).

169. Feng, B., Chen, L. & Ilham, S. J. A Review on Ultrasonic Neuromodulation of the Peripheral Nervous System: Enhanced or Suppressed Activities? *Appl. Sci.* **9**, 1637 (2019).

170. Wright, C. J., Rothwell, J. & Saffari, N. Ultrasonic stimulation of peripheral nervous tissue: an investigation into mechanisms. *J. Phys. Conf. Ser.* **581**, 012003 (2015).

171. Yoo, S., Mittelstein, D. R., Hurt, R. C., Lacroix, J. & Shapiro, M. G. Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nat. Commun.* **13**, 493 (2022).

172. Oberg, P. A. Magnetic stimulation of nerve tissue. 10 (1973).

173. Barker, A. T. An introduction to the basic principles of magnetic nerve stimulation. *J. Clin. Neurophysiol. Off. Publ. Am. Electroencephalogr. Soc.* **8**, 26–37 (1991).

174. NON-INVASIVE MAGNETIC STIMULATION OF HUMAN MOTOR CORTEX. *The Lancet* **325**, 1106–1107 (1985).

175. Bischoff, C., Riescher, H., Machetanz, J., Meyer, B. U. & Conrad, B. Comparison of various coils used for magnetic stimulation of peripheral motor nerves: physiological considerations and consequences for diagnostic use. *Electroencephalogr. Clin. Neurophysiol.* **97**, 332–340 (1995).

176. Binkofski, F., Classen, J. & Benecke, R. Stimulation of peripheral nerves using a novel magnetic coil. *Muscle Nerve* **22**, 751–757 (1999).

177. Weber, M., Eisen, A. A., & Aaem. Magnetic stimulation of the central and peripheral nervous systems. *Muscle Nerve* **25**, 160–175 (2002).

178. Colella, M. *et al.* A study of flex miniaturized coils for focal nerve magnetic stimulation. *Med. Phys.* **n/a**,.

179. Lee, S. W., Thyagarajan, K. & Fried, S. I. Micro-Coil Design Influences the Spatial Extent of Responses to Intracortical Magnetic Stimulation. *IEEE Trans. Biomed. Eng.* **66**, 1680–1694 (2019).

180. Thyagarajan, K. *et al.* Micro-coil probes for magnetic intracortical neural stimulation: Trade-offs in materials and design. *APL Mater.* **9**, 011102 (2021).

181. Magnetic Micro-coils. *The BRAIN Initiative Alliance* https://www.braininitiative.org/toolmakers/resources/magnetic-micro-coils/.

182. Ham, C. L., Engels, J. M., van de Wiel, G. T. & Machielsen, A. Peripheral nerve stimulation during MRI: effects of high gradient amplitudes and switching rates. *J. Magn. Reson. Imaging JMRI* **7**, 933–937 (1997).

183. Tan, E. T. *et al.* Peripheral nerve stimulation limits of a high amplitude and slew rate magnetic field gradient coil for neuroimaging. *Magn. Reson. Med.* **83**, 352–366 (2020).

184. Fork, R. L. Laser Stimulation of Nerve Cells in Aplysia. *Science* **171**, 907–908 (1971).

185. Wells, J. *et al.* Optical stimulation of neural tissue in vivo. *Opt. Lett.* **30**, 504–506 (2005).

186. Wells, J., Konrad, P., Kao, C., Jansen, E. D. & Mahadevan-Jansen, A. Pulsed laser versus electrical energy for peripheral nerve stimulation. *J. Neurosci. Methods* **163**, 326–337 (2007).

187. McCaughey, R. G., Chlebicki, C. & Wong, B. J. F. Novel wavelengths for laser nerve stimulation: NOVEL WAVELENGTHS FOR LASER NERVE STIMULATION. *Lasers Surg. Med.* **42**, 69–75 (2010).

188. Teudt, I. U., Nevel, A. E., Izzo, A. D., Walsh, J. T. & Richter, C.-P. Optical Stimulation of the Facial Nerve: A New Monitoring Technique?: *The Laryngoscope* **117**, 1641–1647 (2007).

189. Duke, A. R. *et al.* Combined optical and electrical stimulation of neural tissue in vivo. *J. Biomed. Opt.* **14**, 060501 (2009).

190. Shapiro, M. G., Homma, K., Villarreal, S., Richter, C.-P. & Bezanilla, F. Infrared light excites cells by changing their electrical capacitance. *Nat. Commun.* **3**, 736 (2012).

191. Plaksin, M., Shapira, E., Kimmel, E. & Shoham, S. Thermal Transients Excite Neurons through Universal Intramembrane Mechanoelectrical Effects. *Phys. Rev. X* **8**, 011043 (2018).

