Published in final edited form as:

J Neural Eng. 2017 June ; 14(3): 036011. doi:10.1088/1741-2552/aa65dd.

Imaging Three-dimensional Innervation Zone Distribution in Muscles from M-wave Recordings

Chuan Zhang¹, Yun Peng¹, Yang Liu¹, Sheng Li², Ping Zhou^{2,3}, William Zev Rymer⁴, and Yingchun Zhang^{1,3,*}

¹University of Houston, Department of Biomedical Engineering, Houston, TX 77204, United States

²University of Texas Health Science Center at Houston, Department of Physical Medicine and Rehabilitation, Houston, TX 77030, United States

³Guangdong Work Injury Rehabilitation Center, Guangzhou, Guangdong 510440, China

⁴Rehabilitation Institute of Chicago, Chicago, IL 60611, United States

Abstract

Objective—To localize neuromuscular junctions in skeletal muscles in vivo which is of great importance in understanding, diagnosing and managing of neuromuscular disorders.

Approach—A three-dimensional (3D) global innervation zone imaging technique was developed to characterize the global distribution of innervation zones, as an indication of the location and features of neuromuscular junctions, using electrically evoked high-density surface electromyogram recordings.

Main results—The performance of the technique was evaluated in the biceps brachii of six intact human subjects. The geometric centers of the distributions of the reconstructed innervation zones were determined with a mean distance of 9.4 ± 1.4 cm from the reference plane, situated at the medial epicondyle of the humerus. A mean depth was calculated as 1.5 ± 0.3 cm from the geometric centers to the closed points over the skin. The results are consistent with those reported in previous histology studies. It was also found that the volumes and distributions of the reconstructed innervation zones increased as the stimulation intensities changed until the supramaximal muscle response was achieved.

Significance—Results have demonstrated the high performance of the proposed imaging technique in noninvasively imaging global distributions of the innervation zones in the 3D muscle space in vivo, and the feasibility of its clinical applications, such as guiding botulinum toxin injections in spasticity management, or in early diagnosis of neurodegenerative progression of amyotrophic lateral sclerosis.

Keywords

Innervation Zone; M-wave; Electrical Source Imaging; Spasticity; Neuromuscular Junctions; Botulinum neurotoxin Injection

* yzhang94@uh.edu.

1. Introduction

There is an unmet need for technology that can characterize the 3-dimensional (3D) distribution of neuromuscular junctions (NMJ) in muscles in vivo, in order to better diagnose and/or treat neuromuscular diseases. This is particularly the case for patients with spasticity who suffer from excessive muscle activities, or in patients with neurodegenerative diseases who suffer from a progressive loss of motor unit (MU) or muscle functions [1, 2].

For instance, botulinum neurotoxin (BTX) is considered as the first-line treatment in focal spasticity management. Intramuscular BTX injection has proven to be a relatively safe procedure [3], but it may still cause side effects or even severe complications, such as regional muscle weakness, blocking of autonomic nerves, and severe muscle atrophy due to anatomical denervation and immunological reactions [4–7]. The occurrence and severity of such side effects are dependent on the delivered dose of toxin [8, 9] and it has been clinically shown that injection of a minimum effective dose reduces the likelihood of such adverse effects [10].

BTX acts on the NMJs to inhibit muscle activity by blocking the release of acetylcholine presynaptically [1, 11, 12], and the effectiveness of the BTX injection is known to depend on the proximity of the injection site to the NMJ [13]. The efficacy of BTX is reported to be suppressed by as much as 46%, if the injection distance is 1 cm away from the NMJ indicated by innervation zone (IZ) [14]. Unfortunately, IZ locations vary radically between muscles and between individuals [15, 16]. Therefore, it is of clinical importance to accurately image NMJ distribution to guide BTX injection. It is also of great clinical value for studying the origin and progression of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) to globally image 3D distribution of functioning NMJs. Previous studies reported ALS as a distal axonopathy in which pathological changes start at the NMJs prior to CNS motor neuron degeneration [17, 18]. The spatial distribution of NMJs within the muscle obtained through our approach could potentially provide a valuable biomarker of disease progression, especially in the early stage, and could make early intervention feasible.