192. Plasma membrane nanoporation as a possible mechanism behind infrared excitation of cells - IOPscience. https://iopscience.iop.org/article/10.1088/1741-2560/11/6/066006/meta.

193. Albert, E. S. *et al.* TRPV4 channels mediate the infrared laser-evoked response in sensory neurons. *J. Neurophysiol.* **107**, 3227–3234 (2012).

194. Wells, J. D. *et al.* Optically mediated nerve stimulation: Identification of injury thresholds. *Lasers Surg. Med.* **39**, 513–526 (2007).

195. Parker, J. L., Shariati, N. H. & Karantonis, D. M. Electrically evoked compound action potential recording in peripheral nerves. *Bioelectron. Med.* **1**, 71–83 (2017).

196. Lefever, R. S. & De Luca, C. J. A Procedure for Decomposing the Myoelectric Signal Into Its Constituent Action Potentials - Part I: Technique, Theory, and Implementation. *IEEE Trans. Biomed. Eng.* **BME-29**, 149–157 (1982).

197. Stuberg, W. Book Reviews: The Treatment of Gait Problems in Cerebral Palsy, by James R. Gage. London, UK: Mac Keith Press, 2004. *J. Child Neurol.* **22**, 242–242 (2007).

198. Kumar, N. G., Kung, T. A. & Cederna, P. S. Regenerative peripheral nerve interfaces for advanced control of upper extremity prosthetic devices. *Hand Clin.* **37**, 425–433 (2021).

199. Rutkove, S. B. & Sanchez, B. Electrical Impedance Methods in Neuromuscular Assessment: An Overview. *Cold Spring Harb. Perspect. Med.* **9**, (2019).

200. Kural, M. A. *et al.* Diagnostic utility of distal nerve conduction studies and sural near-nerve needle recording in polyneuropathy. *Clin. Neurophysiol.* **128**, 1590–1595 (2017).

201. de Kool, B. S. (Stefan) *et al.* Ultrasound-guided near-nerve neurography for early evaluation of nerve regeneration. *J. Neurosci. Methods* **174**, 265–271 (2008).

202. van Neck, J. W. *et al.* Histological validation of ultrasound-guided neurography in early nerve regeneration. *Muscle Nerve* **40**, 967–975 (2009).

203. Kural, M. A., Pugdahl, K., Fuglsang-Frederiksen, A., Andersen, H. & Tankisi, H. Near-Nerve Needle Technique Versus Surface Electrode Recordings in Electrodiagnosis of Diabetic Polyneuropathy. *J. Clin. Neurophysiol.* **33**, 346–349 (2016).

204. Davis, L. A., Gordon, T., Hoffer, J. A., Jhamandas, J. & Stein, R. B. Compound action potentials recorded from mammalian peripheral nerves following ligation or resuturing. *J. Physiol.* **285**, 543–559 (1978).

205. Hagbarth, K.-E. Microneurography: In Vivo Exploration of Impulse Traffic in Human Peripheral Nerve Fibers. in *Electromyography in CNS Disorders* 19–28 (Elsevier, 1984). doi:10.1016/B978-0-409-95144-8.50006-9.

206. Rich, M. M., Bird, S. J., Raps, E. C., McCluskey, L. F. & Teener, J. W. Direct muscle stimulation in acute quadriplegic myopathy. *Muscle Nerve* **20**, 665–673 (1997).

207. De Luca, C. J. D., Adam, A., Wotiz, R., Gilmore, L. D. & Nawab, S. H. Decomposition of Surface EMG Signals. *J. Neurophysiol.* **96**, 1646–1657 (2006).

208. Merletti, R., Knaflitz, M. & DeLuca, C. J. Electrically evoked myoelectric signals. *Crit. Rev. Biomed. Eng.* **19**, 293–340 (1992).

209. Merletti, R. & Cerone, G. L. Tutorial. Surface EMG detection, conditioning and pre-processing: Best practices. *J. Electromyogr. Kinesiol. Off. J. Int. Soc. Electrophysiol. Kinesiol.* **54**, 102440 (2020).

210. Tang, W. *et al.* Quantitative Assessment of Traumatic Upper-Limb Peripheral Nerve Injuries Using Surface Electromyography. *Front. Bioeng. Biotechnol.* **8**, 795 (2020).

211. Nawab, S. H., Chang, S.-S. & Luca, C. J. D. High-yield decomposition of surface EMG signals. *Clin. Neurophysiol.* **121**, 1602–1615 (2010).

212. Marquez-Chin, C. & Popovic, M. R. Functional electrical stimulation therapy for restoration of motor function after spinal cord injury and stroke: a review. *Biomed. Eng. OnLine* **19**, 34 (2020).

213. Drost, G., Stegeman, D. F., van Engelen, B. G. M. & Zwarts, M. J. Clinical applications of high-density surface EMG: A systematic review. *J. Electromyogr. Kinesiol.* **16**, 586–602 (2006).

214. Kuykendal, M., Potter, S., Grover, M. & DeWeerth, S. Targeted Stimulation Using Differences in Activation Probability across the Strength–Duration Space. *Processes* **5**, 14 (2017).

215. Volk, G. *et al.* Tolerability of facial electrostimulation in healthy adults and patients with facial synkinesis. *Eur. Arch. Otorhinolaryngol.* **277**, (2020).

216. Ibitoye, M. O., Hamzaid, N. A., Zuniga, J. M. & Abdul Wahab, A. K. Mechanomyography and muscle function assessment: A review of current state and prospects. *Clin. Biomech.* **29**, 691–704 (2014).

217. Watakabe, M., Itoh, Y., Mita, K. & Akataki, K. Technical aspects of mechnomyography recording with piezoelectric contact sensor. *Med. Biol. Eng. Comput.* **36**, 557–561 (1998).

218. Orizio, C. Surface Mechanomyogram. in *Electromyography* 305–322 (John Wiley & Sons, Ltd, 2004). doi:10.1002/0471678384.ch11.

219. Wessell, N., Khalil, J., Zavatsky, J., Ghacham, W. & Bartol, S. Verification of nerve decompression using mechanomyography. *Spine J.* **16**, 679–686 (2016).

220. Guerrero, J. R. *et al.* Incorporating Intraoperative Mechanomyography to Peripheral Nerve Decompression Surgery. *Oper. Neurosurg.* 10.1227/ons.0000000000000554 (2022) doi:10.1227/ons.0000000000000554.

221. Shahriari, D., Rosenfeld, D. & Anikeeva, P. Emerging Frontier of Peripheral Nerve and Organ Interfaces. *Neuron* **108**, 270–285 (2020).

222. Lee, S. *et al.* Toward Bioelectronic Medicine—Neuromodulation of Small Peripheral Nerves Using Flexible Neural Clip. *Adv. Sci.* **4**, 1700149 (2017).

223. Rijnbeek, E. H., Eleveld, N. & Olthuis, W. Update on Peripheral Nerve Electrodes for Closed-Loop Neuroprosthetics. *Front. Neurosci.* **12**, (2018).

224. Kim, S. J. *et al.* Stretchable and Transparent Biointerface Using Cell-Sheet–Graphene Hybrid for Electrophysiology and Therapy of Skeletal Muscle. *Adv. Funct. Mater.* **26**, 3207–3217 (2016).

225. Maynard, E. M., Nordhausen, C. T. & Normann, R. A. The Utah Intracortical Electrode Array: A recording structure for potential brain-computer interfaces. *Electroencephalogr. Clin. Neurophysiol.* **102**, 228–239 (1997).

226. Chandrasekaran, S. *et al.* Historical perspectives, challenges, and future directions of implantable brain-computer interfaces for sensorimotor applications. *Bioelectron. Med.* **7**, 14 (2021).

227. Thompson, C. H., Zoratti, M. J., Langhals, N. B. & Purcell, E. K. Regenerative Electrode Interfaces for Neural Prostheses. *Tissue Eng. Part B Rev.* **22**, 125–135 (2016).

228. Seo, D. *et al.* Wireless Recording in the Peripheral Nervous System with Ultrasonic Neural Dust. *Neuron* **91**, 529–539 (2016).

229. Takano, Y. *et al.* A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. *Neurosci. Lett.* **491**, 40–43 (2011).

230. Jackson, M. P. *et al.* Safety parameter considerations of anodal transcranial Direct Current Stimulation in rats. *Brain. Behav. Immun.* **64**, 152–161 (2017).

231. Boonzaier, J. *et al.* Design and Evaluation of a Rodent-Specific Transcranial Magnetic Stimulation Coil: An In Silico and In Vivo Validation Study. *Neuromodulation* **23**, 324–334 (2020).

232. Sykes, M. *et al.* Differences in Motor Evoked Potentials Induced in Rats by Transcranial Magnetic Stimulation under Two Separate Anesthetics: Implications for Plasticity Studies. *Front. Neural Circuits* **10**, (2016).

233. Arnold, W. D. *et al.* Electrophysiological Motor Unit Number Estimation (MUNE) Measuring Compound Muscle Action Potential (CMAP) in Mouse Hindlimb Muscles. *J. Vis. Exp. JoVE* (2015) doi:10.3791/52899.

234. Aravamuthan, B. R., Gandham, S., Young, A. B. & Rutkove, S. B. Sex may influence motor phenotype in a novel rodent model of cerebral palsy. *Neurobiol. Dis.* **134**, 104711 (2020).