Recent advances in high-density (HD) surface electromyogram (sEMG) signal recording and processing techniques have greatly extended their potential applicability to the diagnosis and management of neuromuscular disorders. HD sEMG has been utilized to characterize the locations of the IZs, as an indicator of the NMJ in muscles [13, 19, 20]. One-dimensional (1D) linear electrode arrays [21, 22] and two-dimensional (2D) surface electrode grids [10, 23, 24] have both been employed to identify the locations of IZs by inspecting the reversal of single differential (or bipolar) signal polarities in neighboring channels along the muscle fiber direction [13, 25–27]. Automatic algorithms for IZ identification such as two dimensional correlation [23], Radon Transform [28], template matching [29] and optical flow [30] have also been reported. However, the IZs localized using these methods during voluntary contraction are only based on a sub-sample of the entire IZ pool, and therefore may not provide a comprehensive representation of the global distribution of all IZs of a muscle. Furthermore, the IZ locations identified using these methods are essentially a projection of the IZ distribution in the 3D space of a muscle onto the skin surface, since

previous methods cannot offer important information about the depth or the 3D distribution of the IZs. Currently, there is no technique available that can be used to characterize the distribution of the IZs in the 3D muscle space.

Bioelectrical activity imaging approach has been well developed and successfully used to localize bioelectrical activities in the brain from scalp electroencephalogram (EEG), and in the heart from body surface electrocardiogram (ECG) recordings, respectively [31–35]. Similar to EEG and ECG signals, surface EMG signals are composed of the action potentials of muscle fibers, and theoretically can be utilized to localize internal muscle activity or IZs in the muscles. However, only limited attempts have been made to localize the muscle activity in the muscles using noninvasive sEMG recordings [36–38], and no effort has been made to localize 3D IZ distributions of muscles until a recent report from our group [39].

In this recent study, Liu et al., successfully localized the IZs of individual MUs in the 3D space of the biceps in normal subjects from HD sEMG recordings during voluntary contractions by combining bioelectrical activity imaging and HD sEMG decomposition techniques [39]. However, only the IZs of a sample of the entire MU pool of the biceps, those activated during voluntary contraction and successfully identified with EMG decomposition, were localized. As such, those localized IZs are not necessarily a good representation of the global distribution of IZs of the biceps. In contrast, when the musculocutaneous nerve is maximally stimulated via electrical stimulation (M-wave recordings), the entire pool of MUs is considered to be activated and recorded [40, 41].

In the present study, a 3D global innervation zone imaging technique (GIZI) was developed to characterize the global distributions of innervation zones based on activation of the entire pool of MUs from surface M-wave recordings. The performance of the 3D IZ imaging method was evaluated on the biceps brachii in six healthy subjects.

2. Methods

2.1. Participants and consent

Six intact right-handed subjects (5 male, 1 female, mean age: 31 ± 6 years) participated in this study, with no reported history of neuromuscular diseases. The research protocol was approved by the local ethics committee, and all subjects were fully informed about the purpose of the study and each gave informed consent. The biceps brachii muscle of the dominant arm was investigated.

2.2. Experiment configuration

Subjects were seated with the tested arm supported by a horizontal surface. The subjects were asked to keep the hand in neutral position, wrist joint naturally rested and the elbow joint at approximately 120° of flexion. HD sEMG was recorded using two 64-channel electrode grids (TMSi, Enschede, the Netherlands), with an inter-electrode distance of 8.5 mm and a diameter of 4.5 mm for each individual electrode. These two grids were placed against each other on the biceps of the tested arm. As such there were 16 columns of electrodes along the arm axial direction (figure 1(a)). The grid position was adjusted to fully cover the muscle belly where NMJs are most densely distributed [13]. The skin over the

biceps brachii muscle was prepared with abrasive gel and alcohol prior to the electrode placement. A reference electrode (channel 1) was attached to the lateral condyle of the humerus where it is assumed to be electro-physiologically quiet, while the ground electrode was placed on the wrist of the idle arm using a highly conductive wrist strap.

Muscle responses were evoked and recorded by applying electrical stimulation to the proximal arm, with tests performed by an experienced physician (S.L.). Single square pulses (pulse width 200 μ s) were manually triggered using a muscle current stimulator DS7A (Digitimer, United Kingdom). The stimulating electrode placement was determined according to reported protocols [40]. Stimulation started with an intensity of 10 mA, and increased in 10 mA step until at least two supramaximal responses were observed. Sufficient resting time was taken between consecutive stimulations to avoid muscle fatigue. Monopolar EMG responses were amplified and digitized with a Refa-136 amplifier (TMSi, Enschede, the Netherlands) at a sampling rate of 2048 Hz per channel. All the raw data was saved for offline processing.

2.3. Data Preprocessing

Raw data were notch filtered at 60 Hz and high-pass filtered at 1 Hz with a second order Butterworth filter. The stimulation artefacts were then identified and removed using a recently developed approach [42]. Muscle EMG responses recorded under different stimulation intensities were segmented. Then the bipolar map of the HD sEMG recordings was obtained by calculating the signal difference between each channel and its distal neighbor. At the supramaximal responses, the surface IZ locations were identified from the bipolar map by visually inspecting the reversal of signal polarity in the neighboring channels. Then the time point when the bipolar signal at the identified IZ channel reached first peak value after stimulation onset was detected. And the amplitude profile of the monopolar signals among all channels at this time point was extracted for subsequent inverse calculation for each segment. This time instance belonged to the initiation phase of an M-wave and its corresponding 128-channel surface EMG profile incorporated the spatial information of the muscle innervation.

2.4. Modeling

The contraction of the biceps was assumed to be isometric during the stimulation experiment, since the reported M-wave responses precede the associated muscle contraction [43, 44]. For GIZI calculation, a realistic computational model of the upper right arm reconstructed from magnetic resonance (MR) axial images was modified in Rhinoceros 4.0 (Rhinoceros, Robert McNeel & Associates, Seattle). Solid geometries were meshed into a finite element model consist of 234, 103 tetrahedral elements and 40, 865 nodes, using Abaqus 6.12 (SIMULIA, Providence, RI). The final arm model was composed of the compact bone, cancellous bone, body fill, biceps brachii, triceps brachii, fat and skin, with conductivity assigned as 0.02 S/m, 0.075 S/m, 0.0379 S/m, 0.2455 S/m, 0.2455 S/m, 0.0379 S/m and 0.000455 S/m, respectively [39]. The arm model and meshed biceps part were shown in figure 1(b). A distributed, current dipole source model was introduced considering the irregular shape and sprouting of muscle fibers. A total number of 34, 619 current dipoles,

each with three orthogonal components, were evenly distributed in the biceps muscle volume, with an inter-dipole distance of 2 mm.

2.5. Source localization calculation

In this study, the weighted minimum norm estimation (WMNE) algorithm was employed to solve the inverse problem for source localization [33, 35, 39]. The linear relationship between the source space and the measurement can be described as:

$$\Phi = G \cdot J + n \quad (1)$$

where $\Phi = [\Phi_1(t), \Phi_2(t), \dots, \Phi_s(t)]^T$ represents the time-dependent EMG measurements from s sensors; $J = [j_1(t), j_2(t), \dots, j_{3d}(t)]^T$ denotes the strength of d dipoles in the 3D muscle space, with three directionally orthogonal component for each dipole moment. G is an s by $3 \times d$ lead field matrix, denoting the mapping from source space to the measurement space. n is the noise vector. In our study, the sensor number s is 127 and dipole number d is 34, 619. As $s \ll d$, the inverse calculation is an underdetermined problem, and the solution using minimum norm regularization can be achieved by minimizing Δ , given:

$$\Delta = \|\Phi - G \cdot J\|_2^2 + \lambda \|WJ\|_2^2 \quad (2)$$

where W is a $(3 \times d) \times (3 \times d)$ weighting matrix which modulates the influence of sources at different depth. The regularization parameter λ is determined by the L-curve method [45]. The estimated solution $\hat{J}$ is given by:

$$\hat{J} = (W^T W)^{-1} G^T (G (W^T W)^{-1} G^T + \lambda I)^{-1} \Phi \quad (3)$$

The muscle activity reconstruction results based on the EMG profile extracted (as described in section 2.3) were considered the direct indication of IZ location of the muscle. Dipoles with calculated current density exceeding a threshold were considered active and displayed overlapping with the biceps model for a direct visualization of the 3D distribution. To quantify the distribution of the reconstructed dipole sources within the muscle volume, a reference plane was defined at the medial epicondyle of humerus (EH) perpendicular to the longitudinal direction of the humerus. For each active dipole, two metrics were calculated: the longitudinal distance from the dipole location to the EH plane [46] and the depth of the dipole from the skin surface which was defined as the distance from the closest node on the skin model to the dipole location. The mean longitudinal distance and depth for all active dipoles were calculated.

3. Results

The bipolar map of representative HD sEMG responses under supramaximal stimulation is depicted in figure 2. The IZ of the biceps of this particular subject is roughly identified over

the skin surface via visual inspection. A reversal of signal polarity is observed in the proximal and distal neighbor of the marked IZ channel.

By applying 3D source imaging technique to the M-wave recordings, we were able to estimate the IZ locations and volume in 3D space of the bicep muscle. Figure 3(a)–3(f) shows the M-wave potential maps and the corresponding reconstructed spatial IZ distributions for all six subjects. Each red dot represents the location of an active electrical current dipole within the IZ, and the volume formed by red dot clouds represents the IZ in the 3D space of the biceps. Two separated IZs were found in the results of subjects #4 and #6, while only one IZ was found in the rest of the subjects.