235. Gerasimenko, Y., Roy, R. R. & Edgerton, V. R. Epidural stimulation: Comparison of the spinal circuits that generate and control locomotion in rats, cats and humans. *Exp. Neurol.* **209**, 417–425 (2008).

236. Whelan, P. J. Electromyogram recordings from freely moving animals. *Methods* **30**, 127–141 (2003).

237. English, A. W., Schwartz, G., Meador, W., Sabatier, M. J. & Mulligan, A. Electrical stimulation promotes peripheral axon regeneration by enhanced neuronal neurotrophin signaling. *Dev. Neurobiol.* **67**, 158–172 (2007).

238. Day, S. J. & Hulliger, M. Experimental Simulation of Cat Electromyogram: Evidence for Algebraic Summation of Motor-Unit Action-Potential Trains. *J. Neurophysiol.* **86**, 2144–2158 (2001).

239. Fekete, Z., Horváth, Á. C. & Zátonyi, A. Infrared neuromodulation: a neuroengineering perspective. *J. Neural Eng.* **17**, 051003 (2020).

240. Park, D.-W. *et al.* Graphene-based carbon-layered electrode array technology for neural imaging and optogenetic applications. *Nat. Commun.* **5**, 5258 (2014).

241. Llewellyn, M. E., Thompson, K. R., Deisseroth, K. & Delp, S. L. Orderly recruitment of motor units under optical control in vivo. *Nat. Med.* **16**, 1161–1165 (2010).

242. Xu, X., Mee, T. & Jia, X. New Era of Optogenetics: From the Central to Peripheral Nervous System. *Crit. Rev. Biochem. Mol. Biol.* **55**, 1–16 (2020).

243. Nanivadekar, A. C., Ayers, C. A., Gaunt, R. A., Weber, D. J. & Fisher, L. E. Selectivity of afferent microstimulation at the DRG using epineural and penetrating electrode arrays. *J. Neural Eng.* **17**, 016011 (2020).

244. Happel, L. & Kline, D. Intraoperative neurophysiology of the peripheral nervous system. in *Neurophysiology in Neurosurgery* 413–429 (Elsevier, 2020). doi:10.1016/B978-0-12-815000-9.00030-7.

245. Kline, D. G. & Happel, L. T. A Quarter Century's Experience with Intraoperative Nerve Action Potential Recording. *Can. J. Neurol. Sci. J. Can. Sci. Neurol.* **20**, 3–10 (1993).

246. Robert, E. G., Happel, L. T. & Kline, D. G. INTRAOPERATIVE NERVE ACTION POTENTIAL RECORDINGS: TECHNICAL CONSIDERATIONS, PROBLEMS, AND PITFALLS. *Neurosurgery* **65**, A97–A104 (2009).

247. Crum, B. A., Strommen, J. A. & Stucky, S. C. Peripheral nerve stimulation and monitoring during operative procedures. *Muscle Nerve* **35**, 159–170 (2007).

248. Strommen, J. A., Skinner, S. & Crum, B. A. Neurophysiology during peripheral nerve surgery. in *Handbook of Clinical Neurology* vol. 186 295–318 (Elsevier, 2022).

249. Simon, N. G., Spinner, R. J., Kline, D. G. & Kliot, M. Advances in the neurological and neurosurgical management of peripheral nerve trauma. *J. Neurol. Neurosurg. Psychiatry* **87**, 198–208 (2016).

250. Wu, G. *et al.* Solutions to the technical challenges embedded in the current methods for intraoperative peripheral nerve action potential recordings. *J. Neurosurg.* 1–10 (2019) doi:10.3171/2019.5.JNS19146.

251. Saponaro-González, Á. & Pérez-Lorensu, P. J. Novel approach to continuous neurophysiological monitoring during surgery of peripheral nerve tumors. *Surg. Neurol. Int.* **8**, 184 (2017).

252. Assmus, H. [Somatosensory evoked cortical potentials (SSEP) in regenerating nerves following suture (author's transl)]. *EEG EMG Z. Elektroenzephalogr. Elektromyogr. Verwandte Geb.* **9**, 167–171 (1978).

253. ASSMUS, H. SOMATO-SENSORY EVOKED-POTENTIALS (SSEP) IN PERIPHERAL-NERVE LESIONS AND DURING NERVE REGENERATION. *Electroencephalogr. Clin. Neurophysiol.* **48**, P21–P21 (1980).

254. Kline, D. G. & DeJonge, B. R. Evoked potentials to evaluate peripheral nerve injuries: *Surg. Gynecol. Obstet. Int. Abstr. Surg.* **127**, 1239–1248 (1968).