Figure 4 shows the changes of the reconstructed IZ distributions and volumes under different stimulation intensities in two representative subjects. The stimulation intensity employed was increased from 10 mA to 50 mA (figure 4(a)–4(e) and figure 4(f)–4(j)), and the supramaximal stimulation threshold was found as 30 mA for both subjects. The corresponding changes of the reconstructed IZ distributions were shown in figure 4(a)–4(e) for the first subject, and figure 4(f)–4(j) for the second one. Once the stimulation intensities reach the threshold (subfigures in shaded region show the reconstructed IZs with supramaximal stimulation intensities), the reconstructed IZ volume does not increase any more as the stimulation intensity increases. With submaximal intensities, the reconstructed IZ volume decreases compared with that under supramaximal intensities in both subjects.

Table 1 summarizes the mean and standard deviation of the longitudinal distances towards medial EH and depths of all active dipoles in the reconstructed IZs for each subject. For all six subjects, the mean longitudinal distance and depth of each reconstructed IZ volume (mean $\pm$ standard deviation) is 9.4 ± 1.4 cm and 1.5 ± 0.3 cm, respectively. These results are calculated based on the IZ results at stimulation intensity of 50 mA (highlighted in dark rectangular in Table 1). The source depth varies from approximately 0.5 cm to 3 cm from the skin surface across the 6 subjects. These results were calculated from the IZ reconstruction results achieved at the supramaximal intensity of 50 mA. Again, once the stimulation intensities reach the threshold (values in shaded region show the reconstructed IZs with supramaximal stimulation intensities), the IZ-to-EH distances and depths of the reconstructed IZs do not change any more as the stimulation intensity increases. With submaximal intensities, different IZ-to-EH distances and depths of the reconstructed IZs were achieved at different stimulation intensities. The results achieved at supramaximal stimulation intensities (shaded region in table 1) and submaximal intensities were summarized in table 1.

4. Discussion

In this study, a novel 3D GIZI technique was developed to characterize the spatial distribution of IZ in the 3D muscle space from HD sEMG recordings of M-waves induced by nerve stimulation. The proposed M-wave based GIZI technique is based on the 3D IZ imaging technique which was developed by our group and validated with fine wire EMG signals simultaneously recorded with the high-density surface EMG signals [39]. The performance of the M-wave based GIZI was evaluated with the biceps brachii muscles in six

healthy subjects. The mean longitudinal IZ-to-EH distances achieved using the developed GIZI technique is 9.4 ± 1.4 cm across all six subjects, which is consistent to the findings in previous reports [46, 47]. Amirali et al (2007) used a nerve staining method to track the motor endplate region and reported a mean distance of 7 to 11 cm away from the olecranon. Lee et al. (2010) used a surgical microscope to observe the intra-muscular endings and reported a distance approximately 8 to 10 cm away from the EH. A mean depth of 1.5 ± 0.3 cm which was calculated from the geometric center of the reconstructed IZs to the closest skin surface points was achieved for the IZ distributions characterized using the GIZI technique.

A remarkable inter-subject variability in IZ locations, volume sizes and shapes, was found across the 6 subjects, which is in agreement with previous findings [48]. Interestingly, our GIZI results achieved in subject #4 and #6 showed two distinguishable IZ clusters, which could not be identified at all from the surface EMG maps as shown in figure 2. The separation of IZ clusters may be explained by the anatomical separation of biceps short head and long head distributions [46] and a subject-specific pattern in the spatial distribution of MUs. This finding suggests that our M-wave based GIZI technique can provide detailed information of the spatial distributions of IZs in the 3D space of the muscles.

Most of currently available surface EMG-based IZ detection techniques are essentially based on the 2D projection of the internal muscle activities onto the skin surface. These 2D IZ detection techniques suffer from the lack of depth information of the IZs in the muscle volume. Note that the IZ distributions identified using the proposed M-wave based GIZI technique in the subjects #4 and #5) are more proximally oriented and beyond the area covered by the EMG grids. Obviously there is an incomplete surface electrode coverage of the IZs, as reflected in the surface EMG potential maps in figure 2. Fortunately, the M-wave based GIZI technique can mathematically connect all the internal muscle activity to EMG measurements at any location over the skin surface of the upper arm via the volume conductor of the entire upper arm (for the biceps study) to solve this ‘mismatch’ or ‘incomplete coverage’ issue.

The changes of the reconstructed IZ distributions were investigated with respect to different stimulation intensities as shown in table 1 and figure 3. A consistent IZ distribution was observed in all subjects at supramaximal stimulation levels. The intra-subject variability of the proposed 3D GIZI approach has been tested with two individual trials on the same subjects performed separately at the same visit. Very similar reconstructed dipole density results were achieved from the individual trials. Different supramaximal stimulation intensities were also tested in each participants and the reconstructed current density results were also similar and consistent, as shown in Figure 4 and Table 1. The well proved robustness of M-wave recordings also braces the reliability of proposed M-wave based GIZI technique [49]. Nonetheless, a demonstration of reliability at two separate visits will further enhance the potential applicability of GIZI in clinics and is under our consideration in our future study. With stimulation at submaximal levels, the volume of reconstructed IZs decreased as we expected as the stimulation intensity decreased as shown in table 1 and figure 3(a)–3(e), since only a portion of the MUs in the biceps was recruited. However, the changes of volume sizes and shapes of the reconstructed IZs vary from subjects to subjects

as indicated in table 1 and figure 3(f)–3(j) which may reflect their subject-specific MU recruitment patterns.

The proposed 3D GIZI technique was applied to M-wave recordings in this study to characterize the global innervation of the biceps. It may not be appropriated to apply this technique to EMG data acquired under voluntary contractions, especially strong contractions. Muscle deformation, non-uniform spatial muscle behavior and IZ shift often accompanies with relatively strong contractions [20, 50], consequently, the static finite element model which is employed in the implementation of the 3D GIZI technique will no longer be a good approximation. Moreover, sufficient MU spatial-temporal information cannot be secured under voluntary contractions, where only part of the MU pool may be recruited. The IZs localized from this incomplete information may not be a good presentation of the global IZ pool.

The current study presents the first effort to prove the concept and feasibility of applying the source localization to investigate the global muscle innervation in vivo, with results consistent with previous anatomical reports. Imaging results of single MUs using similar isotropic model have been validated with intramuscular recordings in a previous study [39]. Despite of these satisfactory results proved, it should be noted that the isotropic model employed was a less ideal approximation of the electrical properties of muscles. It remains our ongoing effort to incorporate anisotropic properties of muscles into our model to optimize the IZ imaging accuracy.

Electrophysiological parameters remain the most important approach for reducing the risk of neuromuscular damage during surgery, treating muscle hyperactivity by providing guidance of BTX injection and tracing progressive neurodegenerative disorders such as ALS. With the use of surface EMG measurements and electrical stimulation to elicit the M-wave, our procedure can be conveniently completed within 10 to 20 minutes for each subject visit. The employment of M-wave is also helpful when dealing with patients suffering from high level motor or cognitive deficits by avoiding necessity of performing voluntary contractions.

5. Conclusion

In our study, the proposed GIZI method has been proved to be a novel and stable technique to noninvasively characterize the comprehensive spatial distribution of IZs in the biceps brachii, by combining source localization and M-wave recordings. The GIZI results revealed a marked inter-subject variability of IZ distributions and further consolidated the significance of this technique. This approach would be useful in clinical applications such as guidance of BTX injection and early diagnosis of ALS disease. The well-reconstructed depth information further extends its applicability in clinics and research.

Acknowledgments

The author thanks to the support from NIH DK082644, NIH HD050821 and the University of Houston.

References

1. Breidenbach MA, Brunger AT. New insights into clostridial neurotoxin–SNARE interactions. *Trends in molecular medicine*. 2005; 11(8):377–81. [PubMed: 16006188]
2. Dupuis L, Loeffler J-P. Neuromuscular junction destruction during amyotrophic lateral sclerosis: insights from transgenic models. *Current opinion in pharmacology*. 2009; 9(3):341–6. [PubMed: 19386549]
3. Klein AW. Complications and adverse reactions with the use of botulinum toxin. *Disease-A-Month*. 2002; 48(5):336–56. [PubMed: 12195264]
4. Simpson D, Blitzer A, Brashear A, Comella C, Dubinsky R, Hallett M, et al. Assessment: Botulinum neurotoxin for the treatment of movement disorders (an evidence-based review) Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology*. 2008; 70(19):1699–706. [PubMed: 18458230]
5. Warner SE, Sanford DA, Becker BA, Bain SD, Srinivasan S, Gross TS. Botox induced muscle paralysis rapidly degrades bone. *Bone*. 2006; 38(2):257–64. [PubMed: 16185943]
6. Borodic GE, Joseph M, Fay L, Cozzolino D, Ferrante RJ. Botulinum A toxin for the treatment of spasmodic torticollis: dysphagia and regional toxin spread. *Head & neck*. 1990; 12(5):392–9. [PubMed: 2211099]
7. Eleopra R, Tugnoli V, Caniatti L, De Grandis D. Botulinum toxin treatment in the facial muscles of humans Evidence of an action in untreated near muscles by peripheral local diffusion. *Neurology*. 1996; 46(4):1158–60. [PubMed: 8780112]
8. Brans J, De Boer I, Aramideh M, Speelman J, de Visser BO. Botulinum toxin in cervical dystonia: low dosage with electromyographic guidance. *Journal of neurology*. 1995; 242(8):529–34. [PubMed: 8530982]
9. Jahn R. A neuronal receptor for botulinum toxin. *Science*. 2006; 312(5773):540–1. [PubMed: 16645086]
10. Lapatki B, Van Dijk J, Van de Warrenburg B, Zwarts M. Botulinum toxin has an increased effect when targeted toward the muscle's endplate zone: a high-density surface EMG guided study. *Clinical Neurophysiology*. 2011; 122(8):1611–6. [PubMed: 21195024]
11. Simpson DM, Gracies J-M, Yablon SA, Barbano R, Brashear A, Team BTS. Botulinum neurotoxin versus tizanidine in upper limb spasticity: a placebo-controlled study. *Journal of Neurology, Neurosurgery & Psychiatry*. 2009; 80(4):380–5.
12. Albanese A, Asmus F, Bhatia K, Elia AE, Elibol B, Filippini G, et al. EFNS guidelines on diagnosis and treatment of primary dystonias. *European Journal of Neurology*. 2011; 18(1):5–18. [PubMed: 20482602]
13. Beretta Piccoli M, Rainoldi A, Heitz C, Wüthrich M, Boccia G, Tomasoni E, et al. Innervation zone locations in 43 superficial muscles: toward a standardization of electrode positioning. *Muscle & nerve*. 2014; 49(3):413–21. [PubMed: 24741685]
14. Boccia G, Rainoldi A. Innervation zones location and optimal electrodes position of obliquus internus and obliquus externus abdominis muscles. *Journal of Electromyography and Kinesiology*. 2014; 24(1):25–30. [PubMed: 24280244]
15. Merletti R, Conte LL, Avignone E, Guglielminotti P. Modeling of surface myoelectric signals. I. Model implementation. *IEEE transactions on biomedical engineering*. 1999; 46(7):810–20. [PubMed: 10396899]
16. Merletti R, Bottin A, Cescon C, Farina D, Gazzoni M, Martina S, et al. Multichannel surface EMG for the non-invasive assessment of the anal sphincter muscle. *Digestion*. 2004; 69(2):112–22. [PubMed: 15087578]
17. Wong M, Martin LJ. Skeletal muscle-restricted expression of human SOD1 causes motor neuron degeneration in transgenic mice. *Human molecular genetics*. 2010; 19(11):2284–302. [PubMed: 20223753]
18. Mancuso R, Navarro X. Amyotrophic lateral sclerosis: Current perspectives from basic research to the clinic. *Progress in Neurobiology*. 2015; 133:1–26. [PubMed: 26253783]

19. Masuda T, Sadoyama T. Topographical map of innervation zones within single motor units measured with a grid surface electrode. *Biomedical Engineering, IEEE Transactions on*. 1988; 35(8):623–8.
20. Piitulainen H, Rantalainen T, Linnamo V, Komi P, Avela J. Innervation zone shift at different levels of isometric contraction in the biceps brachii muscle. *Journal of electromyography and kinesiology*. 2009; 19(4):667–75. [PubMed: 18387314]
21. Merletti R, Farina D, Gazzoni M. The linear electrode array: a useful tool with many applications. *Journal of Electromyography and Kinesiology*. 2003; 13(1):37–47. [PubMed: 12488085]
22. Yao B, Zhang X, Li S, Li X, Chen X, Klein CS, et al. Analysis of linear electrode array EMG for assessment of hemiparetic biceps brachii muscles. *Frontiers in human neuroscience*. 2015:9.
23. Ullah K, Cescon C, Afsharipour B, Merletti R. Automatic detection of motor unit innervation zones of the external anal sphincter by multichannel surface EMG. *Journal of Electromyography and Kinesiology*. 2014; 24(6):860–7. [PubMed: 24948528]
24. Peng Y, He J, Khavari R, Boone TB, Zhang Y. Functional mapping of the pelvic floor and sphincter muscles from high-density surface EMG recordings. *International urogynecology journal*. 2016:1–8.
25. Saitou K, Masuda T, Michikami D, Kojima R, Okada M. Innervation zones of the upper and lower limb muscles estimated by using multichannel surface EMG. *Journal of human ergology*. 2000; 29(1/2):35–52. [PubMed: 12696320]
26. Barbero M, Gatti R, Conte LL, Macmillan F, Coutts F, Merletti R. Reliability of surface EMG matrix in locating the innervation zone of upper trapezius muscle. *Journal of Electromyography and Kinesiology*. 2011; 21(5):827–33. [PubMed: 21752668]
27. Guzmán-Venegas RA, Araneda OF, Silvestre RA. Differences between motor point and innervation zone locations in the biceps brachii. An exploratory consideration for the treatment of spasticity with botulinum toxin. *Journal of Electromyography and Kinesiology*. 2014; 24(6):923–7. [PubMed: 25138645]
28. Cescon, C., editor. *Medical Measurement and Applications, 2006 MeMea 2006 IEEE International Workshop on*. IEEE; 2006. Automatic location of muscle innervation zones from multi-channel surface EMG signals.
29. Mesin L, Gazzoni M, Merletti R. Automatic localisation of innervation zones: a simulation study of the external anal sphincter. *Journal of Electromyography and Kinesiology*. 2009; 19(6):e413–e21. [PubMed: 19269857]
30. Östlund N, Gerdle B, Karlsson JS. Location of innervation zone determined with multichannel surface electromyography using an optical flow technique. *Journal of Electromyography and Kinesiology*. 2007; 17(5):549–55. [PubMed: 16890457]
31. Mosher JC, Spencer ME, Leahy RM, Lewis PS. Error bounds for EEG and MEG dipole source localization. *Electroencephalography and clinical Neurophysiology*. 1993; 86(5):303–21. [PubMed: 7685264]
32. Pascual-Marqui RD, Michel CM, Lehmann D. Low resolution electromagnetic tomography: a new method for localizing electrical activity in the brain. *International Journal of psychophysiology*. 1994; 18(1):49–65. [PubMed: 7876038]
33. Zhang Y, Ding L, van Drongelen W, Hecox K, Frim DM, He B. A cortical potential imaging study from simultaneous extra-and intracranial electrical recordings by means of the finite element method. *NeuroImage*. 2006; 31(4):1513–24. [PubMed: 16631381]
34. He B, Liu C, Zhang Y. Three-dimensional cardiac electrical imaging from intracavity recordings. *IEEE transactions on biomedical engineering*. 2007; 54(8):1454–60. [PubMed: 17694866]
35. Zhang Y, van Drongelen W, Kohrman M, He B. Three-dimensional brain current source reconstruction from intra-cranial ECoG recordings. *NeuroImage*. 2008; 42(2):683–95. [PubMed: 18579412]
36. Jesinger, RA., Stonick, VL., editors. *Digital Signal Processing Workshop, 1994, 1994 Sixth IEEE. IEEE; 1994. Processing signals from surface electrode arrays for noninvasive 3D mapping of muscle activity*.
37. Van Den Doel K, Ascher UM, Pai DK. Source localization in electromyography using the inverse potential problem. *Inverse Problems*. 2011; 27(2):025008.

38. Zhang YC, Wang D, Timm GW. A three-dimensional muscle activity imaging technique for assessing pelvic muscle function. *Inverse Problems*. 2010; 26(11):115018.
39. Liu Y, Ning Y, Li S, Zhou P, Rymer WZ, Zhang Y. Three-dimensional innervation zone imaging from multi-channel surface EMG recordings. *International journal of neural systems*. 2015; 25(06):1550024. [PubMed: 26160432]
40. Li S, Liu J, Bhadane M, Zhou P, Rymer WZ. Activation deficit correlates with weakness in chronic stroke: evidence from evoked and voluntary EMG recordings. *Clinical Neurophysiology*. 2014; 125(12):2413–7. [PubMed: 24747057]
41. Li S, Latash ML, Yue GH, Siemionow V, Sahgal V. The effects of stroke and age on finger interaction in multi-finger force production tasks. *Clinical neurophysiology*. 2003; 114(9):1646–55. [PubMed: 12948793]
42. Liu J, Li S, Li X, Klein C, Rymer WZ, Zhou P. Suppression of stimulus artifact contaminating electrically evoked electromyography. *NeuroRehabilitation*. 2014; 34(2):381–9. [PubMed: 24419021]
43. Place N, Bruton JD, Westerblad H. Mechanisms of fatigue induced by isometric contractions in exercising humans and in mouse isolated single muscle fibres. *Clinical and Experimental Pharmacology and Physiology*. 2009; 36(3):334–9. [PubMed: 18671711]
44. Millet G, Lepers R, Maffiuletti N, Babault N, Martin V, Lattier G. Alterations of neuromuscular function after an ultramarathon. *Journal of applied physiology*. 2002; 92(2):486–92. [PubMed: 11796655]
45. Hansen PC. Analysis of discrete ill-posed problems by means of the L-curve. *SIAM review*. 1992; 34(4):561–80.
46. Lee J-H, Kim H-W, Im S, An X, Lee M-S, Lee U-Y, et al. Localization of motor entry points and terminal intramuscular nerve endings of the musculocutaneous nerve to biceps and brachialis muscles. *Surgical and radiologic anatomy*. 2010; 32(3):213–20. [PubMed: 19779662]
47. Amirali A, Mu L, Gracies J-M, Simpson DM. Anatomical localization of motor endplate bands in the human biceps brachii. *Journal of clinical neuromuscular disease*. 2007; 9(2):306–12. [PubMed: 18090684]
48. Masuda T, Sadoyama T. Distribution of innervation zones in the human biceps brachii. *Journal of Electromyography and Kinesiology*. 1991; 1(2):107–15. [PubMed: 20870500]
49. Calder KM, Hall L-A, Lester SM, Inglis JG, Gabriel DA. Reliability of the biceps brachii M-wave. *Journal of neuroengineering and rehabilitation*. 2005; 2(1):33. [PubMed: 16332261]
50. DeFreitas JM, Costa PB, Ryan ED, Herda TJ, Cramer JT, Beck TW. An examination of innervation zone movement with increases in isometric torque production. *Clinical Neurophysiology*. 2008; 119(12):2795–9. [PubMed: 18996046]

Figure 1.

Experiment diagram and an example of the realistic finite element model. (a) The placement of high-density electrode grids over the skin which covers the biceps. The red and green line marks the locating region of two 8*8 recording electrode grids in the magnified photo. The white arrow marks the stimulation site. (b) Seven anatomical structures incorporated in the finite element model of the upper arm (top), and the placement of electrode grids relative to the biceps (bottom), the blue dots indicate the location of 128 sensors.

Figure 2.

Bipolar map of the surface EMG responses in one subject under the stimulation intensity of 50 mA. The purple circle marks the visually identified IZ.

Figure 3.

3D GIZI results of all six subjects. The three subplots in the left panel for each subject display the distal, top and side view of reconstructed imaging results in the biceps model, respectively. The red dots represent the active current dipoles in the IZ and the blue stars indicate the locations of EMG electrodes. The subplot in the right panel box shows the corresponding surface EMG potential distributions.

Figure 4.

Reconstructed IZ distributions from the M-wave recordings induced by the musculocutaneous nerve stimulation with a series of incremental stimulation intensities in Subject#1 (a)–(e) and Subject #2 (f)–(j). The red dots represent the active current dipoles in the IZ and the blue stars indicate the locations of EMG electrodes. The shaded regions highlight the imaging result under supramaximal stimulation intensities.

Table 1

Mean distances towards medial EH and depths of all active dipoles in the reconstructed IZ.

SN ^a	Metrics(cm)	Stimulation Intensity (mA)					
		60	50	40	30	20	10
01	Dist.	-	8.6(1.4) ^c	8.5(1.4)	8.4(1.4)	8.5(1.2)	8.6(0.7)
	Depth	-	1.9(0.6)	1.9(0.6)	1.9(0.6)	1.8(0.5)	1.6(0.3)
02	Dist. ^b	-	7.5(0.9)	7.5(0.9)	7.5(0.9)	7.8(0.8)	8.0(1.2)
	Depth	-	1.4(0.4)	1.4(0.4)	1.4(0.4)	1.3(0.4)	1.5(0.4)
03	Dist.	8.6(0.8)	8.6(0.8)	8.8(0.9)	9.5(1.2)	9.8(1.5)	10.1(0.7)
	Depth	1.5(0.4)	1.5(0.4)	1.5(0.4)	1.7(0.4)	1.7(0.5)	1.5(0.3)
04	Dist.	-	10.4(1.8)	10.3(1.7)	7.6(0.8)	7.6(0.9)	7.5(0.6)
	Depth	-	1.2(0.3)	1.2(0.3)	1.6(0.4)	1.7(0.4)	1.3(0.4)
05	Dist.	-	11.4(1.2)	10.5(1.2)	11.2(0.6)	10.4(0.7)	10.5(0.7)
	Depth	-	1.2(0.3)	1.2(0.2)	1.2(0.2)	1.4(0.3)	1.4(0.3)
06	Dist.	10.1(1.7)	10.0(1.7)	10.5(1.6)	10.3(1.7)	13.4(0.7)	13.0(1.1)
	Depth	1.6(0.3)	1.6(0.3)	1.6(0.3)	1.6(0.3)	1.8(0.3)	1.9(0.5)

^aSN = subject number

^bDist. = Mean distance towards medial EH of all active dipoles in the reconstructed IZ(s)

^cThe shaded values correspond to the GIZI results achieved with supramaximal stimulation intensities.